



SOURCES OF FECAL INDICATOR BACTERIA TO GROUNDWATER, MALIBU LAGOON AND THE NEAR-SHORE OCEAN, MALIBU, CALIFORNIA, USA

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ABSTRACT

Onsite wastewater treatment systems (OWTS) used to treat residential and commercial sewage near Malibu, California have been implicated as a possible source of fecal indicator bacteria (FIB) to Malibu Lagoon and the near-shore ocean. For this to occur, treated wastewater must first move through groundwater before discharging to the Lagoon or ocean. In July 2009 and April 2010, $\delta^{18}\text{O}$ and δD data showed that some samples from water-table wells contained as much as 70% wastewater; at that time FIB concentrations in those samples were generally less than the detection limit of 1 Most Probable Number (MPN) per 100 milliliters (mL). In contrast, Malibu Lagoon had total coliform, *Escherichia coli*, and enterococci concentrations as high as 650,000, 130,000, and 5,500 MPN per 100 mL, respectively,

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and as many as 12% of samples from nearby ocean beaches exceeded the U.S. Environmental Protection Agency single sample enterococci standard for marine recreational water of 104 MPN per 100 mL. Human-associated *Bacteroidales*, an indicator of human-fecal contamination, were not detected in water from wells, Malibu Lagoon, or the near-shore ocean. Similarly, microarray (PhyloChip) data show *Bacteroidales* and *Fimicutes* Operational Taxonomic Units (OTUs) present in OWTS were largely absent in groundwater; in contrast, 50% of *Bacteroidales* and *Fimicutes* OTUs present in the near-shore ocean were also present in gull feces. Terminal-Restriction Length Fragment Polymorphism (T-RFLP) and phospholipid fatty acid (PLFA) data showed that microbial communities in groundwater were different and less abundant than communities in OWTS, Malibu Lagoon, or the near-shore ocean. However, organic compounds indicative of wastewater (such as fecal sterols, bisphenol-A and cosmetics) were present in groundwater having a high percentage of wastewater and were present in groundwater discharging to the ocean. FIB in the near-shore ocean varied with tides, ocean swells, and waves. Movement of water from Malibu Lagoon through the sand berm at the mouth of the Lagoon contributed FIB to the adjacent beach at low tide. Similar increases in FIB concentrations did not occur at beaches adjacent to unsewered residential development, although wastewater indicator compounds and radon-222 (indicative of groundwater discharge) were present. High FIB concentrations at high tide were not related to groundwater discharge, but may be related to FIB associated with debris accumulated along the high-tide line.

Keywords: fecal indicator bacteria, microbial source tracking, surface water, groundwater, coastal water, wastewater indicators

1. INTRODUCTION

Each year more than 550 million people visit California's public beaches [1]. To protect beachgoers from exposure to waterborne disease, California state law requires water-quality monitoring for fecal indicator bacteria (FIB), such as enterococci and *Escherichia coli* (*E. coli*) at beaches with more than 50,000 visitors [2]. Although not typically disease causing, FIB are used to assess the microbiological quality of recreational waters because high FIB concentrations are correlated with the occurrence of certain waterborne diseases [3-10]. FIB are used as a

surrogate for fecal contamination because they are 1) present at high concentrations in human waste and 2) relatively easily and inexpensively measured using standardized tests. Although not necessarily fecal in origin, total coliform bacteria also are commonly used with FIB to assess microbial quality of recreational waters.

The use of FIB to determine the health risk associated with recreational waters is complicated by the presence of FIB in warm-blooded animals other than humans, including farm animals, pets, and rodents. Seabirds living along shorelines also may be sources of FIB [11-15]. In addition to contamination by human and animal feces, extended survival or regrowth of FIB can occur in streambed and lagoonal sediments [16-20], biofilms along stream channels and in urban storm drains [20-22], beach sand [23-32], coastal wetlands and intertidal zones [11,15,33,34], and in kelp and other debris accumulated along the high tide line (wrack line) at ocean beaches [29,35,36].

Recent studies have implicated groundwater discharge as a possible source of fecal contamination to recreational ocean beaches [37-39]. However, the survival and transport of FIB in groundwater is limited [40]. In typical soils, 90% of FIB die within 3 to 13 days [41], with the longer survival times occurring during cooler periods and in moist, organic-rich soils [42]. Although FIB may survive from 2 to 4 months after they reach the water table [43], transport of bacteria through saturated porous media is limited by the slow movement of groundwater, and by physical filtration and adsorption of bacteria onto aquifer materials [43,44].

In recent years a wide range of genetic and chemical tracer techniques have become available to supplement traditional measurements of FIB in water to aid in determining the source of fecal contamination in recreational waters. These techniques include genetic and molecular characterization of microbial communities associated with different fecal sources [45-51] or measurement of specific microorganisms that can be linked to animal or human fecal sources, such as human-associated *Bacteroidales*, enteroviruses, or adenoviruses [52-60]. Recently developed microarray techniques (also known as Phylochip) that use genetic probes embedded on silicon chips to identify the presence of genetic material from almost 60,000 bacterial taxa may prove useful in the identification of microorganisms associated with fecal sources and for characterization of microbial populations [61,62]. Other approaches rely on measurement of low concentrations of chemicals

commonly associated with human wastewater [63-67], including fecal sterols [68-75].

The use of tracer techniques, especially genetically-based tracers, to determine the source(s) of fecal contamination has expanded rapidly in recent years [76,77]. Because each technique has limitations, studies of fecal contamination increasingly use multiple tracers coupled with hydrologic data to constrain the source of FIB in complex settings. As hydrologic controls on the occurrence of FIB and their source are increasingly recognized, the need for detailed spatial and temporal sample collection to characterize relations to streamflow, tides, wave action, and other hydrologic or meteorological processes is increasing [29,78-81].

This study uses multiple hydrologic, microbiological, and chemical tracers to examine the potential sources of FIB in a complex coastal setting, considering the source and hydrologic history of the water, timing of groundwater discharge, tidal and wave action, and other coastal processes.

1.1. Study Area

The study area is the Civic Center area of Malibu, about 40 km west of downtown Los Angeles, California (Figure 1). The climate is Mediterranean, with cool wet winters and warm dry summers. Average annual precipitation, falling mostly as rain during winter storms between November and March, is about 340 mm. The data for this study were collected in 2009 and 2010 following an extended drought. Precipitation in the study area during the 2009 rainy season was about 60% of normal, and during the 2010 rainy season about 110% of normal.

The area contains alluvial deposits about 3.4 km² in extent [82], having a maximum depth of approximately 60 m below sea level [83]. Malibu Lagoon and associated wetlands occupy about 9 hectares near the eastern edge of the alluvium. The alluvial deposits are surrounded and underlain by low-permeability consolidated marine, non-marine, and volcanic rock that compose the Santa Monica Mountains [82].

Land use in the Civic Center area includes undeveloped land (including parkland surrounding Malibu Lagoon) and low-density and high-density residential and commercial uses. The area is unsewered. Residential and commercial wastewater is treated by onsite wastewater-treatment systems (OWTS) prior to discharge to shallow groundwater. More than 400 OWTS were identified in and near the Civic Center area; 49 of these systems served

commercial properties [84]. Most systems were traditional septic systems; however, almost 30 advanced systems were in use by 2010. Most of the advanced systems served newer residential properties in Malibu Colony and commercial properties [84]. The advanced systems commonly contain multiple treatment processes intended to reduce fecal bacterial and nutrient concentrations, and some of the advanced systems disinfect prior to discharge.

Historically, depth to water in the alluvial deposits ranged from 0 m near the Lagoon and ocean to 10 m below ground surface in upland areas [83,85-87]. Prior to the importation of water, the alluvial deposits were a source of public supply. These deposits are not presently pumped for supply, and water from northern California and the Colorado River is imported for public supply. Groundwater recharge occurs as infiltration of streamflow from Malibu Creek, infiltration of runoff from the surrounding uplands, direct infiltration of precipitation and as groundwater movement from surrounding consolidated rock. Additional recharge also occurs from infiltration of water imported for public supply and discharged through OWTS or from infiltration of landscape irrigation water. Most landscape irrigation occurs in the low-density residential areas in the

northern part of the study area. Recharge to the alluvial aquifer from OWTS discharges has been estimated to be about 1,050 cubic meters per day (m^3/d), which is about 28% of the total recharge [88]. Discharge from the alluvial aquifer occurs to Malibu Lagoon and to the near-shore ocean [83,88].

During the dry season, a berm develops at the mouth of Malibu Lagoon separating the Lagoon from the ocean. As a consequence, water levels in the Lagoon and parts of the surrounding alluvial aquifer are higher during the dry season than in the wet season. Median depth to water in sampled wells was 1.8 m during July 2009, when the berm in the Lagoon was closed. Minimum depth to water for discharge from OWTS is commonly 1 m for areas having percolation rates of 2 min/cm or greater [89,90]. During the wet season, streamflow in Malibu Creek prevents the development of a berm and water levels in the Lagoon vary with the daily tidal cycle. Median depth to water in sampled wells was 2.5 m during April 2010, when the berm in Malibu Lagoon was open. During the wet season a greater fraction of groundwater discharges to the Lagoon and flows to the ocean with the outgoing tide, and a smaller fraction of groundwater discharges directly to the Pacific Ocean [83,88] (Figure 2).



Figure 1 Study area location

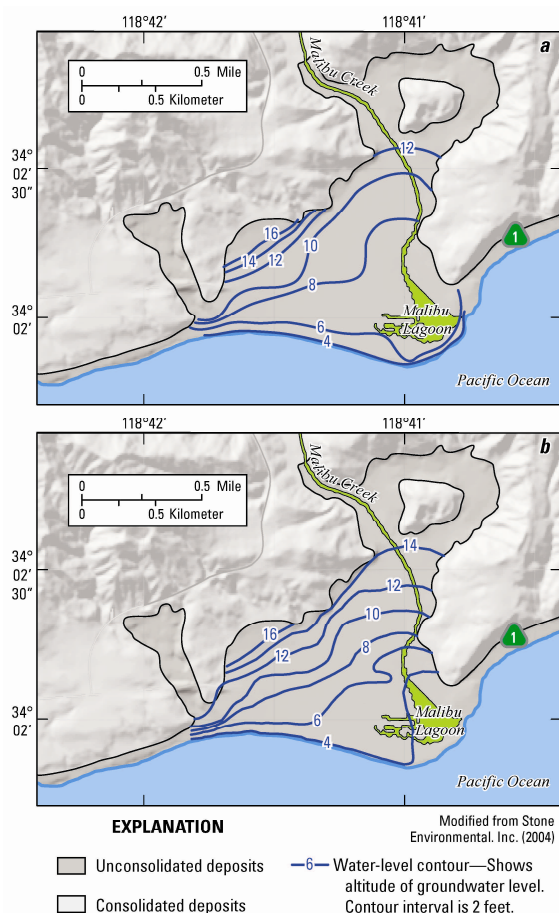


Figure 2 Water-table contours in the alluvial deposits near the Civic Center area, Malibu California, September 2003 and March 2004

1.2. History of Fecal Indicator Bacteria Occurrence

The occurrence of FIB at concentrations above standards for recreational water has been a long-standing concern in Malibu Lagoon and the nearby recreational ocean beaches [91]. An association between FIB in Malibu Lagoon with human waste and the presence of human-enterovirus (Coxsackie B) was first reported by Gold et al. [92]. Although the source of the human-enterovirus was not determined, upstream wastewater discharges were considered unlikely, and more diffuse local sources such as incidental human releases or OWTS were implicated [92]. Although the concern that improperly treated human waste may reach groundwater and subsequently discharge to sensitive receiving waters is greater when the Lagoon is full and groundwater

levels are high, Gold et al. [92] were unable to demonstrate a link between Lagoon water levels and FIB or enterovirus concentrations.

FIB have been reported in water from wells in the alluvial aquifer underlying the Civic Center area [83] and high FIB concentrations were measured in the discharge from some OWTS [93]. Fecal sterols, possibly associated with OWTS discharges, were reported in surface drains that cross the alluvial deposits and flow into Malibu Lagoon [94]. It has been suggested by regulatory agencies [93,95] that high concentrations of FIB in groundwater may be responsible for FIB in the near-shore ocean and Malibu Lagoon. However, other work did not detect human-enterovirus in Malibu Lagoon [96] and concluded that OWTS discharges could not explain the high concentrations of FIB in Malibu Lagoon and nearby ocean beaches [94]. Concern over FIB concentrations and nutrients in groundwater led to a ban on the onsite disposal of human waste in the Malibu Civic Center area [93]. The ban was intended to protect sensitive receiving waters such as Malibu Lagoon and recreational beaches from human fecal contamination. Epidemiological studies were designed to assess the health risk to swimmers at Surfrider Beach adjacent to Malibu Lagoon and the Civic Center area [97].

1.3. Purpose and Scope

The purpose of this study was to determine the source of FIB to shallow groundwater, Malibu Lagoon and the near-shore ocean near Malibu, California. The scope of the study included collection of more than 450 samples, primarily during the dry season (July 21-27, 2009) and near the end of the rainy season (April 17-22, 2010). FIB in water from wells, Malibu Lagoon, Malibu Creek, and the near-shore ocean were analyzed in a temporary, onsite laboratory, generally within 6 hours of collection. The wastewater history of samples was evaluated on the basis of the stable oxygen (delta oxygen-18) and hydrogen (delta deuterium) isotopic composition of the water molecule. Groundwater exchange with Malibu Lagoon and with the near-shore ocean was evaluated on the basis of hydrologic, geophysical, and radon-222 data. Potential sources of FIB were evaluated using genetic (Terminal-Restriction Fragment Length Polymorphism, human-associated *Bacteroidales*, and microarray data), molecular (phospholipid fatty acid) and selected organic compound (wastewater indicator) data from more than 50 samples.

2. METHODS

Sample collection and analysis was distributed spatially and temporally to provide data on seasonally- and tidally-driven changes in selected hydrologic processes that may affect FIB concentrations in the study area (Table 1). Samples from wells, which were presumed to be less variable than surface water or the near-shore ocean, were collected once during each sample period to assess seasonal differences in FIB concentrations and water quality. In contrast, samples from the near-shore ocean were collected at high tide, low tide, mid-high, and mid-low tides each day during a falling tidal cycle from spring to neap tide to address the effect of tidally driven changes in sea level on FIB concentrations. Detailed sample collection (including continuous measurement of radon-222 activity) from the near-shore ocean during falling daily tidal cycles (not shown on Table 1) was done hourly adjacent to Malibu Lagoon and adjacent to unsewered residential development in Malibu Colony to identify rapid changes in FIB concentrations associated with groundwater discharge at lower tidal stands, or with wave action on the beach and the wrack line at higher tidal stands. It was not possible to analyze all samples for all constituents, and less expensive analyses such as field parameters or FIB were conducted at higher frequency on more samples than more expensive genetic, molecular or chemical analysis.

2.1. Field Methods

Monitoring wells. Eleven existing monitoring wells were sampled in July, 2009. Fifteen monitoring wells were sampled in April, 2010. Additional wells in the commercial district near Malibu Lagoon were sampled in April 2010 to provide more data in that area (Figure 3). Well construction data, including depth and screened interval, and water-level data are provided in Table 2. Wells were purged to remove at least 3 casing volumes prior to sample collection. Field parameters (pH, temperature, and specific conductance) were monitored during purging. Purging continued until field parameters stabilized. Samples were collected using peristaltic pumps. Pump tubing, including tygon tubing at the pump head, was disposed of after sample collection from each well and not reused. Samples were analyzed for various constituents as described in Table 1.

Malibu Lagoon and Malibu Creek. Samples were collected from seven sites in Malibu Lagoon during the July and April sample periods and from Malibu

Creek upstream of the Civic Center area (Figure 4). At three locations (ML-Upper, ML-Middle, and ML-Lower, respectively, Figure 4) samples were collected at selected depths during the July and April sample periods to evaluate the effect of stratification within the Lagoon on FIB concentrations. At one site on the berm of the Lagoon (ML-Berm, Figure 4), grab samples were collected in July and April at high, low, mid-high, and mid-low tidal stands during a falling monthly tidal cycle from spring to neap tide, and hourly during a falling daily tidal cycle from high to low tide. Samples also were collected from the discharge of the Lagoon (ML-discharge, Figure 4) at the various tidal stands from spring to neap tide during April 2010 when the Lagoon was open to the ocean, and hourly during a falling daily tidal cycle. Samples were analyzed for field parameters and FIB. Selected samples from within the Lagoon (ML-Comm and ML-West), Malibu Creek, and samples collected during the falling daily tidal cycles at ML-Berm (near high and low tide), and ML-Discharge (low tide) were analyzed for genetic, molecular and chemical tracers (Table 1).

Near-shore ocean. Samples were collected during a falling tidal cycle from spring to neap tide at high, low, mid-high, and mid-low tides in the swash zone (approximately ankle to mid-calf depth) from three sites: 1) west of the residential development (Puerco Beach), 2) near Malibu Colony and 3) east of Malibu Lagoon (Surfrider Beach) (Figure 4). Timing of sample collection at these three sites was concurrent with similar samples collected at ML-Berm and ML-Discharge. These samples were analyzed for field parameters and FIB (Table 1).

Detailed sample collection was done at hourly intervals during falling daily tidal cycles (high to low tide) in July and November 2009 and in April 2010 on the berm adjacent to Malibu Lagoon, and on the beach adjacent to unsewered residential development in Malibu Colony. (Because of high surf conditions in July 2009, hourly sample collection adjacent to Malibu Colony was done during a rising tidal cycle.) Sample collection at these sites was from the swash zone, sample intakes just above the seafloor attached to buoys just outside the surf zone, piezometers driven into the beach at different depths, and seepage samplers located just below the mid-low and just above the low tide line (Figure 5). Hourly samples were analyzed for field parameters and FIB. Selected samples (generally coinciding with high tide and low tide) were chemically analyzed and for genetic, molecular and chemical tracers of FIB sources (Table 1).

Table 1 Sample collection and analysis plan for monitoring wells, Malibu Creek, Malibu Lagoon, and the near-shore ocean, Malibu, California.

Sites	Field parameters	Major and minor ions, and selected trace elements	Nutrients	Optical properties	Stable isotopes of oxygen and hydrogen (d ¹⁸ O and dD)	Continuous radon-222	Fecal indicator bacteria	Human-associated <i>Bacteroidales</i>	Genetic data				Molecular data	
									Terminal-Restriction Length Fragment Polymorphism (T-RLFP)	Microarray (PlyloChip)	Phospholipid fatty acids (PLFA)	Fecal sterols	Selected organic compounds	
Monitoring wells		Each monitoring well sampled once during July, 2009 and during April, 2010												
July, 2009														
Low-intensity wells	pH, ec, alk, DO	x	x	x	x		x							
High-intensity wells	pH, ec, alk, DO	x	x	x	x		x	x		x		x	x	
April, 2010														
Low-intensity wells	pH, ec, alk, DO	x	x	x	x		x							
High-intensity wells	pH, ec, alk, DO	x	x	x	x		x	x		x	x	x	x	
Malibu Lagoon and Malibu Creek		Each site sampled once during July, 2009 and April, 2010												
July, 2009	pH, ec, alk, DO	x	x	x	x		x	x		x		x	x	
April, 2010	pH, ec, alk, DO	x	x	x	x		x	x		x	x	x	x	
Continuous Malibu Lagoon monitoring sites	pH, ec, alk, DO			x		x	x							
Malibu Lagoon discharge		Samples collected daily at high tide, low tide, mid-high tide, and mid-low tide during falling monthly tidal sequence from spring to neap tide												
July, 2010		Not flowing / not sampled												
April, 2010	pH, ec, alk, DO			x			x							
Near-shore ocean		Samples collected daily at high tide, low tide, mid-high tide, and mid-low tide during falling monthly tidal sequence from spring to neap tide												
July, 2009	pH, ec			x			x							
April, 2010	pH, ec			x			x							
Onsite wastewater treatment systems		One sample from a conventional and advanced system collected during October, 2009 and during April, 2010												
October, 2009	pH, ec, alk	x	x	x	x		x	x		x	x	x	x	
July, 2010	pH, ec, alk	x	x	x	x		x	x		x	x	x	x	
Kelp and sand extracts														
July, 2010	pH, ec		x	x	x		x	x		x		x	x	
April, 2010	pH, ec		x	x	x		x	x		x		x	x	



Figure 3 Location of sampled wells, piezometers, seepage samplers and onsite wastewater treatment systems (OWTS), Malibu, California, July 2009 to April 2010.

Table 2 Well construction, fecal indicator bacteria (FIB) concentrations, and percent imported water having a wastewater history in water from sampled wells, Malibu California, July 2009 and April 2010

Station name (Location shown on Figure 3)	USGS well number	Well depth (m)	Top and bottom of screened interval	Nearby land use	Date (m/dd/yyyy)	Depth to water (m)	Sample type	Fecal Indicator					Percent Imported water
								Specific conductance (μS/cm)	Bacteria Total coliform (MPN/100mL)	Enter- Coccus (MPN/100mL)	<i>Escherichia coli</i> (MPN/ 100mL)		
SMBRP-3c	340242118410501	9.1	4.5 - 9.1	residential	4/21/2010	5.6	high	1,545	1,700	< 1	< 1	19	
SMBRP-6	340230118410401	7.6	3.0 - 7.6	residential	4/17/2010	4.5	high	1,540	1	< 1	< 1	9	
SMBRP-11	340159118414601	6.1	1.5 - 6.1	residential	7/21/2009	2.6	low	2,960	< 10	< 10	< 10	76	
SMBRP-11	340159118414601	6.1	1.5 - 6.1	residential	4/21/2010	2.5	high	2,100	< 1	< 1	< 1	46	
SMBRP-12	340158118412401	7.6	3.0 - 7.6	residential	7/22/2009	2.1	high	3,820	< 1	< 1	< 1	60	
SMBRP-12	340158118412401	7.6	3.0 - 7.6	residential	4/21/2010	2.1	high	2,600	< 1	< 1	< 1	75	
SMBRP-13	340156118411401	6.1	1.5 - 6.1	residential	7/22/2009	2.3	high	2,450	< 1	< 1	< 1	39	
SMBRP-13	340156118411401	6.1	1.5 - 6.1	residential	4/20/2010	2.5	high	18,400	--	< 10	< 10	<1	
C-1	340155118410201	4.4	1.2 - 4.4	residential	7/26/2009	1.4	high	22,300	< 10	< 10	< 10	<1	
C-1	340155118410201	4.4	1.2 - 4.4	residential	4/19/2010	2.0	high	8,260	3	< 1	< 1	39	
CCPC	340207118410401	6.9		commercial	7/23/2009	1.8	low	2,020	< 1	< 1	< 1	22	
CCPC	340207118410401	6.9		commercial	4/18/2010	2.5	high	2,040	> 2,400	1	< 1	19	
CCPE	340208118410101	16.1	3.7 - 15.8	commercial	7/23/2009	1.5	low	10,820	1,600	11	65	3	
CCPE	340208118410101	16.1	3.7 - 15.8	commercial	4/17/2010	2.7	high	26,000	> 2,400	5	< 1	17	
CCPNE	340209118410301	7.6		commercial	7/23/2009	1.8	low	1,960	8	1	< 1	23	
CCPNE	340209118410301	7.6		commercial	4/18/2010	2.8	high	1,901	1	< 1	< 1	26	
CCPSW	340206118410701	6.2		commercial	4/18/2010	2.6	high	1,900	3	< 1	< 1	27	
CCR-1	340208118410701	5.9		commercial	7/24/2009	1.7	low	2,080	2	2	< 1	19	
CCR-1	340208118410701	5.9		commercial	4/18/2010	2.4	high	2,000	< 1	< 1	< 1	21	
CCSC-1	340203118410701	4.5		commercial	4/18/2010	2.2	high	1,500	220	1	< 1	71	
P-9	340205118410101	4.2	1.3 - 4.2	commercial	7/22/2009	1.3	high	2,000	< 1	< 1	< 1	30	
P-9	340205118410101	4.2	1.3 - 4.2	commercial	4/18/2010	2.6	high	1,950	< 1	< 1	< 1	69	
SMBRP-10C	340207118413301	7.6	3.0 - 7.6	undevelope	7/21/2009	1.9	low	12,710	< 10	< 10	< 10	3	
SMBRP-10C	340207118413301	7.6	3.0 - 7.6	undevelope	4/17/2010	1.3	high	8,900	3	< 1	< 1	<1	
SMBRP-2	340210118405401	7.6	3.0 - 7.6	undevelope	7/22/2009	1.6	high	3,360	< 1	< 10	< 10	13	
SMBRP-2	340210118405401	7.6	3.0 - 7.6	undevelope	4/19/2010	2.7	high	2,340	2,400	96	< 1	3	

[Residential and commercial land uses are unsewered. Well depths below land surface. Sample type: low--sample not analyzed for genetic, molecular, or selected organic compounds; high-- sample analyzed for genetic, molecular, and selected organic compounds (Table 1). --, not determined; m, meters; m/dd/yyyy, month/day/year; mS/cm, microSiemens per centimeter; <, less than; >, greater than; MPN/100ml, Most Probable Number per 100 milliliters. Percent wastewater calculated from delta deuterium data]

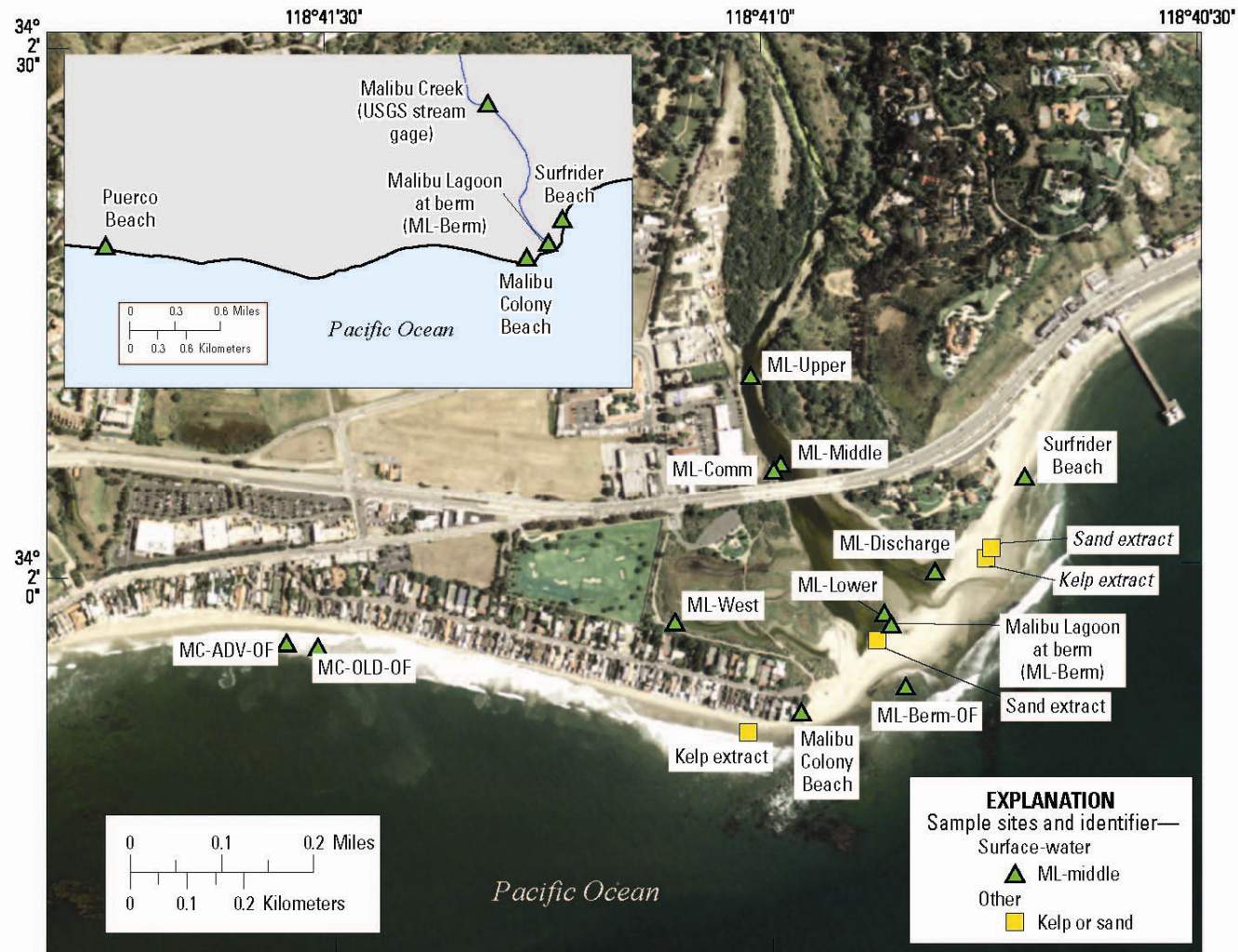


Figure 4 Location of surface water sample sites and sites for collection of kelp and sand for water extract analysis, Malibu California, July 2009 to April 2010.

In November 2009 and April 2010, hourly data were supplemented with radon-222 data collected from the near-shore ocean and from selected piezometers. Radon-222 data from the near-shore ocean were collected from sample intakes just above the seafloor attached to buoys just outside to surf zone (Figure 5) to minimize Rn-222 losses to the atmosphere prior to sample collection. Radon-222 was measured continuously using a water/air exchanger and a radon-in-air monitor [98-100]. Water level, air and water temperature, specific conductance and pH were monitored continuously while radon-222 data were collected.

Detailed sample collection also was supplemented with direct-current (DC) resistivity data collected at high and low tide on the berm adjacent to Malibu Lagoon, and at low tide on the beach adjacent to unsewered residential development in Malibu Colony in July 2009 and April 2010. DC resistivity data were collected according to manufacturer's specifications

[101] using a dipole-dipole array with a 1-m electrode spacing. DC resistivity data were interpreted using the computer program EarthImaGer [102].

Onsite wastewater treatment systems. Samples were collected from within conventional residential treatment systems (septic systems), advanced residential treatment systems, and from commercial treatment systems in October 2009 and July 2010 (Figure 3). Water samples from the conventional and commercial systems were collected from within the septic tank prior to discharge. Advanced systems in the study area differ in design and construction. The sampled systems contained multiple treatment chambers including a traditional anaerobic chamber, an activated biological growth chamber, an oxidizing chamber, and a settling chamber with ultraviolet disinfection. Water samples from the advanced systems were collected from the settling chamber prior to UV disinfection and discharge.

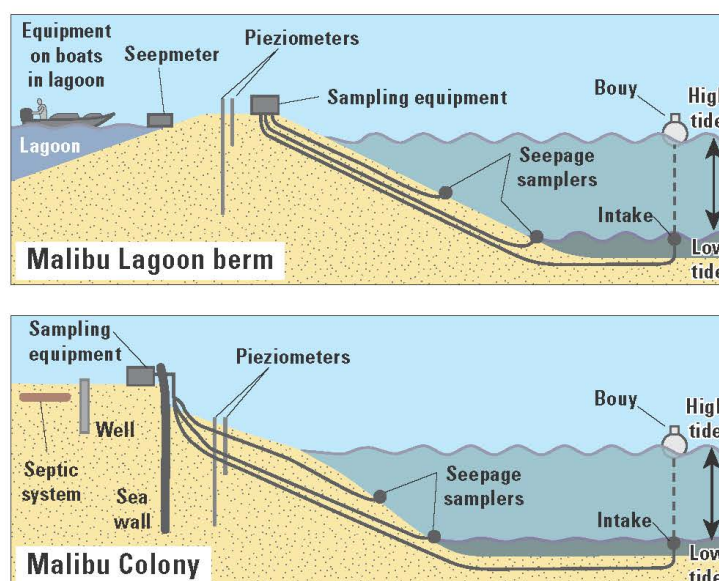


Figure 5 Diagram of sample collection sites adjacent to the berm at Malibu Lagoon and Malibu Colony, near Malibu, California, July 2009 to April 2010.

Water extractions. Kelp and sand from the upper 0.5 cm were collected near the high tide line at selected locations (Figure 4). Samples were collected with sterile stainless steel implements and placed in sterile stainless steel buckets. The sample implements were cleaned and rinsed with organic-free water between sample collection and buckets were not reused for sampling. The mass of the sample was measured in the field by subtracting the weight of the bucket from

the weight of the sample and 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 for shipment to various

laboratories for analysis. The procedure was similar to that used by Izbicki et al. [29].

2.2. Laboratory Methods

Fecal indicator bacteria. Total coliform and *E. coli* were analyzed by Colilert-18 for water having salinity greater than 5 parts per thousand, and by Colitert-18 for water having salinity less than 5 parts per thousand. Enterococci were analyzed using Enterolert (IDEXX, Westbrook MN). Samples collected during July 2009 and April 2010 were analyzed in temporary laboratories set up by the U.S. Geological Survey within the study area. A range of dilutions was used to ensure proper quantification of samples in accordance with the manufacturers' specifications.

Most samples, including all groundwater samples, were analyzed within 6 hours after collection, the recommended holding time for samples collected for regulatory purposes [103]. Some samples, primarily hourly samples collected late at night during falling tidal cycles, were held for slightly longer prior to analysis. However, all samples were analyzed within 24 hours of collection, the recommended holding time for samples collected for scientific purposes [104]. During July 2009 and April 2010, selected FIB samples analyzed within 6 hours after collection were re-analyzed 24 hours after collection. Results of the re-analysis of the samples for *E. coli* and enterococci were within the 95% confidence levels of the original value for 6 of the 7 samples. Results of the re-analysis of samples for total coliforms were within of the 95% confidence levels for 3 of the 7 samples. The differences in total coliform concentrations were random and concentrations increased or decreased after 24 hours irrespective of the initial concentration. The results suggest an increased variability in total coliform numbers from samples collected late at night during falling tidal cycles. Enterococcus and *E. coli* data appear to be only marginally affected by sample holding times up to 24 hours.

Internal quality control in the field laboratory consisted of daily laboratory blanks and positive and negative control cultures for each method. All laboratory blanks for total coliforms, *E. coli* and enterococci were non-detects. Results from the positive and negative control cultures analyzed using each lot of Colilert-18, Enterolert and membrane filtration media indicated the methods performed satisfactorily.

Samples collected at times other than July 2009 or April 2010 were hand delivered to the U.S. Geological Survey office in San Diego, California for

analysis within 24 hours of collection using the procedures described above.

Genetic data. Genetic data analyzed as part of this study included Terminal-Restriction Fragment Length Polymorphism (T-RFLP), human-associated *Bacteroidales* (HF183 SYBR) and microarray (PhyloChip) analyses.

Terminal-Restriction Fragment Length Polymorphism. Samples for T-RFLP were processed by the University of California at Santa Barbara. Samples were preserved on ice and delivered to the lab by courier on the day of collection. Immediately on arrival at the lab, samples were filtered through 0.22 µm filters and stored at -20°C. DNA was extracted using commercially available kits (Power-Water® DNA Isolation Kit, MO BIO Laboratories, Inc., Carlsbad, CA) as specified by the manufacturer, followed by ethanol precipitation. Total DNA was quantified on a BioTek Synergy 2 plate reader (Winooski, VT) using a commercially available kit (Quant-iT™ dsDNA BR Assay Kit, Invitrogen-Molecular Probes, Eugene, OR). Results were related to the volume of water filtered for reporting DNA concentration (Total DNA). 16s rRNA genes from the purified DNA were amplified using the polymerase chain reaction (PCR) with eubacterial primers 8F hex (fluorescently labeled forward primer) and 1389R on a PCR Sprint thermal cycler (Hybaid US, Franklin, MA) [105]. PCR products were purified with the QIAquick PCR purification kit (Qiagen, Valencia, CA), and ca. 300 ng was digested separately with H-hal and M-spl enzymes (New England Biolabs, Ipswich, MA). The restriction enzymes were inactivated by heating (H-hal: 65°C for 20 min, M-spl: 80°C for 20 min) and the length of the fluorescently labeled fragments was determined with an Applied Biosystems Instruments PRISM® 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA) at the Genomics Technology Support Facility (Michigan State University, East Lansing, MI).

Human-associated Bacteroidales. Human-associated *Bacteroidales* (HF183 SYBR) were analyzed by the University of California, Santa Barbara on the same DNA extracts as the T-RFLP analysis. Prior to HF 183 SYBR qPCR assays, samples were diluted at least five-fold and inhibition was assessed using the salmon testes qPCR assay [106,107]. HF183 SYBR qPCR [108] were run in triplicate using SYBR green I chemistry as reported previously [107,109], with an iQ5 real-time PCR detection system (Bio-Rad, Hercules, CA). Melt curves were validated for all sample replicates that

amplified within the quantification range. The HF183 SYBR assay is more specific to human fecal *Bacteroidales* than results from the BacHum assay [107].

Microarray data (PhyloChip). DNA extracted and purified from selected samples also was analyzed by Lawrence Berkeley National Laboratories using microarray (PhyloChip) technology (U.S. Patents WO/2008/130394 and US-2009-0291858-A1). The microarray technique uses oligonucleotide probes embedded on a silicon chip to identify the presence of gene sequences (referred to as Operational Taxonomic Units or OTUs, rather than species) from almost 60,000 bacteria and archaea [110]. Each chip contains more than 1.1 million oligonucleotide probes, providing multiple levels of confirmation and increased accuracy in the identification of DNA from different organisms. The probes fluoresce under laser light when the target gene sequence is present, and differences in fluorescence are related to abundance [62]. Each probe is accompanied by a mismatched control that fluoresces if gene sequences differing by as little as one base-pair are present to provide for identification of interference from non-specific hybridization. Inhibition is evaluated through addition and recovery of non-16S rRNA genes [110]. The microarray method is rapid and repeatable, and is capable of detecting gene sequences that constitutes as little as 10^{-5} of the sample 16S rRNA gene pool.

Phospholipid fatty acids. Samples for phospholipid fatty acids (PLFAs) were analyzed by Microbial Insights in Rockford, Tennessee. Samples were preserved on ice 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 [111]. 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) [112]. The detection limit is 50 picomoles per liter (pm/L) and the quantification limit is 150 pm/L [113].

Selected organic compounds. Samples for selected organic compounds, collectively known as wastewater indicators, were analyzed by the U.S. Geological Survey National Water Quality Laboratory (NWQL)

in Denver, Colorado. Samples were preserved on ice at the time of collection and shipped in coolers on the day of collection for overnight delivery to the lab. Organic compounds in whole-water samples were extracted using continuous liquid-liquid extractors and methylene chloride solvent. After extraction, samples were preserved by adding 60 grams of sodium chloride and stored at 4°C. Analysis of the extract was done within 14 days by continuous liquid-liquid extraction and capillary-column gas chromatography/mass spectrometry [114]. Compounds analyzed using this method include alkylphenol ethoxylate nonionic surfactants, food additives, fragrances, antioxidants, flame retardants, plasticizers, industrial solvents, disinfectants, fecal sterols, polycyclic aromatic hydrocarbons and high-use domestic pesticides.

Isotopic data. Isotopic analysis included measurement of delta Oxygen-18 and delta Deuterium. The radioactive isotope radon-222 was measured in the field using methods described previously. Samples for analysis of the stable isotopes of oxygen and hydrogen in water were shipped to the U.S. Geological Survey Reston Stable Isotope Laboratory (RSIL) in Reston, VA for analysis by mass spectrometry. The ratio of oxygen-18(^{18}O) to the more common isotope oxygen-16(^{16}O) was measured using the carbon dioxide (CO_2) equilibration technique [115]. The ratio of deuterium (^2H) to the more common isotope hydrogen (^1H) was measured using a hydrogen equilibration technique at 30°C [116]. Oxygen and hydrogen isotopic results are reported in delta notation (δ) as per mil (‰) differences relative to VSMOW (Vienna Standard Mean Ocean Water) according to the following equation,

$$\Delta^{18}\text{O} \text{ or } \delta\text{D} = [(R_{\text{sample}} - R_{\text{standard}}) / R_{\text{standard}}] \times 1,000$$

where R_{sample} and R_{standard} refer to the ratio in the sample and the standard, respectively. By convention, the value of VSMOW (R_{standard}) is 0 per mil [117]. Values were normalized on scales such that the oxygen and hydrogen isotopic values of SLAP (Standard Light Antarctic Precipitation) are -55.5 per mil and -428 per mil, respectively [118-121]. $\delta^{18}\text{O}$ and δD ratios can be measured more accurately than absolute abundances and analytical precision is about ± 1 per mil and ± 0.1 per mil, respectively.

2.3. Statistical Analysis

Principal component analysis (PCA) was used to

analyze genetic, molecular and organic tracers (wastewater indicators) of sources of FIB. PCA is a multivariate statistical technique that transforms a set of intercorrelated variables into to a new coordinate system [122,123]. The transformed variables known as principal components are uncorrelated linear combinations of the original data. The principal components have a mean of zero and the same variance as the original data set. The values of the principal components are known as scores. The magnitude and direction (positive or negative) of the contribution of each variable to the principal component score is described by an eigenvector [124]. Data were rank-ordered prior to analysis and PCA was done on the ranked values rather than on concentration data. Estimated concentrations (organic tracer analysis only) were rank-ordered by numerical value, and less-than values were assigned the lowest rank. The use of ranked data minimized the effect of non-normally distributed data and extreme values on the magnitude of the PCA scores [125]. However, comparison of PCA results on ranked and unranked data showed that the relative distribution of the PCA scores and interpretations derived from the data were similar.

3. Results

3.1. Source of Groundwater and Malibu Lagoon Water

The source of groundwater and water in Malibu Lagoon is important in understanding the hydrologic history of sample water with respect to OWTS discharges and the percentage of imported water having a wastewater history. 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 global meteoric water line [126]. Atmospheric and hydrologic processes combine to produce broad global and regional differences in the $\delta^{18}\text{O}$ and δD composition of 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 [127].

In the study area, all water used for public supply, and ultimately discharged from OWTS, is imported from northern California or from the Colorado River. Water sampled from OWTS has $\delta^{18}\text{O}$ and δD values near -9.6 and -75 per mil. These values are more

negative than the $\delta^{18}\text{O}$ and δD composition of precipitation along the California coast near Santa Maria, California, about 200 km northwest of the study area [128] and more negative than the $\delta^{18}\text{O}$ and δD composition of water from Malibu Creek (Figure 6).

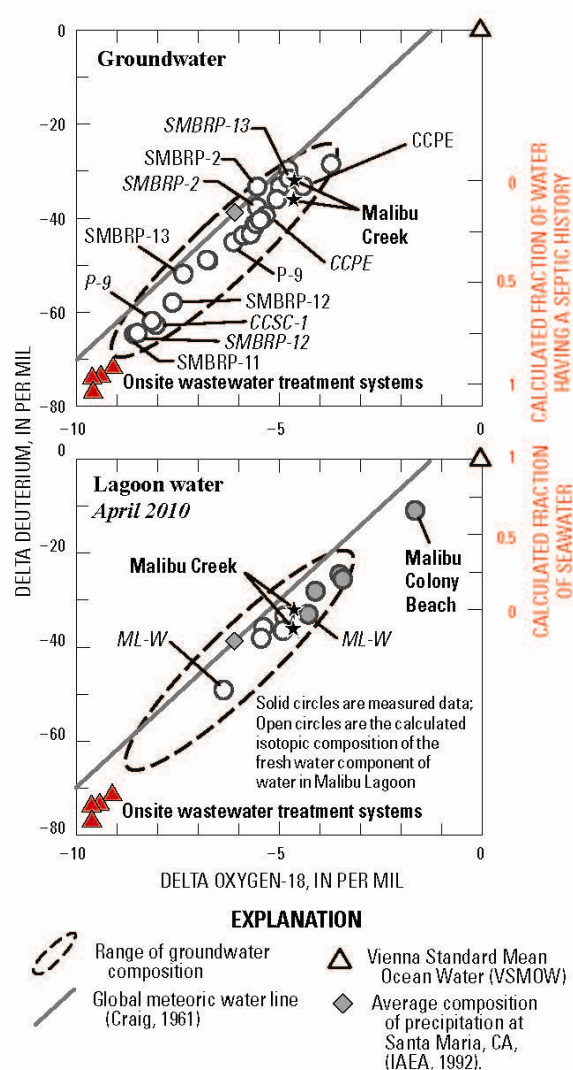


Figure 6 delta Oxygen-18 and delta Deuterium composition of wastewater, groundwater, and water from Malibu Lagoon, Malibu, California, July 2009 to July 2010.

The $\delta^{18}\text{O}$ and δD composition of water from 15 sampled wells ranged from -4.4 to -8.6 and -28 to -64.7 per mil, respectively (Figure 6). Given the measured $\delta^{18}\text{O}$ and δD composition of water sampled from within OWTS and the $\delta^{18}\text{O}$ and δD composition

of native water from Malibu Creek, the average percentage of imported water in sampled wells was 25%. This value is similar to a value of 28% of recharge to the alluvial deposits in the Civic Center area from OWTS estimated using model analysis [88].

Water from some wells in residential areas near Malibu Colony, such as SMBRP-11 and SMBRP-12, had high percentages of imported water, as high as 76% (Table 2). Isotopic data indicate that imported water was less than 30% of water from wells in the commercial area near Malibu Lagoon during July 2009. However, percentages of imported water were approximately 70% in wells CCSC-1 and P-9 in the commercial area during April 2010 (Table 2). This seasonal difference may occur because higher water levels in the Lagoon when the berm of the Lagoon was closed in summer results in flow of water from the Lagoon into the groundwater system; in contrast, lower water levels in the Lagoon in the winter when the berm was open result in flow of recharge derived from imported water from the commercial area towards the Lagoon (Figure 2).

Water in Malibu Lagoon is a three-part mixture of seawater, having $\delta^{18}\text{O}$ and δD compositions near 0 per mil and fresh water derived from streamflow, and groundwater discharged to the Lagoon. Given a specific conductance of 53,000 $\mu\text{Siemens}$ per centimeter for seawater and a specific conductance of 1,150 $\mu\text{Siemens}$ per centimeter in Malibu Creek, the $\delta^{18}\text{O}$ and δD composition of the fresh water component (streamflow and groundwater) of samples from the Lagoon can be calculated (Figure 6). On the basis of these data, the fresh water component of most samples from Malibu Lagoon was isotopically heavy and contained small amounts of imported water, typically less than 3%. However, the $\delta^{18}\text{O}$ and δD composition of the fresh water component calculated from the ML-West sample in April 2010 was -6.4 and -49 per mil, respectively (Figure 6). Consequently, almost 50% of the fresh water component of this sample was imported water and the total sample contained about 11% imported water. The higher percentage of imported water in this sample is consistent with the location of ML-West in the western arm of the Lagoon near Malibu Colony away from the main channel of the Lagoon (Figure 4), and with increased groundwater discharge to the Lagoon during the April sample period when the berm of the Lagoon was open to the ocean.

3.2. Fecal Indicator Bacteria

More than 450 samples collected from wells,

piezometers, Malibu Lagoon (including inflow from Malibu Creek), near-shore ocean water, within OWTS, and water extractions from sand and kelp were analyzed for FIB concentrations.

Onsite wastewater treatment systems. FIB were highest in samples from within OWTS (Figure 7). Total coliform bacteria concentrations ranged from 16,000 to >2,400,000 MPN per 100 mL. *E. coli* and enterococci concentrations ranged from 1,400 to 2,000,000 and 52 to 240,000 MPN per 100 mL, respectively. Median total coliform, *E. coli*, and enterococci concentrations were 920,000, 220,000, and 7,300 MPN per 100 mL, respectively. *E. coli* and enterococci concentrations were lower in the samples from within the advanced OWTS and higher in the sample from within the commercial OWTS.

Monitoring wells. FIB concentrations were lowest in water from wells (Figure 7). Total coliform bacteria concentrations ranged from <1 to >2,400 MPN per 100 mL. *E. coli* and enterococci concentrations ranged from <1 to 65 and <1 to 96 MPN per 100 mL, respectively (Table 2).

Total coliform were present in water from 29% of samples in unsewered residential areas and 64% of samples in the unsewered commercial area. Total coliforms were detected in 27% of samples in July 2009 and 71% of samples in April 2010 (Table 2). During April 2010, concentrations in some wells exceeded the upper reporting limit of 2,400 MPN per 100 mL (Table 2); however, the median total coliform concentration at that time was 3 MPN per 100 mL. *E. coli* and enterococci were not detected in water from wells in unsewered residential areas, even though water from some of these wells contained more than 70% imported water, potentially indicating a wastewater history. *E. coli* and enterococci were present in 7 and 43% of samples, respectively, in the unsewered commercial area near Malibu Lagoon. Within the commercial area, *E. coli* and enterococci concentrations were as high as 65 and 11 MPN per 100 mL, respectively, in water from well CCPE during July 2009 (Table 2). This well is adjacent to Malibu Lagoon and is saline (specific conductance greater than 10,000 $\mu\text{S}/\text{cm}$). The sample had a low percentage of imported water and the specific conductance of the water suggests that water from the Lagoon was present in the well at this time. *E. coli* and enterococci concentrations decreased and the percentage of imported water increased, in water from this well in April 2010 when water levels in the Lagoon were lower (Table 2).

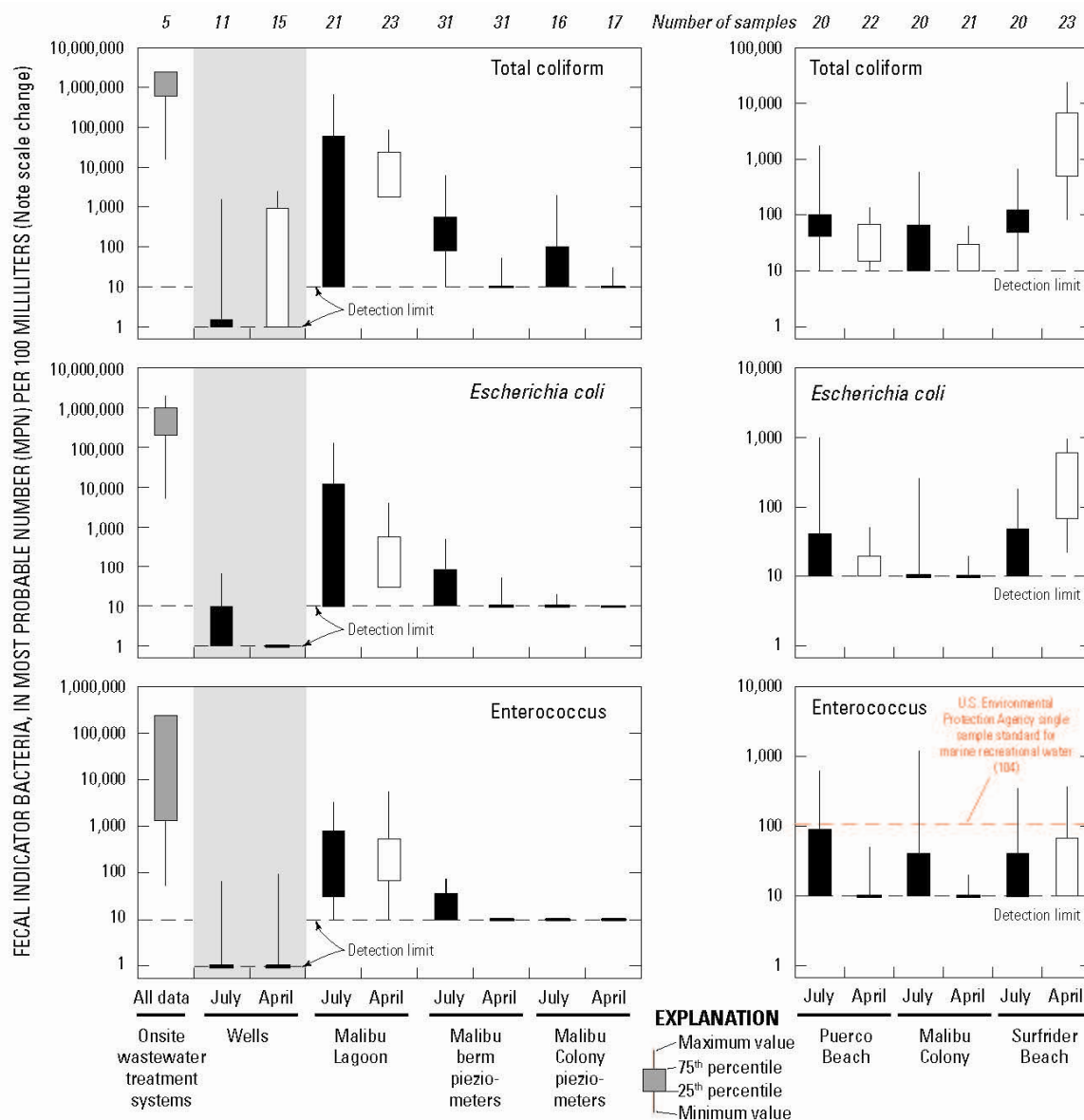


Figure 7 Fecal indicator bacteria concentrations (FIB) in water from onsite wastewater treatment systems (OWTS), existing monitoring wells, Malibu Lagoon, piezometers and the near-shore ocean near Malibu, California, July 21-27, 2009 and April 16-23, 2010.

FIB detections and concentrations were not correlated with the percentage of imported water having a wastewater history estimated from $\delta^{18}\text{O}$ and δD data (Figure 8). High concentrations of total coliform and enterococci, (2,400 and 96 MPN per 100 mL, respectively) were present during April 2010 in water from well SMBRP-2 (Figure 8) in an undeveloped area east of Malibu Lagoon. SMBRP-2 along the east side of Malibu Lagoon is removed from

any known wastewater discharges, and in April 2010 had a $\delta^{18}\text{O}$ and δD composition consistent with local water and a low percentage of imported water (Table 2). The lack of correlation between even low concentrations of FIB and the percentage of imported water and the presence of FIB in water from wells, where imported water having a wastewater history is absent, suggests that OWTS may not be the sole source of FIB in water from most wells.

Malibu Lagoon and Malibu Creek. Total coliform concentrations collected at high, low, mid-high, and mid-low tides near the berm of the Lagoon (ML-Berm) during the July 2009 and April 2010 sample periods ranged from <10 to 650,000 MPN per 100 mL. *E. coli* and enterococci concentrations at that site ranged from <10 to 130,000 and <10 to 5,500 MPN per 100 mL, respectively (Figure 7). Fifty-seven percent of the samples exceeded the U.S.

Environmental Protection Agency single sample standard for enterococci in marine recreational water of 104 MPN per 100 mL [9]. Samples collected at other locations in the Lagoon (Figure 4) fell within those ranges. FIB concentrations within the Lagoon were highly variable during both the July sample period when the Lagoon was closed to the ocean, and the April sample period when the Lagoon was open to the ocean and flushed twice daily by tides (Figure 7).

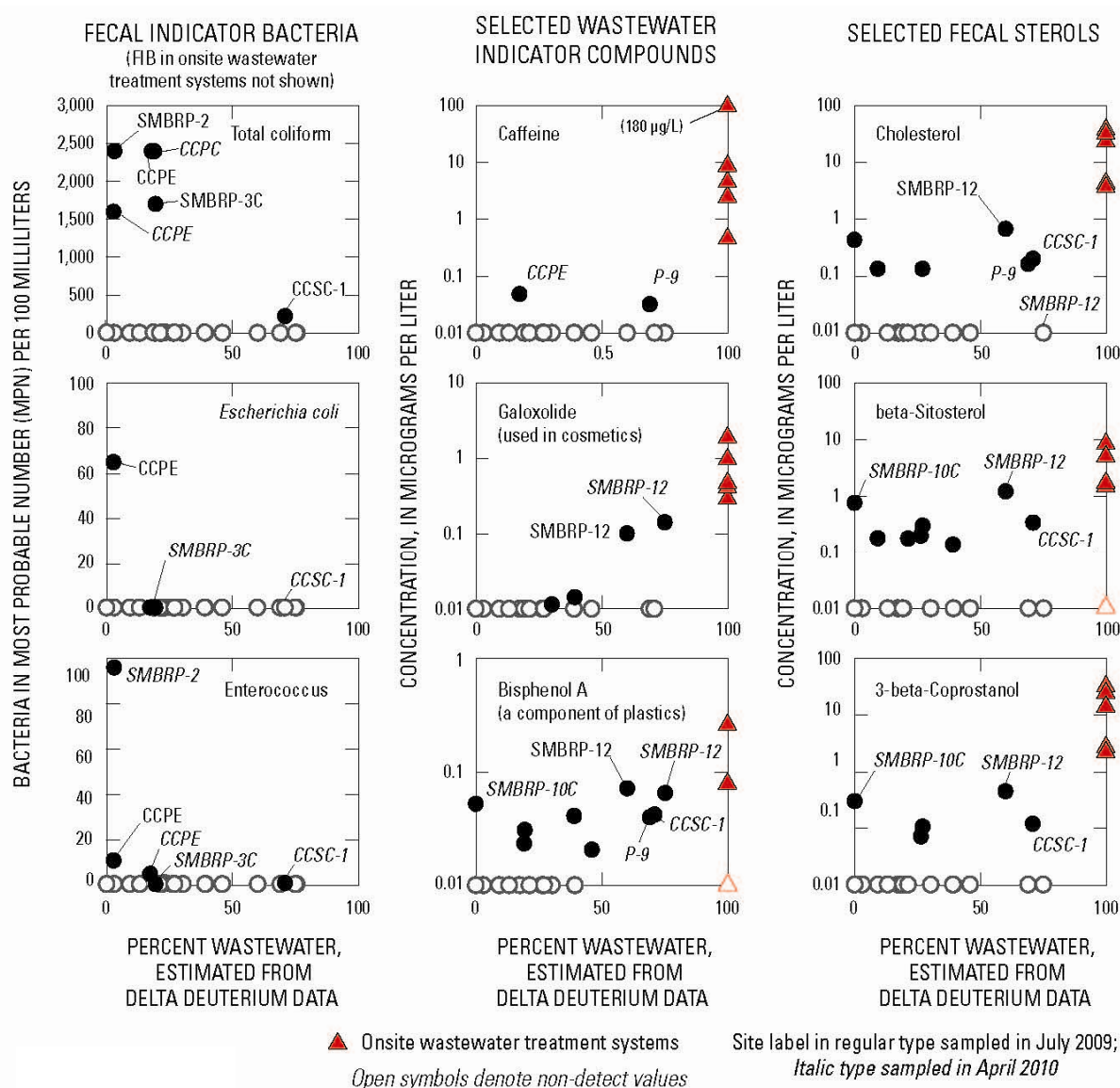


Figure 8 Fecal indicator bacteria (FIB), selected wastewater indicator compounds, and selected fecal sterols as a function of percent of imported water having a wastewater history in water from wells and onsite wastewater treatment systems (OWTS), Malibu California, July 2009 to July 2010.

Total coliform, *E. coli* and enterococci concentrations in Malibu Creek were 14,000, 10, and 280 MPN per 100 mL in July 2009 and 4,100, 17, and 9 MPN per 100 mL in April 2010. In July 2009, the sample was collected from a pool in the stream channel because Malibu Creek was not flowing.

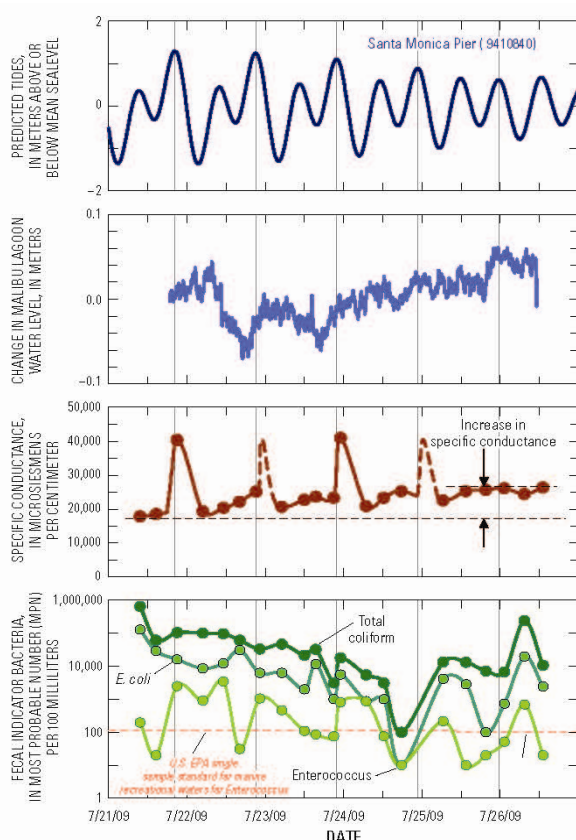


Figure 9 Ocean tides, water levels, specific conductance, and fecal indicator bacteria (FIB) concentrations in Malibu Lagoon, near Malibu, California. July 21-27, 2009.

During the July 2009 sample period, Malibu Lagoon was not open to the ocean. However, ocean water overtopped the berm separating the Lagoon from the ocean during high tides at the beginning of the sample period, causing water levels and specific conductance in the Lagoon to increase and FIB concentrations to decrease during the initial part of the sample period (July 21-24, Figure 9). FIB concentrations increased during the later part of the sample period (July 25-26, Figure 9) after the magnitude of the tides decreased and ocean water no longer overtopped the berm and entered the Lagoon. In addition to dilution by ocean water, enterococci

concentrations also show a diurnal pattern with lower concentrations in samples collected later in the day during the mid-low tidal stand (Figure 9). This may occur as a result of inactivation of bacteria by ultraviolet (UV) radiation in sunlight after clouds associated with the morning marine layer dissipated. Inactivation of bacteria and viruses by UV radiation in sunlight has been observed previously at marine and freshwater beaches [129-133].

Ocean water entering Malibu Lagoon as a result of high tides during the July sampling period had a higher salinity and lower temperature than Lagoon water. As a consequence, this water was denser and sank to the bottom of the Lagoon, stratifying water in the Lagoon (Figure 10). Initially it was expected that the saline water near the bottom of the Lagoon would have low bacteria concentrations. However, the deeper saline water generally had higher bacteria concentrations than near surface water (Figure 10).

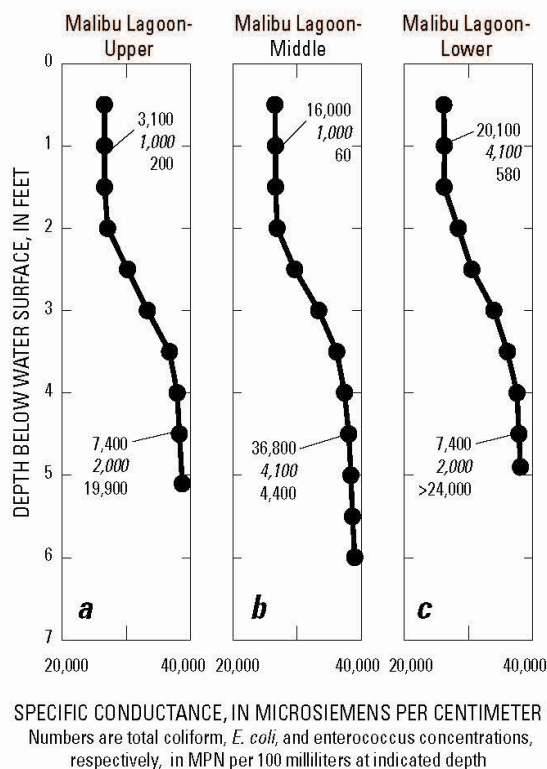


Figure 10 Specific conductance and fecal indicator bacteria (FIB) concentrations with depth in Malibu Lagoon, July 23, 2009.

Although UV radiation may have reduced daytime enterococci concentrations in near-surface water, total coliform and *E. coli* bacteria seem to be

less affected by sunlight (Figure 9). One possible explanation is that as denser ocean water entered the Lagoon during high tide and sank to the bottom, bacteria were mobilized from bottom sediments into the water column. Summer temperatures in Malibu Lagoon commonly reach 31°C, and sediments in warm, eutrophic, nutrient-rich environments such as Malibu Lagoon have been shown to be a reservoir for FIB, promoting extended survival and in cases where water is sufficiently warm, regrowth of bacteria [16-19, 134] which could be mobilized into the water. Additional data collection would be required to confirm or reject this hypothesis.

During the April 2010 sample period, Malibu Creek was flowing and Malibu Lagoon was open to the ocean. Although discharge of treated wastewater from the Tapia Wastewater Treatment Plant, about 6 km upstream, had ceased on April 15 (Jan Dougall, Las Virgenes Water District, written communication, May 2010), streamflow showed a diurnal pattern consistent with upstream discharges (Figure 11). Seawater flowed into the Lagoon during high tide, and water flowed from the Lagoon into the ocean during low tide. Low FIB concentrations were measured at the mouth of the Lagoon (ML-Discharge) during high tide when seawater entered the Lagoon, and high FIB concentrations were measured during low tide as water in the Lagoon flowed to the ocean (Figure 11). FIB concentrations were higher at the sample site on the berm within the Lagoon compared to FIB concentrations at the Lagoon mouth, and tidally driven changes in specific conductance and FIB concentrations within the Lagoon (ML-Berm, Figure 4) were damped and lagged those measured at the mouth of the Lagoon (Figure 11).

The last stormflow of the winter rainy season, generated by precipitation in the watershed upstream from the study area, occurred during the sample period on April 19-21 (Figure 11). The timing of the stormflow coincided with hourly sample collection in the Lagoon and at the Lagoon mouth. FIB concentrations at the berm and at the mouth of the Lagoon were high during stormflow (April 19-21) and the concentrations were independent of tidal fluctuations as the increased volume of water discharging to the ocean prevented seawater from entering the Lagoon (Figure 11). Because the inflow to the Lagoon from Malibu Creek was not sampled, it was not possible to determine if FIB discharged from the mouth of the Lagoon during stormflow were from upstream sources or were mobilized from sources within the Lagoon. High specific conductance values measured at the berm within the Lagoon during stormflow may have

resulted from wave-driven seawater overtopping the berm during the storm (Figure 11).

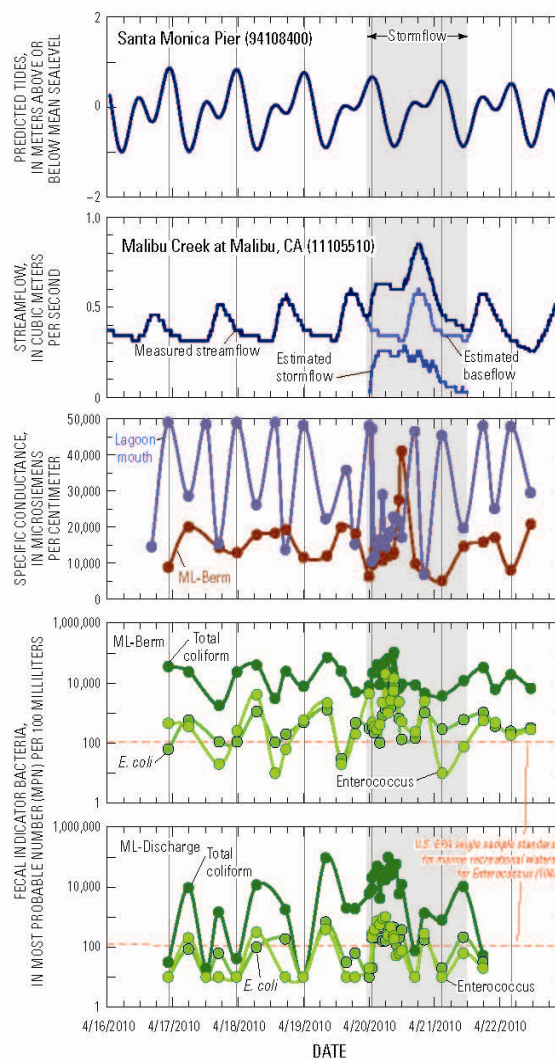


Figure 11 Ocean tides, streamflow in Malibu Creek, specific conductance, and fecal indicator bacteria (FIB) concentrations in Malibu Lagoon, near Malibu, California. April 16-23, 2010.

Near-shore ocean. Total coliform concentrations collected at high, low, mid-high, and mid-low tides from beaches west of the Civic Center area (Puerco Beach), adjacent to unsewered residential development in Malibu Colony and east of Malibu Lagoon at Surfrider Beach ranged from <10 to >24,200 MPN per 100 mL (Figure 7). *E. coli* and enterococci concentrations ranged from <10 to 1,010 and <10 to 1,210 MPN per 100 mL, respectively, (Figure 7).

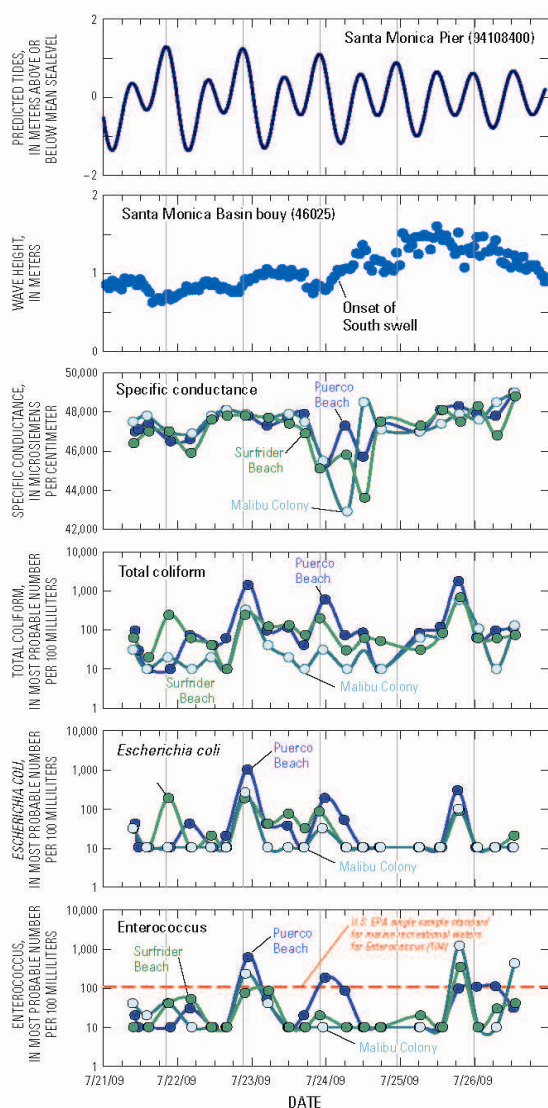


Figure 12 Tide, ocean swell, specific conductance, and FIB concentrations in the near-shore ocean at Puerco, Malibu Colony, and Surfrider beaches, near Malibu California, July 21-27, 2009.

Although FIB concentrations were generally low in the near-shore ocean, exceedances of the U.S. Environmental Protection Agency single-sample enterococci standard for marine recreational water of 104 MPN per 100 mL [9] occurred in 9, 7 and 12% of samples at Puerco, Malibu Colony and Surfrider beaches, respectively. FIB concentrations at the three sampled beaches showed seasonal differences (Figure 7), and variations in FIB concentrations with daily tidal cycles and ocean waves (Figures 12 and 13).

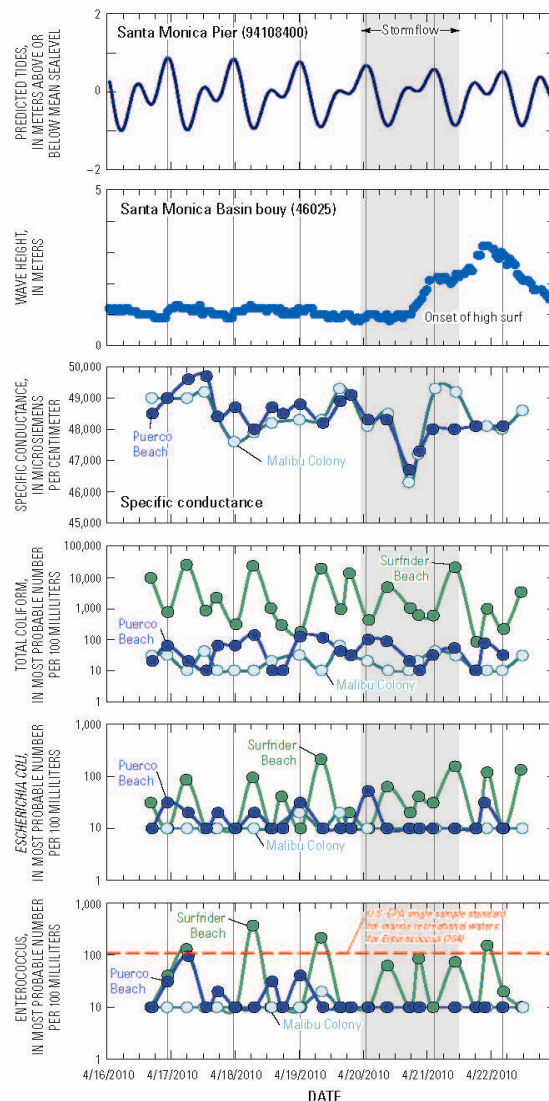


Figure 13 Tide, ocean swell, streamflow, specific conductance, and FIB concentrations in the near-shore ocean at Puerco, Malibu Colony, and Surfrider beaches, near Malibu California, April 16-23, 2010.

During the July sample period, FIB concentrations at all Puerco and Malibu Colony beaches were higher at high tide than at other tidal stands (Figure 14), and 30% of the high tide samples exceeded the single sample enterococci standard (Figure 14). This pattern is not consistent with FIB from groundwater discharge, which would be greater at low tide. Similar increases in FIB concentrations during high tides have been observed at coastal California beaches [29,74,135] and attributed to wave run-up on the beach washing FIB from the wrack line or from beach sand [29].

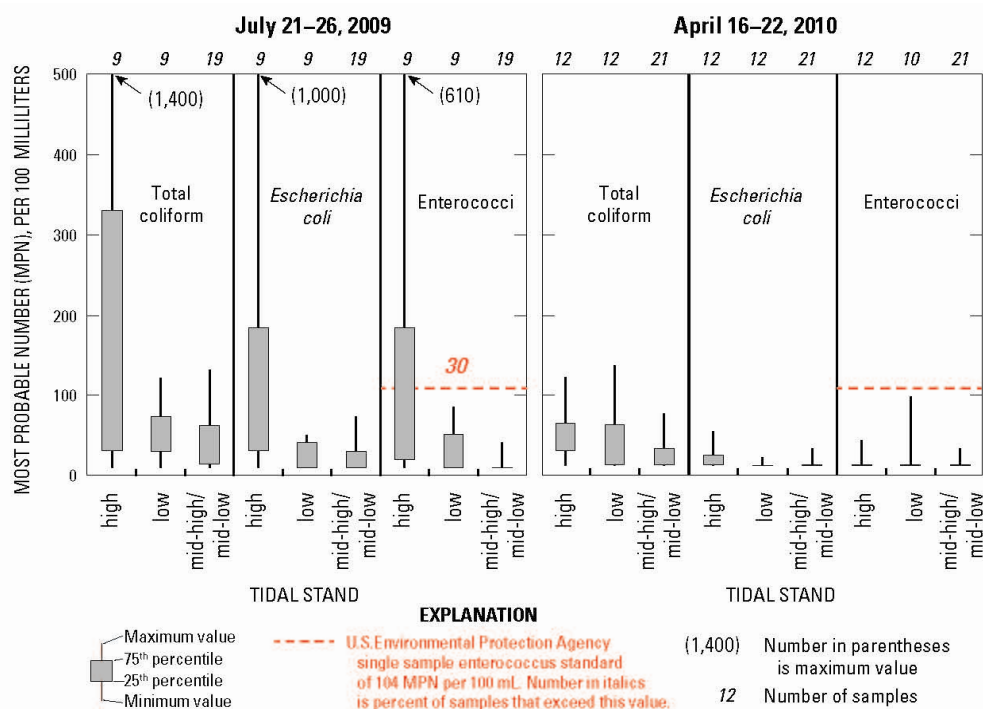


Figure 14 Fecal indicator bacteria (FIB) concentrations at high, low, mid-high, and mid-low tides at Puerco, and Malibu Colony Beaches, near Malibu, California, July 21-26, 2009, and April 16-22, 2010.

Table 3 Fecal indicator bacteria concentrations (FIB) in water extractions from kelp, and beach sands, near Malibu, California, July 2009 to April, 2010.

Sample	Date	Mass of sample in kg	Mass of extractant in kg	Extract					
				Fecal indicator bacteria in MPN per 100 mL			Fecal indicator bacteria in MPN per kg of sample		
				Total coliform	<i>Escherichia coli</i>	Enterococci	Total coliform	<i>Escherichia coli</i>	Enterococci
Kelp	7/24/09	5.3	7.3	20,000	140	11,000	280,000	1,900	150,000
	4/22/09	21.3	12.6	2,800	330	2,100	17,000	1,900	12,000
	4/16/07	12	7	12,000	8,600	24,200	70,000	50,000	140,000
	4/16/07	12	7	9,800	7,270	24,200	57,000	42,000	140,000
	4/19/07	12	11.4	15,500	7,270	3,450	150,000	69,000	33,000
	4/19/07	12	11.4	17,300	8,160	2,480	160,000	78,000	24,000
Sand	10/1/09	0.5	7	10	10	230	1,400	1,400	32,000
	4/22/09	0.5	7	20	10	10	2,800	1,400	1,400
	4/16/07	0.5	7	15,000	3,450	9,800	2,100,000	480,000	1,400,000

[Data in *italics* from West Beach in Santa Barbara, California (Izbicki, et al., 2009 [29]). kg, kilograms; MPN, Most Probable Number; ml, milliliters]

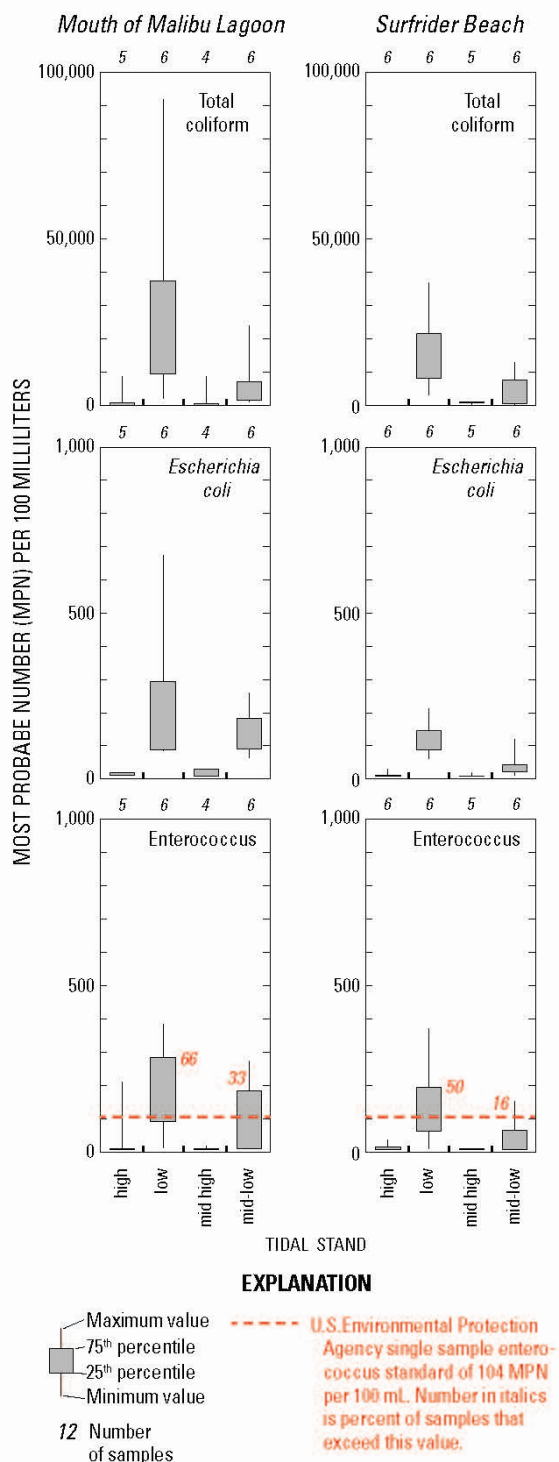


Figure 15 Fecal indicator bacteria concentrations (FIB) at high, low, mid-high, and mid-low tides in the discharge from Malibu Lagoon and Surfrider Beach, near Malibu, California, April 16-23, 2010.

Large waves associated with a strong south swell beginning on July 24 (Figure 12) disturbed the tidally driven differences in FIB concentrations at all three beaches. The swell also affected the specific conductance of the near-shore ocean, which may reflect a change in the tidally driven pattern of groundwater discharge to the ocean. Remobilization of FIB from beaches by wave action has been documented [136] and may be responsible for enterococci concentrations in excess of the single sample standard for marine recreational water measured at low, mid-high, and mid-low tidal stands during the swell (Figure 12). FIB data collected during the swell are not included in Figure 14.

During the April sample period, FIB concentrations were lower at Puerco and Malibu Colony Beaches than during the July sample period. Increased FIB concentrations at high tide were less pronounced than during July and there were no exceedances of the single sample enterococci standard at either beach during this time (Figure 14).

In contrast to Puerco and Malibu Beaches, exceedances of the single sample enterococci standard for marine recreational water were present in 17% of samples collected to the east of Malibu Lagoon at Surfrider Beach. These exceedances all occurred at low or mid-low tide when the Lagoon was discharging to the ocean (Figure 15). At this time, total coliform, *E. coli* and enterococci concentrations at Surfrider Beach were highly correlated with concentrations in the discharge at the mouth of the Lagoon, with Spearman rank correlations coefficients of 0.66, 0.74, and 0.74, respectively.

Kelp and sand extracts. FIB concentrations associated with kelp were as high as 280,000 MPN per kg for total coliforms; *E. coli* and enterococci were as high as 1,900 and 150,000 MPN per kg, respectively (Table 3). Total coliform and enterococci concentrations were similar to samples collected at West Beach in Santa Barbara, California about 90 km northwest of Malibu[29]; *E. coli* values were lower (Table 3). FIB concentrations in sand were lower than kelp, with total coliform concentrations as high as 1,400 MPN per kg, and *E. coli* and enterococci concentrations as high as 1,400 and 32,000 MPN per kg, respectively (Table 3). These data indicate the potential for kelp accumulated along the wrack line to contribute FIB to the near-shore ocean during high tide, ocean swells or storms.

High FIB concentrations in extracts from kelp on California beaches also were obtained by Imamura et al. [36]. Sand extract values are lower than those obtained from similar samples in Santa Barbara,

California [29]. These lower values are consistent with previous work that suggests that FIB accumulate to higher concentrations in sand on sheltered beaches, such as those sampled in Santa Barbara, and do not accumulate to high concentrations on more exposed, higher-energy beaches such as those found in Malibu [25, 136].

3.3. Genetic Data

More than 50 samples from wells, piezometers, Malibu Lagoon (including inflow from Malibu Creek), the near-shore ocean, within OWTS, and water extractions from sand and kelp were analyzed for genetic data. In this study, differences in microbial

communities were evaluated using T-RFLP data, and the presence of human fecal material was assessed on the basis of human-associated *Bacteroidales* data. DNA obtained from the qPCR amplification from selected samples collected during April 2010 also was analyzed using microarray techniques (PhyloChip).

Terminal-Restriction Fragment Length Polymorphism. T-RFLP data provide information on the diversity of microbial communities and the presence or absence of microorganisms within those communities [45-51]. T-RFLP uses restriction enzymes to break fluorescently-labeled DNA into smaller fragments known as amplicons (Figure 16).

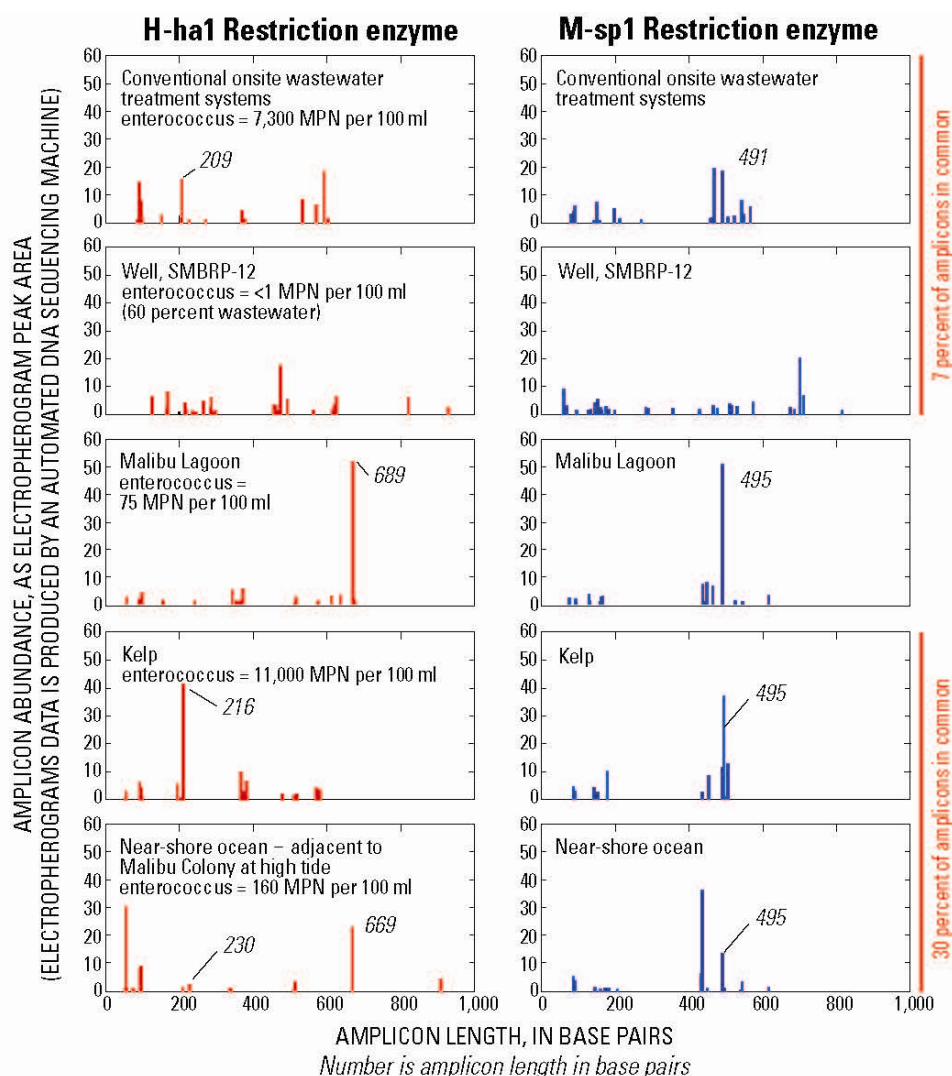


Figure 16
Amplicons from selected samples from a conventional onsite wastewater treatment system (OWTS), well, Malibu Lagoon, water extracts from kelp, and the near-shore ocean analyzed using T-RFLP with H-ha1 and M-sp1 restriction enzymes, Malibu, California, July 2009.

Two enzymes, H-ha1 and M-sp1, were used in this study. Each breaks DNA at different locations (corresponding to different sequences of base pairs), and produces a different assemblage of amplicons. Amplicons having different numbers of base pairs (amplicon length) represent different microorganisms. However, the sequence of base pairs within amplicons having the same length may be different, and more than one type of microorganism may be represented by an amplicon. The electropherogram peak area of the labeled amplicons (Figure 16) provides a measure of the abundance of an amplicon and the microorganism(s) it represents. The specific microorganism(s) represented by an amplicon is(are) not known without additional analysis.

Comparison of amplicons from samples collected from different locations provides information on the diversity and abundance of microbial communities. For example, T-RFLP data from a conventional OWTS, a well SMBRP-12 (which on the basis of oxygen-18 and deuterium data has a high percentage of wastewater), Malibu Lagoon, a kelp extract and the near-shore ocean show similarities and differences in the microbial communities present in the study area in July 2009 when the berm of the Lagoon was closed (Figure 16).

In this example, there is a low diversity of microorganisms in Malibu Lagoon. Although difficult to generalize, microbial diversity often decreases as eutrophication increases [137,138]. The T-RFLP data obtained using the H-ha1 restriction enzyme are dominated by one amplicon composed of 689 base pairs. Similarly, the M-sp1 restriction enzyme data also is dominated by one amplicon composed of 495 base pairs. These paired amplicons compose more than 50% of the labeled DNA obtained from each enzyme and are probably from the same microorganism(s). These paired amplicons also were present at high concentrations in other samples from Malibu Lagoon in July 2009 and contributed to a lack of diversity in those samples.

T-RFLP data from the near-shore ocean have paired H-ha1 and M-sp1 amplicons similar to the dominant amplicons present in Malibu Lagoon and likely represent the same microorganism(s) (Figure 16). In contrast, although the T-RFLP data from the kelp extract has an M-sp1 amplicon having 495 base pairs, the absence of the 669 base pair H-ha1 amplicon suggests the presence of a different microorganism, possibly the organism responsible for the H-ha1 amplicon at 216 base pairs, (Figure 16). Similarly, an M-sp1 amplicon having 491 base pairs was present in the sample from the conventional OWST; however,

the near-absence of the 669 base pair H-ha1 amplicon (present at 0.3% of the total peak area) suggests the presence of a different microorganism from the dominant microorganism in Malibu Lagoon. The H-ha1 amplicon at 209 base pairs suggests that this could be the same microorganism present in the kelp extract (Figure 16).

The lowest abundance but highest diversity of amplicons was in samples from wells, such as SMBRP-12 (Figure 16). Both the H-ha1 and M-sp1 amplicons that dominate microbial communities in Malibu Lagoon were absent in well SMBRP-12. Seven percent of the amplicons present in samples from the conventional OWTS also were present in well SMBRP-12 (Figure 16). The low percentage of common microorganisms (and the lack of FIB in the groundwater sample) is consistent with the changes in microbial populations that would be expected from filtration, sorption, death, predation and other processes that act on microbes as water moves through groundwater to discharge areas.

In contrast, there is similarity in the DNA from microbial communities in kelp and the near-shore ocean (Figure 16), where 30% of the amplicons were present in both samples. Processes affecting microbial populations as groundwater slowly moves through aquifers would not be expected to occur as tides and waves wash the wrack line. Although T-RFLP data track the more abundant members of the microbial community, these data are consistent with the possibility that high FIB concentrations in kelp (Table 3) are a source of FIB to the near-shore ocean at high tide or during ocean swells when kelp and other debris accumulated along the wrack line is washed by the ocean [29,36].

Although instructive of the general procedure for interpreting T-RFLP data, individual comparisons of the abundance, diversity and presence or absence of specific amplicons from more than 50 samples collected under different hydrologic conditions as part of this study was not feasible. Instead a multivariate statistical technique PCA was used to interpret the data. PCA of T-RFLP data was done for amplicons obtained using both the H-ha1 and M-sp1 restriction enzymes. PCA results for H-ha1 data showed the first, second, and third principal components explained 8, 7, and 6% of the variability in the data, respectively, for a cumulative percentage of 20%. PCA results for M-sp1 data showed the first, second, and third principal components explained 7, 5, and 5% of the variability in the data, respectively, for a cumulative percentage of 17%. PCA results for the two restriction enzymes were similar, and results for PCA of M-sp1 are

presented in Figure 17.

The first and second PCA scores for T-RFLP data closely grouped samples from OWTS, Malibu Lagoon, and the near-shore ocean (Figure 17). If OWTS discharged directly to the Lagoon or the ocean, this would be evidence of fecal contamination from OWTS. However, OWTS do not discharge directly to the Lagoon or ocean; instead, OWTS discharge to

groundwater, which subsequently discharges to the Lagoon or ocean. PCA results for sampled wells were independent of the fraction of water having a wastewater history (Figure 17). For example, PCA scores from some wells in the commercial area having a high percentage of wastewater such as CCSC-1 were similar to PCA scores from OWTS.

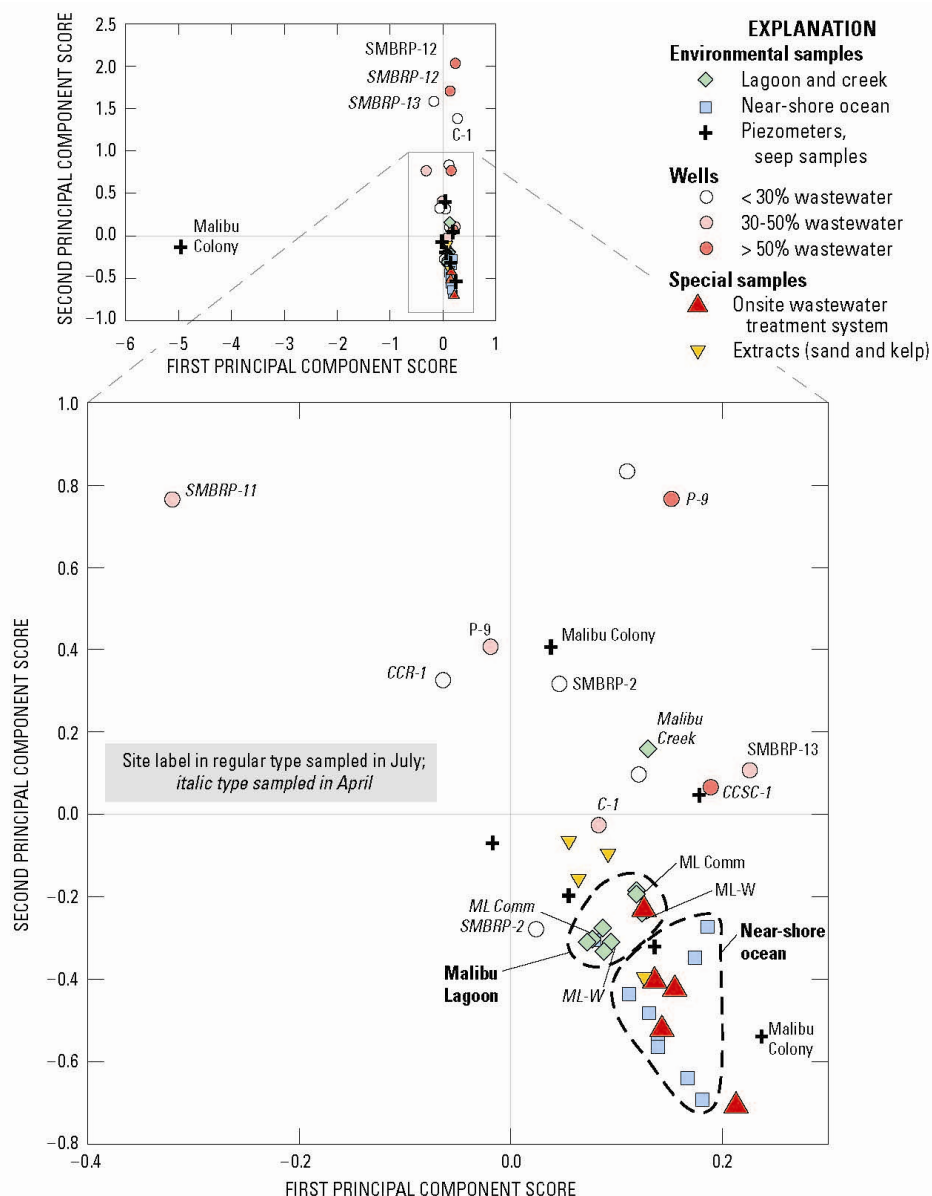


Figure 17 Results of Principal Component Analysis (PCA) for T-RFLP data with M-sp1 restriction enzyme digestion, from samples from onsite wastewater treatment systems (OWTS), water from wells, Malibu Lagoon, Malibu Creek, and the near-shore ocean, near Malibu, California, July 2009 and April 2010.

In contrast, PCA scores from some wells in the residential area having a similar high percentage of wastewater, such as SMBRP-12, were greatly different from scores from OWTS (Figure 17). The low cumulative percent of the data explained by PCA of T-RFLP data and the PCA scores from sampled wells do not support a cause and effect relationship linking microbial communities in OWTS and groundwater with those in Malibu Lagoon or the near-shore ocean. Instead, PCA results suggest that the similarity between samples collected from within OWTS and the samples from Malibu Lagoon and the near-shore ocean may reflect microbial communities that have developed independently in response to similar environments.

PCA scores from piezometers and seep samplers installed on the berm of Malibu Lagoon were similar to those from the Lagoon and ocean (Figure 17). This similarity in PCA results reflects the short travel time and distance for microbial communities in water moving from the Lagoon through the berm to the ocean to change through sorption, filtration, death, predation, regrowth or other processes. In contrast to Malibu Lagoon, PCA scores for piezometers in the beach adjacent to Malibu Colony (an unsewered residential development) showed little similarity to OWTS or water from other wells.

PCA scores were similar for samples extracted from kelp, sand, and other debris collected from wrack near the high-tide line with samples from the near-shore ocean (Figure 17). PCA scores from samples collected in the ocean at high tide were shifted toward the composition of extracts from kelp compared to samples collected at low tide (Figure 18). Washing of abundant microorganism from the wrack line by the ocean at high tide may explain this shift in PCA scores. In addition to the abundant microorganisms measured using T-RFLP, kelp on the beach also is a reservoir of FIB (Table 3), and contributions of FIB from beach wrack may partly explain increases in FIB concentrations in the near-shore ocean at high tide.

Human-Associated *Bacteroidales*. Human-associated *Bacteroidales* (HF183 SYBR) are an indicator of human fecal contamination. Human-associated *Bacteroidales* concentrations in OWSTs ranged from 4.2×10^4 to 5.3×10^8 copies per liter. Human-associated *Bacteroidales* concentrations were higher in the commercial and conventional treatment systems and were lower in the advanced systems, suggesting removal of *Bacteroidales* along with FIB within the advanced treatment systems. For comparison, human-associated *Bacteroidales* concentrations in untreated

inflow to a southern California wastewater treatment plant were about 10^6 copies per liter [29].

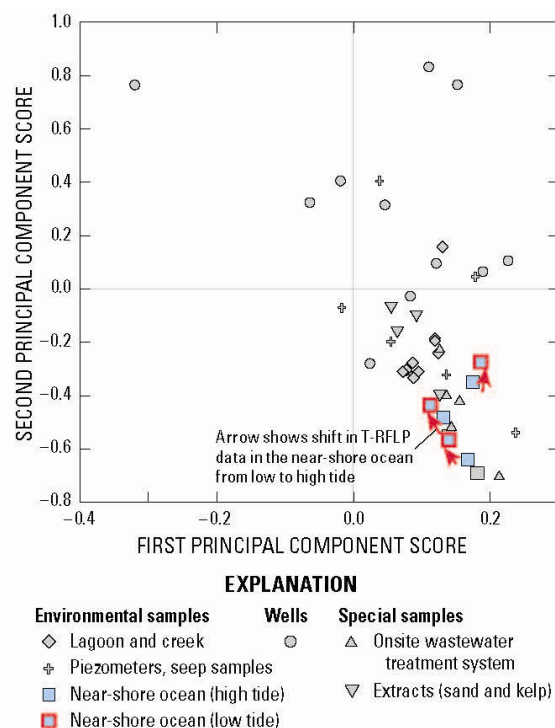


Figure 18 Results of PCA for T-RFLP data with Msp1 restriction enzyme digestion, showing the shift in principal component scores with tidal stand for samples from the near-shore ocean, July 2009 and April 2010.

Human-associated *Bacteroidales* were not detected in water from wells, Malibu Lagoon, the near-shore ocean, or in extracts from kelp or sand. The absence of human-associated *Bacteroidales* in wells, Malibu Lagoon, and the near-shore ocean is consistent with either dilution to low levels, or removal of microorganisms associated with human fecal material through filtration, sorption, death or predation.

Microarray (PhyloChip) data. The microarray (PhyloChip) technique can identify gene sequences (referred to as Operational Taxonomic Units or OTUs, rather than species) in DNA from almost 60,000 bacteria and archaea. Although the technique is less sensitive than qPCR analysis for specific microorganisms such as human-associated *Bacteroidales*, the large number of microorganisms that can be identified provides information on microbial communities present in different environmental

settings and the potential sources of those microorganisms [61,62].

Selected samples collected in April 2010 were analyzed using the microarray technique. More than 7,440 microbial OTUs were identified in 19 water samples from the study area (Figure 19) (Eric Dubinsky and Gary Andersen, Lawrence Berkeley National Laboratory, written communication, May 4, 2011). The number of OTUs present in an individual water sample ranged from 239 to 5,194. The largest number was in the sample from the conventional OWTS, followed by Malibu Creek and well SMBRP-2. The number of OTUs present was positively correlated with total coliform but not with *E. coli* or enterococci. For comparison, a composite of gull feces from eight samples collected in the Malibu area (Eric Dubinsky, Lawrence Berkeley National Laboratory, written communication, 2011) contained 2,130 OTUs (Figure 19). Four groups of microorganisms are discussed in this section *Bacteroidales*, *Firmicutes*, *Enterobacteriales* and *Pseudomonadaceae*. The

occurrence and distribution of specific microorganisms, including potential pathogens identified using microarray data, is beyond the scope of this paper.

Bacteria from the order *Bacteroidales* and the phylum *Firmicutes* dominate human and animal feces [139-141]. Almost half of the OTUs in the sample from the conventional OWTS and about 25% of the OTUs in gull feces were from these two groups (Figure 19). Although most *Bacteroidales* and *Firmicutes* are not specific indicators of the source of fecal contamination, their occurrence and distribution in wells, Malibu Lagoon and the near-shore ocean was examined to determine if there were similarities in microorganisms from these sources with microorganisms in OWTSs and gull feces. In contrast to water from OWTSs and gull feces. In contrast to water from OWTSs where *Bacteroidales* and *Firmicutes* dominate, *Bacteroidales* were not present and *Firmicutes* compose less than 3% of the OTUs in water from most sampled wells (Figure 19).

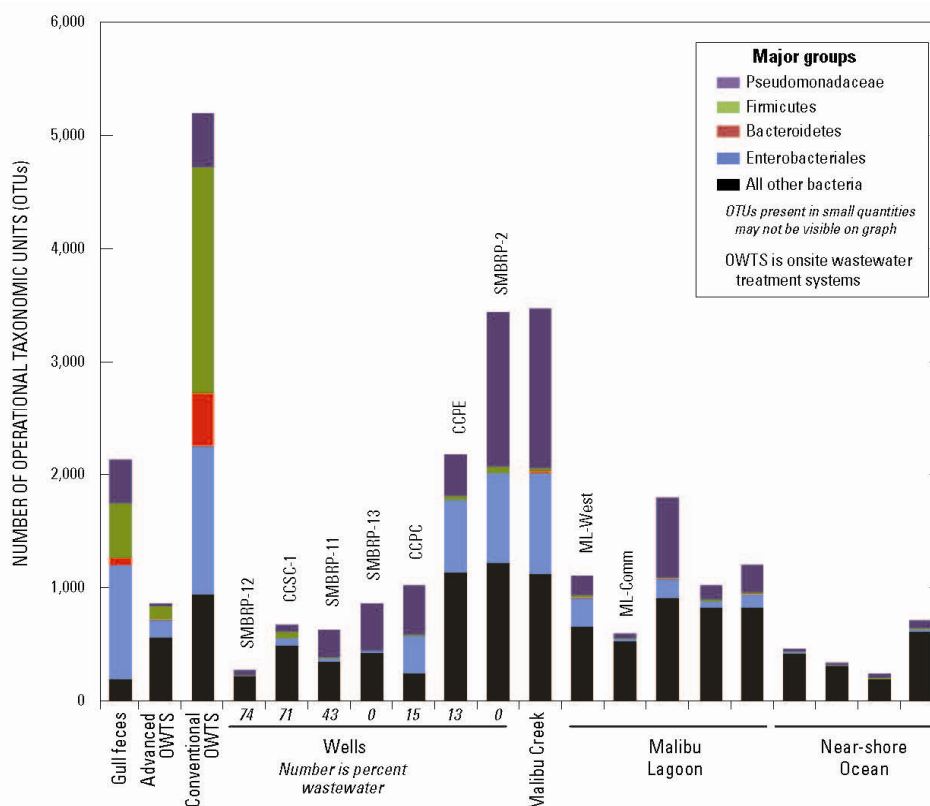


Figure 19 Operational taxonomic units (OTUs) of bacteria and archaea identified in selected samples from onsite wastewater treatment systems (OWTS), bird feces, existing monitoring wells, Malibu Lagoon, and the near-shore ocean near Malibu, Calif., April 17-22, 2010.

The low occurrence of these microorganisms within groundwater is consistent with the low occurrence of FIB in water from wells. The exception is well CCSC-1 in the commercial area near Malibu Lagoon, where *Bacteroidales* and *Firmicutes* compose almost 9% of the total OTUs. Well CCSC-1 had 71% imported water, total coliform concentrations of 220 MPN per 100 mL and enterococci concentrations of 1 MPN per 100 mL (Table 2). However, the *Bacteroidales* or *Firmicutes* OTUs identified in water from well CCSC-1 were not present in water from the sampled OWTS. *Bacteroidales* were absent in other wells having a high percent of imported water, such as SMBRP-12 in the residential area near Malibu Colony, and *Firmicutes* present in water from this well were not present in water from the sampled OWTS.

Bacteroidales and the *Firmicutes* comprise slightly more than 1% of the OTUs in water from Malibu Creek (Figure 19). The large number of OTUs present in water from Malibu Creek (3,469 OTUs) means a large number of *Bacteroidales* and the *Firmicutes* OTUs were present in the sample (23 and 26 OTUs, respectively). In contrast to water from wells, about 90% of *Bacteroidales* and the *Firmicutes* OTUs present in Malibu Creek were present in the sampled OWTS. *Bacteroidales* and *Firmicutes* OTUs associated with human fecal material from upstream discharges would be present in Malibu Creek.

Bacteroidales and the *Firmicutes* comprise less than 2% of the OTUs in water from Malibu Lagoon (Figure 19). Thirty percent of the *Bacteroidales* and *Firmicutes* OTUs present in Malibu Lagoon also were present in the sampled OWTS, and 40% of the *Bacteroidales* and *Firmicutes* OTUs present also were present in the composite sample of gull feces. A mixture of microbial OTUs associated with different sources is not surprising given the human fecal signal in Malibu Creek and the presence of birds in the Lagoon.

Bacteroidales were not present in any samples from the near-shore ocean and the *Firmicutes* comprised less than 4% of the OTUs in the near-shore ocean (Figure 19). Less than 3% of the *Firmicutes* OTUs present in the near-shore ocean also were present in the sampled OWTS, while on average 50% of the *Firmicutes* OTUs present also were present in the composite sample of gull feces. 90% of the *Firmicutes* OTUs present at high tide in the near-shore ocean adjacent to the berm of Malibu Lagoon also were present in gull feces. These data are consistent with increases in FIB concentrations (Figure 14) and with shifts in microbial community composition in the

near-shore ocean at high tide shown by PCA of T-RFLP data (Figure 18). They indicate that OWTS discharges are not the source of high FIB concentrations in the near-shore ocean.

Microorganisms within the order *Enterobacteriales* and *Pseudomonadaceae* also are commonly associated with human and animal feces, but also occur in non-fecal environments. *Enterobacteriales* and *Pseudomonadaceae* are abundant in samples from the conventional OWTS and in gull feces. These microorganisms also are abundant in water from some wells, especially wells having a low percentage of wastewater such as SMBRP-2, CCPE, and CCPC (Figure 19). Of the samples analyzed, these wells had the highest total coliform concentrations (Table 2) and the microbial OTUs in these wells are more similar to the OTUs from Malibu Creek than from the conventional OWTS (Figure 19).

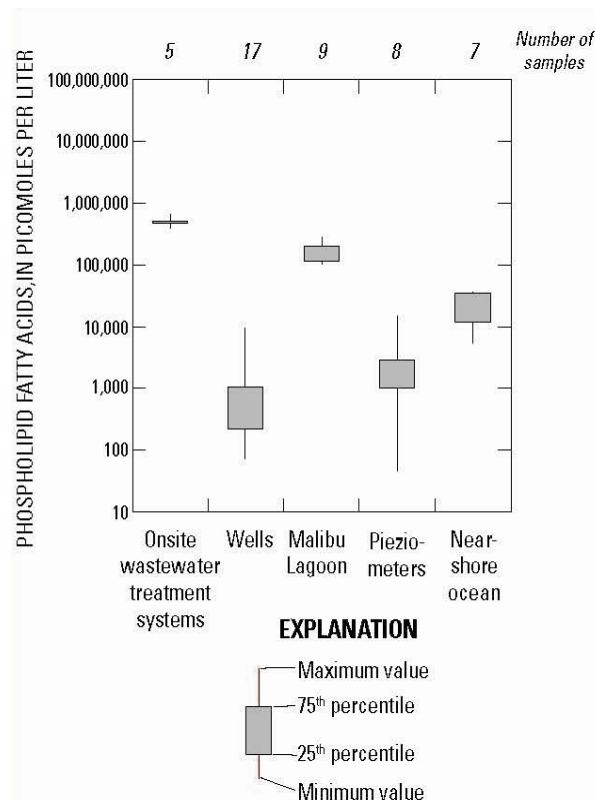


Figure 20 Phospholipid fatty acid (PLFA) concentrations in samples from onsite wastewater treatment systems (OWTS), wells, Malibu Lagoon, and the near-shore ocean, near Malibu California July 2009 to April 2010.

3.4. Phospholipid Fatty Acids

More than 50 samples from wells, piezometers, Malibu Lagoon (including inflow from Malibu Creek), the near-shore ocean, within OWTS and water extractions from sand and kelp were analyzed for phospholipid fatty acids (PLFAs). PLFAs provide energy, structural material for cellular membranes and facilitate biochemical reactions during cellular metabolism. Unlike genetic material that can be used

to identify individual microorganisms present in a sample, PLFA data can be used to evaluate biochemical processes in which microorganisms are engaged [142-146]. PLFAs degrade rapidly after cell death and are often more reflective of living (or recently living) microorganisms than DNA based techniques [111,147,148]. Higher PLFA concentrations reflect larger microbial communities and greater biomass. Lower PLFA concentrations reflect smaller microbial communities and smaller biomass.

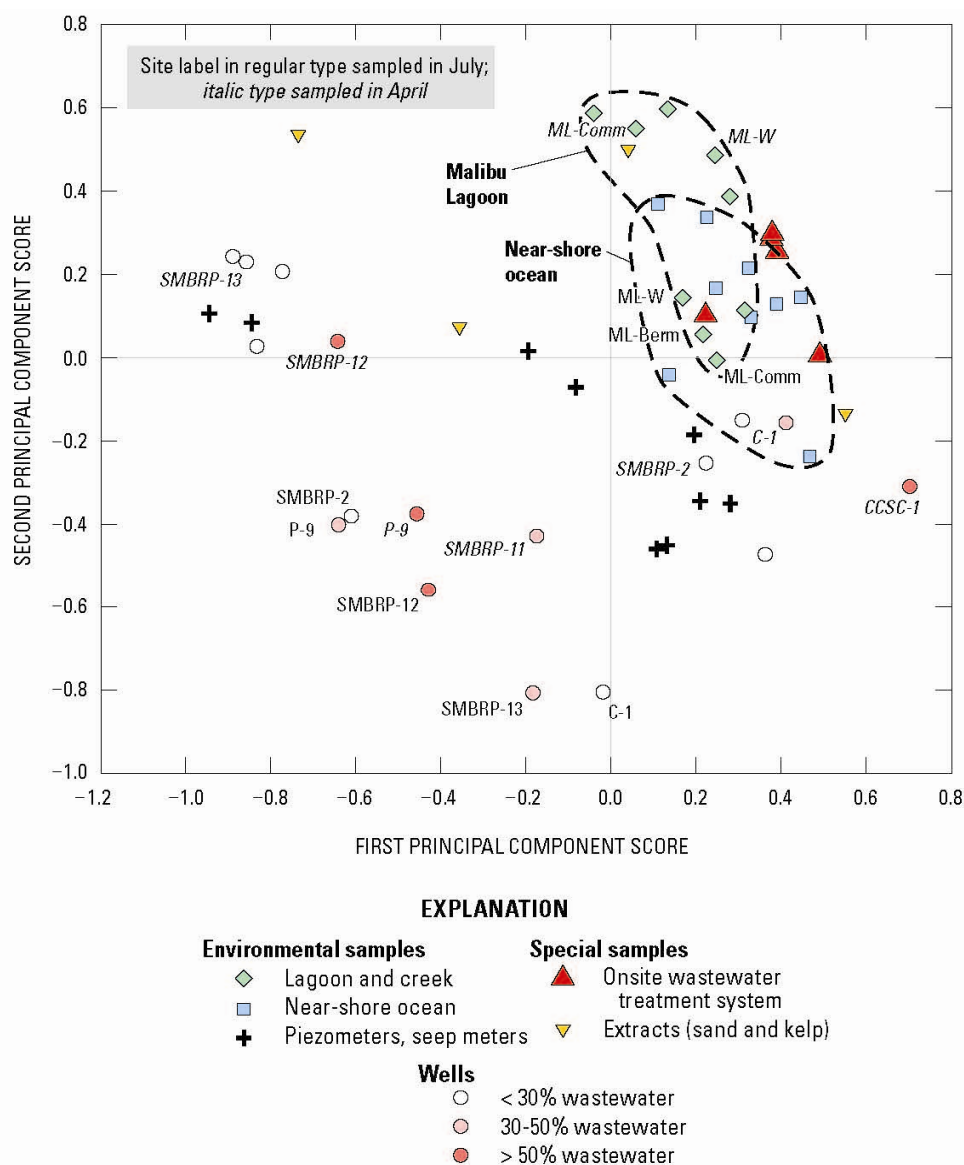


Figure 21 Results of PCA for phospholipid fatty acids (PLFAs) in samples from onsite wastewater treatment systems (OWTS), water from wells, Malibu Lagoon, Malibu Creek, the near-shore ocean, and extracts from kelp and sand, near Malibu, California, July 2009 and April 2010.

Thirty-seven PLFAs were identified in 53 samples from OWTS, wells, piezometers, Malibu Creek, Malibu Lagoon, the near-shore ocean and in water extracts from sand and kelp. Samples from OWTS had high total PLFA concentrations, ranging from 3.9×10^5 to 6.6×10^5 pmol/L (Figure 20). PLFA concentrations were higher in the commercial treatment system, followed by the advanced systems, with lower concentrations in the conventional systems. PLFA concentrations in Malibu Lagoon ranged from 1.0×10^5 to 2.9×10^5 pmol/L and in the near-shore ocean concentrations ranged from 5.4×10^3 to 3.7×10^4 pmol/L. Water samples from monitoring wells had the lowest PLFA concentrations, ranging from 72 to 9.6×10^3 pmol/L (Figure 20).

PCA was used to interpret PLFA data in the same manner as T-RFLP data. PCA of PLFA data showed that the first, second, and third principal components explained 19, 13, and 8% of the total variability in the data, for a cumulative percentage of 41%.

Similar to PCA results for T-RFLP data, the distribution of first and second principal component scores for PLFA data from OWTS, Malibu Lagoon, and the near-shore ocean were similar (Figure 21). PLFA data showed seasonal differences in PCA scores, possibly reflecting differences in microbial metabolism (Figure 21). However, similar to PCA results for T-RFLP data, water from wells had a wide range of PCA scores that were independent of the fraction of water having an imported water history (Figure 21). Piezometers and seep samples from the berm adjacent to Malibu Lagoon and adjacent to Malibu Colony plot between the composition of groundwater and the near-shore ocean (Figure 21) and appear to represent a transition between groundwater and ocean water consistent with their physical setting.

Even though FIB and microorganisms associated with human fecal material were not generally present in water from wells, discharges from OWTS may fertilize and alter microbial communities [51]. Comparison of DNA used for T-RFLP analysis and PLFA data was made to assess effects associated with OWTS discharges. DNA and PLFA concentrations in water from sampled wells were lower than concentrations within OWTS, Malibu Lagoon or the near-shore ocean (Figure 22).

Samples from wells collected in July 2009 plot along a line between groundwater and OWTS (Figure 22), consistent with fertilization of microbial communities in groundwater by nutrients discharged from OWTS. However, FIB were absent in the wells showing the greatest shift (C-1, SMBRP13 and SMBRP-12), consistent with die-off of these

microorganisms and the regrowth of other microorganisms in groundwater. In contrast, in April 2010 there was a shift in DNA and PLFA concentrations in water from wells toward the composition of Malibu Creek. At that time, recharge from Malibu Creek appears to have exerted a greater influence on DNA and PLFA concentrations than discharges from OWTS. Microarray (PhyloChip) data show a strong similarity in the microorganisms present in wells near Malibu Creek and the micro-organisms present within the Creek, especially well SMBRP-2 (Figure 19), and water recharged from Malibu Creek may be the source of higher total coliform concentrations measured in April 2010 compared to July 2009.

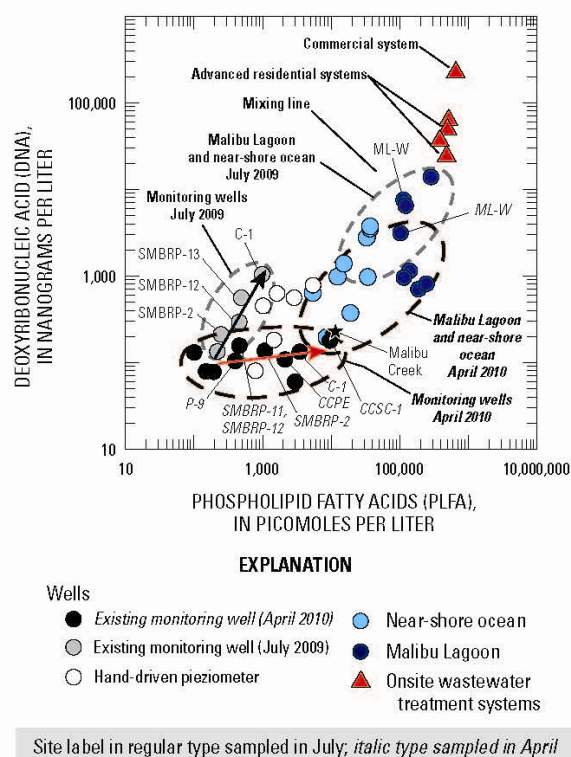


Figure 22 Deoxyribonucleic acid (DNA) as a function of phospholipid fatty acid (PLFA) concentrations in water from onsite wastewater treatment systems (OWTS), wells, Malibu Lagoon, and the near-shore ocean, near Malibu California, July 2009 to April 2010.

3.5. Selected Organic Compounds

More than 50 samples from wells, piezometers, Malibu Lagoon (including inflow from Malibu

Creek), the near-shore ocean, within OWTS and water extractions from sand and kelp were analyzed for a suite of 69 organic compounds. These compounds known as wastewater indicators can be divided into a number of categories depending on their use and origin (Table 4) [29]. Reporting limits for most compounds were in the parts per trillion range, and measured concentrations were below thresholds for public health or environmental concerns.

At least one wastewater compound was detected in 95% of all samples. The most commonly detected compound was cholesterol, which was present in 67% of all samples (Figure 23). Caffeine, commonly used as an indicator of wastewater from human sources, was detected in 19% of samples. One-third of the compounds analyzed were either not detected (9 compounds) or detected only once (14 compounds) (Figure 23).

Wastewater indicator compounds were detected at higher concentrations and with greater frequency in samples from OWTS than in water from other sources (Figure 23). For example, caffeine, indole (associated with the odor of fecal material), cholesterol (which may be dietary or fecal in origin), 3β -coprostanol (a form of cholesterol associated with fecal material from carnivorous mammals including humans), and triclosan (an antimicrobial) were present in samples collected within OWTS at concentrations typically 2 orders of magnitude greater than the concentrations from other samples where these compounds were detected.

Detergents and their metabolites, commonly excellent tracers of residential human waste [63,149], were largely absent in samples from residential OWTS within the study area (Table 4), possibly reflecting public awareness that certain chemicals interfere with the operation of OWTS.

As many as 25 compounds were detected in water from wells such as SMBRP-12 in Malibu Colony, which contains as much as 75% imported water having a wastewater origin. In contrast, no wastewater compounds were detected in water from other wells, such as SMBRP-2 in an undeveloped area east of Malibu Lagoon that is removed from OWTS discharges. Bisphenol-A and 3-methyl-¹H-indole (a degradate of indole) were the most commonly detected compounds in wells, present in 45% of sampled wells. Cholesterol, the most frequently occurring compound overall, was detected in 35% of samples from wells (Table 4). Detergent metabolites and flame retardants (used to treat children's clothing and other fabrics and commonly present in laundry

wastewater), which were not especially abundant in sampled OWTS, were detected frequently in wells (Table 4), suggesting that samples from OWTS may not have completely characterized the use and release of wastewater indicator compounds in the study area.

Caffeine, indole and 3-methyl-¹H-indole, which were present at high concentrations in wastewater but have a low number of detections in water from wells, may be degraded in the environment after discharge from the OWTS. In contrast, several man-made compounds, such as galaxolide (a synthetic musk used in cosmetics) and bisphenol-A (used in the production of polycarbonate plastic and epoxy resins) were frequently detected in wastewater and groundwater, with increasing concentrations as the fraction of wastewater increased (Figure 8). Similarly, fecal sterol concentrations generally increased with an increasing fraction of wastewater, consistent with human fecal sources for these compounds in water from wells (Figure 8).

The absence of some fecal sterols in water from some wells having a high fraction of wastewater is consistent with sorption or degradation of these compounds, similar to caffeine and 3-methyl-¹H-indole.

Water from Malibu Lagoon had fewer compounds detected than wells, with a maximum of 10 compounds present in any one sample (Figure 23). The most commonly detected compounds in Malibu Lagoon were cholesterol and tribromoethane (also known as bromoform, a common disinfection byproduct that can occur naturally in marine environments), which were present in 100 and 71% of samples from the Lagoon, respectively (Table 4).

Water from the near-shore ocean contained fewer compounds than either wells or Malibu Lagoon (Figure 23). Similar to Malibu Lagoon, cholesterol and tribromomethane were the most commonly detected compounds present in 89 and 100% of samples from the near-shore ocean (Table 4). Some personal care products such as galaxolide and DEET (an insect repellent) were detected during the July 2009 sample period when recreational use in the ocean was higher, but were not present during the April 2010 sample period when recreational use was lower. The number of wastewater compounds in piezometers and seepage samplers in the beach at Malibu Colony also was greater in July 2009 than in April 2010 (Figure 23). This is consistent with increased groundwater discharge containing treated wastewater from OWTS to the ocean when the berm of the Lagoon was closed and water levels in the Lagoon were high.

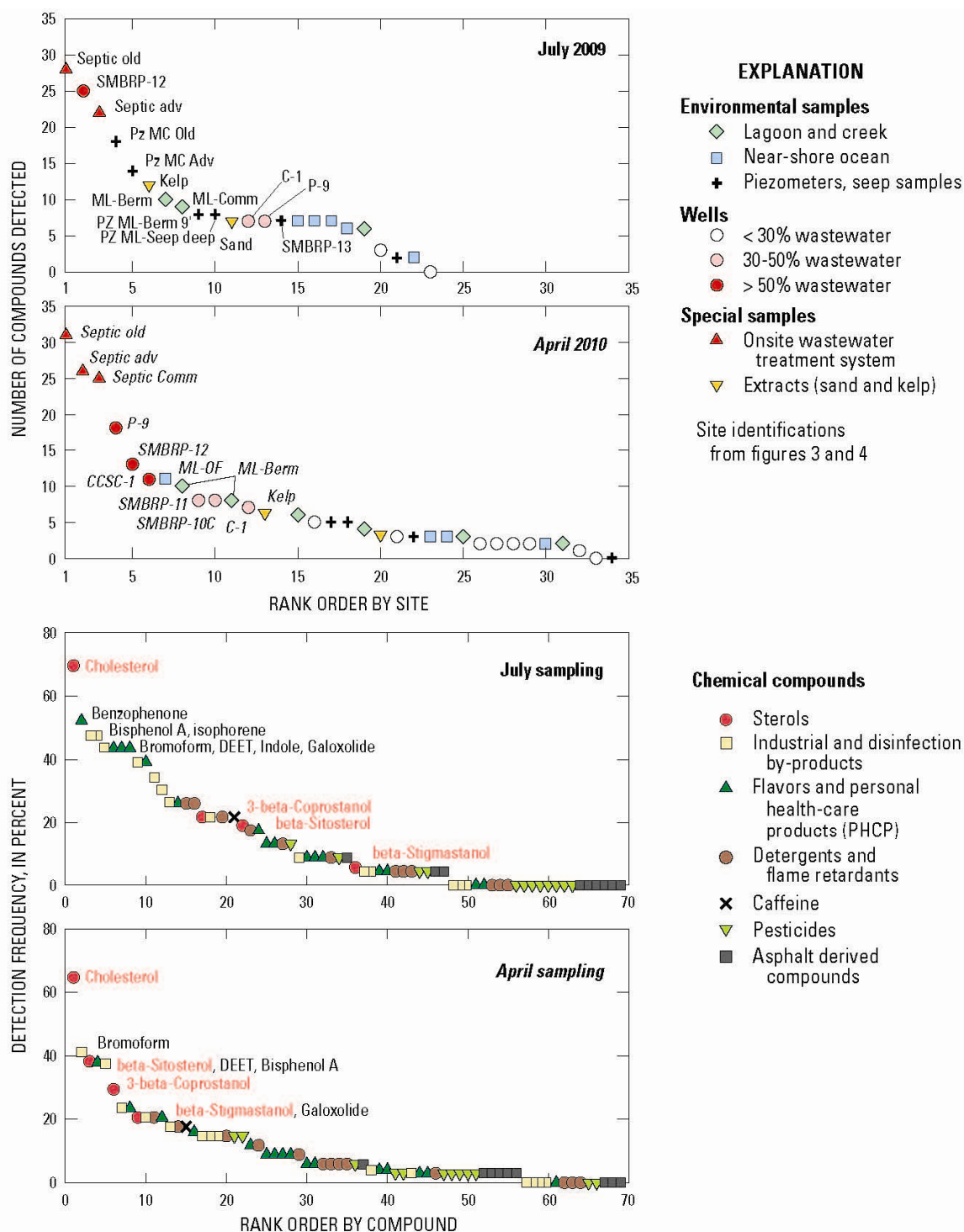


Figure 23 Selected organic compound abundance in samples from onsite wastewater treatment systems (OWTS), existing monitoring wells, Malibu Lagoon, piezometers, near-shore ocean, and extracts from sand and kelp, near Malibu, California, 2009-2010.

Table 4 Trace organic compounds and origin in water from onsite wastewater treatment systems (OWTS), wells, Malibu Lagoon, Malibu Creek, and the near-shore ocean near Malibu, California, 2009-2010.

Compound	Reporting limit	Onsite wastewater treatment systems (5)	Wells (17)	Malibu Lagoon (7)	Malibu Creek (1)	Piezio- meters (8)	Near-shore ocean (9)	Maximum concentration and where obtained	
Caffeine	0.2	5	2	0	0	2	1	180 OWTS	
Indole	0.2	5	1	3	0	0	4	28 OWTS	
Menthyl-1H-indole	0.2	5	1	2	0	0	1	23 OWTS	
Sterols									
3-beta-Coprostanol	1.6	4	5	2	0	1	2	30 OWTS	
Beta-Sitosterol	1.6	3	8	3	0	0	0	8.4 OWTS	
Beta-Stigmastanol	1.6	3	2	0	0	1	1	2.0 OWTS	
Cholesterol	1.6	5	6	7	0	6	8	38 OWTS	
Flavors, food additives, and fragrances									
3-tert-Butyl-4-hydroxyanisole	0.2	2	0	0	0	0	0	0.40 OWTS	
Acetophenone	0.4	3	0	0	0	1	0	2.3 OWTS	
Acetyl hexamethyl tetrahydro naphthalene	0.2	3	2	0	0	0	0	0.17 OWTS	
Benzophenone	0.2	4	5	1	0	5	1	30 OWTS	
Galoxolide (HHCB)	0.2	5	4	2	0	1	4	3.0 OWTS	
Menthol	0.2	0	0	0	0	0	0	-- --	
Tetrabromodiphenyl ether	0.2	0	0	0	0	0	0	-- --	
Triethyl citrate	0.2	4	1	0	0	0	0	1.2 OWTS	
Personal care products									
1,4-Dichlorobenzene	0.2	0	4	0	0	0	0	0.09 Well	
3,4-Dichlorophenyl isocyanate	1.6	1	0	0	0	0	0	0.016 OWTS	
Camphor	0.2	4	2	5	0	4	2	0.83 OWTS	
Cotinine	0.8	2	0	1	0	0	0	2.8 OWTS	
Carbazole	0.2	2	0	0	0	0	0	0.19 OWTS	
DEET	0.2	2	6	4	0	4	1	0.09 Well	
Isoborneol	0.2	0	0	0	0	0	0	-- --	

[Reporting limit and maximum concentration in micrograms per liter; Maximum concentration italicized where concentration below reporting limit was estimated by laboratory. Number in parenthesis in number of samples.]

Table 4 (cont.)— Trace organic compounds and origin in water from onsite wastewater treatment systems (OWTS), wells, Malibu Lagoon, Malibu Creek, and the near-shore ocean near Malibu, California, 2009-2010

Compound	Reporting limit	Onsite wastewater treatment systems (5)	Wells (17)	Malibu Lagoon (7)	Malibu Creek (1)	Piezio- meters (8)	Near-shore ocean (9)	Maximum concentration and where obtained	
Personal care products									
Isoquinoline	0.2	0	0	0	0	0	0	--	--
Naphthalene	0.2	1	0	0	0	1	0	0.008	OWTS
<i>p</i> -Cresol	0.2	5	2	3	0	4	0	330	OWTS
Pentachlorophenol	1.6	1	1	0	0	0	0	0.25	OWTS
Tricolsan	0.2	5	1	0	0	0	0	6.1	OWTS
Detergent metabolites									
4- <i>n</i> -Octylphenol	0.2	0	0	0	0	1	0	--	OWTS
4-Nonylphenol	1.6	1	4	0	0	0	1	1.0	Well
4-Nonylphenol diethoxylate	3.2	1	4	0	0	0	0	4.4	OWTS
4-Nonylphenol monoethoxylate	1.6	1	0	0	0	1	0	6.2	OWTS
4- <i>tert</i> -Octylphenol	0.4	2	4	0	0	0	0	0.89	OWTS
4- <i>tert</i> -Oxtylphenol diethoxylate	0.5	2	1	0	0	0	1	11	OWTS
4- <i>tert</i> -Oxtylphenol monoethoxylate	1.0	1	1	0	0	0	0	9.1	OWTS
Flame retardants									
Tris-2-butoxyethylphosphate	0.2	1	8	0	0	0	2	6.3	OWTS
Tris-2-chloroethylphosphate	0.2	2	3	0	0	3	0	9.3	Well
Tris-dichloroisopropylphosphate	0.2	0	0	0	0	1	0	--	--
Asphalt derived compounds									
1-Methylnaphthalene	0.2	1	0	0	0	0	0	0.005	OWTS
2-Methylnaphthalene	0.2	1	0	0	0	0	0	0.008	OWTS
2,6-Dimethylnaphthalene	0.2	1	0	3	0	0	0	0.04	Lagoon
Anthracene	0.2	0	1	0	0	0	0	0.008	Well
Benzo(a)pyrene	0.2	0	0	0	0	0	0	--	--
Fluoranthene	0.2	0	0	0	0	0	0	--	--
Phenanthrene	0.2	1	0	0	0	0	0	0.008	OWTS
Pyrene	0.2	0	0	0	0	0	0	--	--

[Reporting limit and maximum concentration in micrograms per liter; Maximum concentration italicized where concentration below reporting limit was estimated by laboratory. Number in parenthesis in number of samples.]

Table 4 (cont.)— Trace organic compounds and origin in water from onsite wastewater treatment systems (OWTS), wells, Malibu Lagoon, Malibu Creek, and the near-shore ocean near Malibu, California, 2009-2010

Compound	Reporting limit	Onsite wastewater treatment systems (5)	Wells (17)	Malibu Lagoon (7)	Malibu Creek (1)	Piezio-meters (8)	Near-shore ocean (9)	Maximum concentration and where obtained	
Pesticides, insecticides, and herbicides									
Bromocil	0.8	0	1	0	0	0	0	0.04	Well
Carbaryl	0.2	0	1	0	0	0	0	0.01	Well
Chlorpyrifos	0.2	0	1	0	0	0	0	0.02	Well
d-Limonene	0.2	4	0	0	0	0	1	1.4	OWTS
Diazinon	0.2	0	1	0	0	0	0	0.03	Well
Dichlorvos	0.2	0	0	0	0	0	0	--	--
Metaloxyl	0.2	0	0	0	0	0	0	--	--
Metolachlor	0.2	0	1	0	0	0	0	0.006	Well
Prometon	0.2	0	3	0	0	0	0	0.03	Well
Industrial compounds									
4-Cumylphenol	0.2	1	0	0	0	1	0	0.06	OWTS
5-Methyl-1H-benzotriazole	1.6	0	0	0	0	0	0	--	--
Anthraquinone	0.2	1	0	1	0	0	0	0.03	Lagoon
Bis-2-ethylhexylphthalate	2.0	2	1	1	1	2	5	1.2	Malibu
Bisphenol-A	0.4	2	9	4	1	4	2	4.1	Piezometer
Diethylphthalate	0.2	5	4	0	0	3	0	3.1	OWTS
Isophorone	0.2	1	1	3	0	4	0	0.08	Lagoon
Isopropyl benzene	0.2	0	0	0	0	0	0	--	--
Methyl salicylate	0.2	3	0	0	0	1	0	1.4	OWTS
Phenol	0.2	5	1	3	0	5	0	120	OWTS
Tetrachloroethene	0.4	1	5	0	0	0	0	0.2	OWTS
Tribromomethane	0.4	4	0	5	1	0	9	3.4	OWTS
Tributyl phosphate	0.2	3	7	0	0	0	1	0.66	OWTS
Triphenyl phosphate	0.2	3	2	0	0	4	1	0.38	OWTS

[Reporting limit and maximum concentration in micrograms per liter; Maximum concentration italicized where concentration below reporting limit was estimated by laboratory. Number in parenthesis in number of samples.]

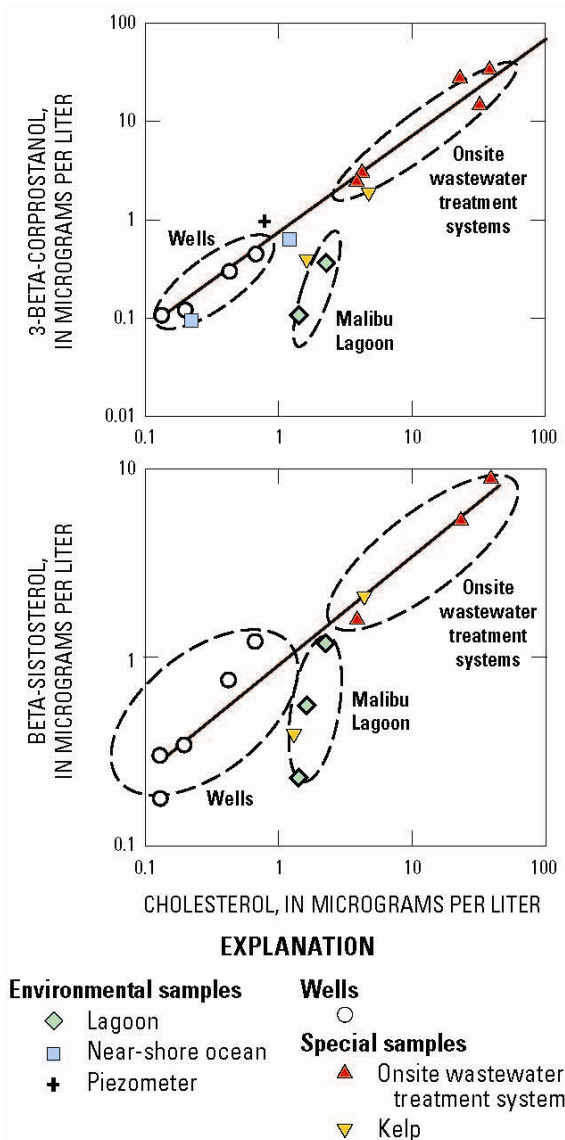


Figure 24 3-beta-coprostanol and beta-sitosterol as a function of cholesterol in water from onsite wastewater treatment systems (OWTS), wells, and Malibu Lagoon, Malibu Calif., July 2009 and April 2010.

The ratio of fecal sterols differs between humans and some animals as a result of metabolic and dietary differences; as a consequence, these compounds have been widely used to determine fecal sources [68-75]. Fecal sterol concentrations were high in wastewater, and present in water from some wells and in water from Malibu Lagoon. The ratio of cholesterol to 3-beta-coprostanol, the form of cholesterol associated

with carnivores and the primary form of human fecal cholesterol, is similar in samples from OWTS and in water from wells (Figure 24). Discharges from OWTS are the likely source of cholesterol and 3-beta-coprostanol in water from wells, consistent with the large amount of groundwater recharge from OWTS. The ratio of cholesterol to 3-beta-coprostanol also is similar for samples collected from piezometers and the near-shore ocean adjacent to Malibu Colony, and suggests the presence of groundwater discharge containing treated wastewater from OWTS to the ocean. However, the fecal sterol ratios differ in samples from Malibu Lagoon (Figure 24). The lower abundance of 3-beta-coprostanol relative to cholesterol suggests fecal inputs from a non-human (or at least non-carnivorous) source to Malibu Lagoon. Similar results were obtained for beta-sitosterol (Figure 24), a form of human dietary cholesterol commonly present in plants. A fourth fecal sterol, beta-stigmastanol was not detected frequently enough to permit interpretation.

PCA was used to further interpret selected organic compound data, including fecal sterol data, in the same manner as T-RFLP and PLFA data. PCA of wastewater indicator data showed that the first, second, and third principal components explained 22, 13, and 10% of the variability in the data, for a cumulative percentage of 45% (Figure 25). This was more than twice the variability explained by PCA of T-RFLP data, and is slightly greater than variability explained by PCA of the PLFA data collected.

In the same manner as PCA results for T-RFLP and PLFA data, PCA scores for the first and second principal components group data from Malibu Lagoon and the near-shore ocean together are combined (Figure 25). The PCA scores for kelp and sand extracts also plot near the range of these data. In contrast to PCA for T-RFLP and PLFA data, PCA scores for wastewater indicator compounds within OWTSs were different from all other data (Figure 25). Samples of groundwater having a low percentage of wastewater plot to the left of samples from Malibu Lagoon and the near-shore ocean. Samples having a high fraction of imported water plot between native groundwater and samples from within OWTSs, consistent with their wastewater history. PCA of wastewater indicator data shows a clearer relationship between OWTSs and groundwater having a high fraction of wastewater than either T-RFLP or PLFA data.

Samples from piezometers driven into the beach adjacent to unsewered residential development in Malibu Colony collected during the July sample

period plot near groundwater samples having a high fraction of wastewater. This is consistent with the discharge of treated wastewater to the near-shore ocean from OWTS when the berm raises water levels in the Lagoon, even though FIB were not present in these samples. Evidence of treated wastewater was not present in water from piezometers sampled during the April sample period when groundwater discharge in the area was less. Similarly, PCA scores for samples from piezometers on the berm near Malibu Lagoon during both the July 2009 and April 2010 sample periods are similar to samples collected within the Lagoon or ocean and do not reflect a wastewater history. In contrast to piezometers adjacent to Malibu Colony, many of the samples collected on the berm of Malibu Lagoon contain high FIB concentrations.

3.6. Groundwater Discharge and Fecal Indicator Bacteria in the Near-Shore Ocean

Changes in FIB concentrations in the near-shore ocean as a result of groundwater discharge were measured at two locations during July 2009 and April 2010: the sand berm at the mouth of Malibu Lagoon and the beach adjacent to unsewered residential development in Malibu Colony (Figure 1). Data collection at each site was during a falling monthly tidal cycle between spring and neap tide to maximize discharge of groundwater to the ocean, while minimizing discharge of ocean water emplaced in beach sands by high tides during the previous rising monthly tidal cycle [29]. Instrumentation at each site is shown in Figure 5.

Adjacent to Malibu Lagoon. A berm consisting of well-sorted, uniform beach sand up to 4 m thick, separates Malibu Lagoon from the ocean (Figure 1). The berm overlies cobbles deposited by Malibu Creek. The cobbles are exposed within the Lagoon and the adjacent ocean at low tide. While the berm is closed during the dry season, discharge from the Lagoon occurs as groundwater movement through the berm. The rate and timing of this discharge varies with the tides as the gradient between the Lagoon and the ocean changes. While the berm is open during the wet season, ocean water circulates in and out of Malibu Lagoon with rising and falling tidal cycles.

During the July 2009 sample period, the berm was overtopped by seawater at high tide, which flowed directly into the Lagoon. Seawater that infiltrated into the berm during high tide drained downward through the sand to the water table, forming an electrically conductive layer at the top of the water table (Figure 26b). This layer intersected the

upper part of the seepage face present along the beach at low tide (Figure 26a). Much of the water that discharged along this seepage face was ocean water infiltrated into the berm during the previous high tide. The underlying more resistive layer contained less saline water from Malibu Lagoon that discharged to the ocean through the lower part of the seepage face that was exposed at low tide (Figure 26).

Median total coliform, *E. coli*, and enterococci concentrations collected within the Lagoon at high, low, mid-high, and mid-low tides during the July 2009 sample period were as high as 650,000, 130,000 and 5,500 MPN per 100 mL, respectively (Figure 7). Total coliform, *E. coli*, and enterococci concentrations in samples from piezometers driven into the berm of the Lagoon at depths of 1.7 and 3 m during the July 2009 sample period were as high as 6,300, 520, and 50 MPN per 100 mL, respectively. FIB concentrations in the piezometers were lower than median concentrations measured in the Lagoon, consistent with removal of FIB in beach sand through sorption, filtration, death and other factors over even short distances.

During the July 2009 sampling period, specific conductance in the near-shore ocean at low tide was 34,300 $\mu\text{S}/\text{cm}$, and total coliform, *E. coli* and enterococci concentrations were 330, 180, and 590 MPN per 100 mL, respectively. At low tide, FIB were not detected and specific conductances in water from the seepage sampler in the upper part of the seepage face were near seawater values, ranging from 43,800 to 47,700 $\mu\text{S}/\text{cm}$, consistent with DC-resistivity data and infiltration of ocean water into the berm during the previous high tide. However, the low specific conductance in the near-shore ocean at low tide is consistent with fresh groundwater discharge (seepage deep on Figure 26a), suggesting that seepage of Lagoon water may be the source of FIB in the ocean under low tide. During high tide, enterococci concentrations of 100 MPN per 100 mL, in excess of the U.S. Environmental Protection Agency single sample standard for marine recreational water [9] also occurred (Figure 27). However, the specific conductance of 45,800 $\mu\text{S}/\text{cm}$ was consistent with seawater, suggesting that seawater washing beach sands, kelp, and other debris on the beach may be the source of enterococci at high tide, rather than water from the Lagoon.

High surf conditions during the July sample period precluded the collection of radon-222 data that would have confirmed the presence and timing of discharge from the Lagoon through the sand berm to the ocean during low tide.

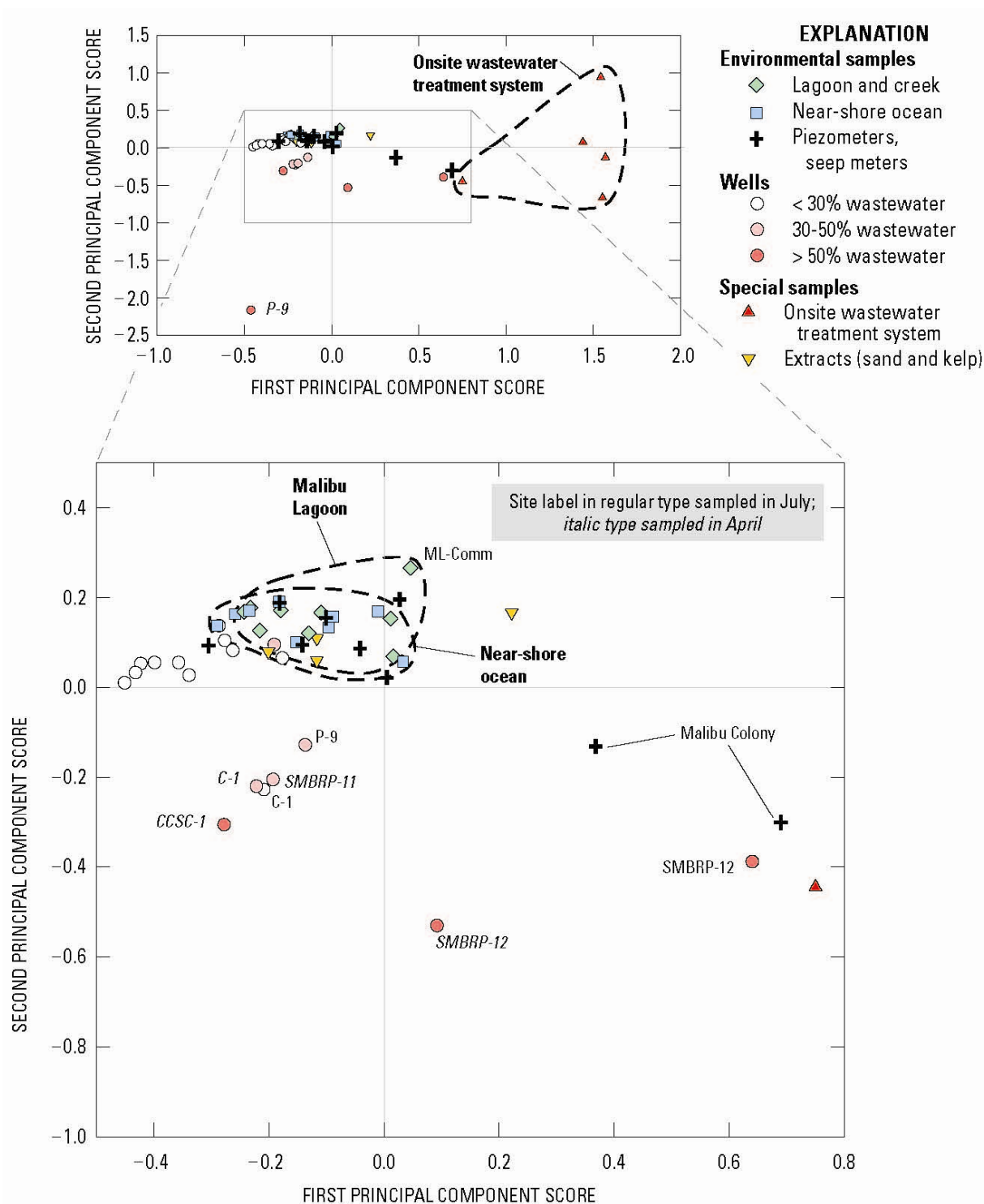


Figure 25 Results of Principal Component Analysis (PCA) for selected trace organic compounds in samples from onsite wastewater treatment systems (OWTS), water from wells, Malibu Lagoon, Malibu Creek, and the near-shore ocean, near Malibu, California, July 2009 and April 2010.

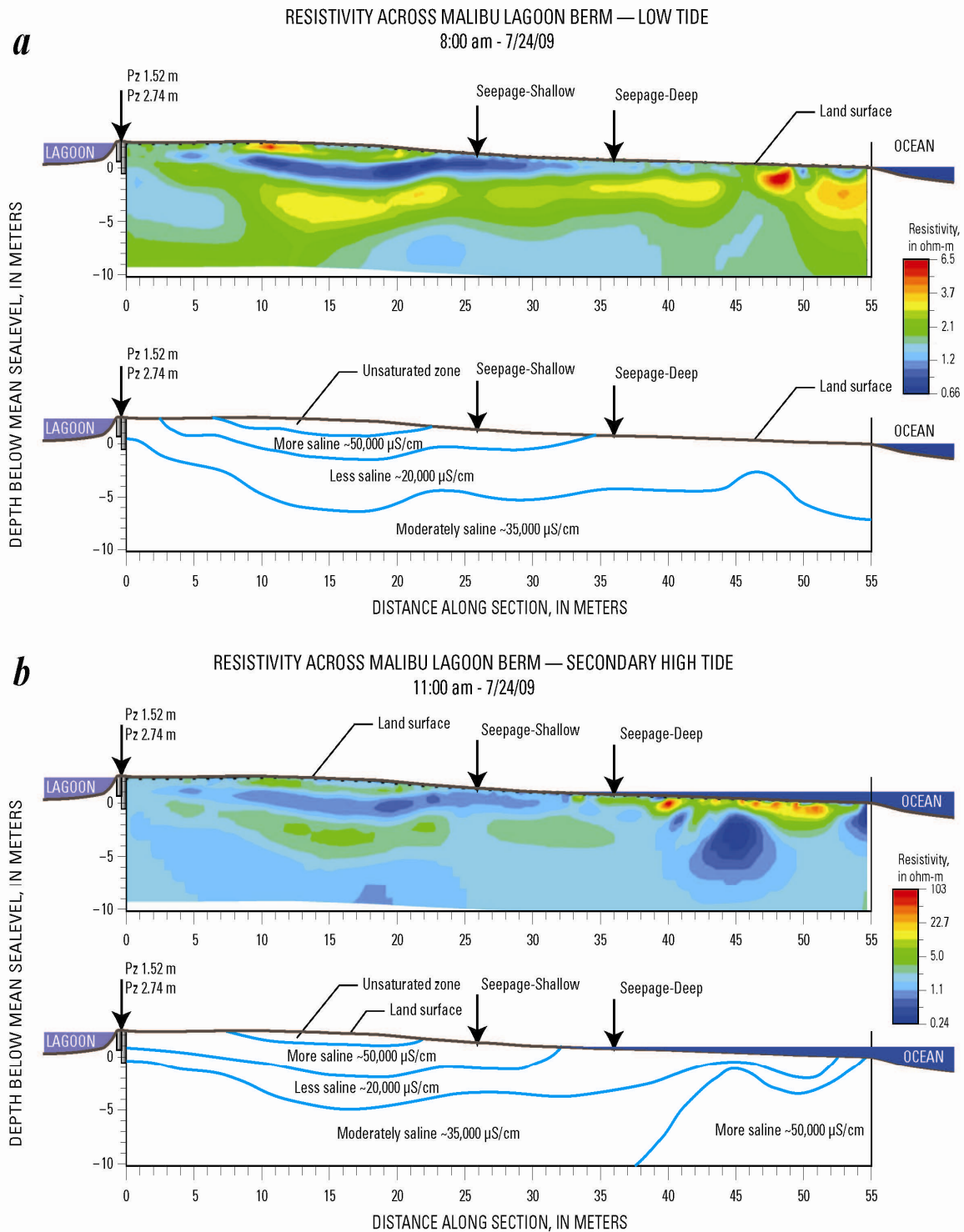


Figure 26 Direct-current (DC) resistivity data collected during low tide along a section perpendicular to the sand berm separating Malibu Lagoon from the Pacific Ocean, Malibu Calif., July 24, 2009.

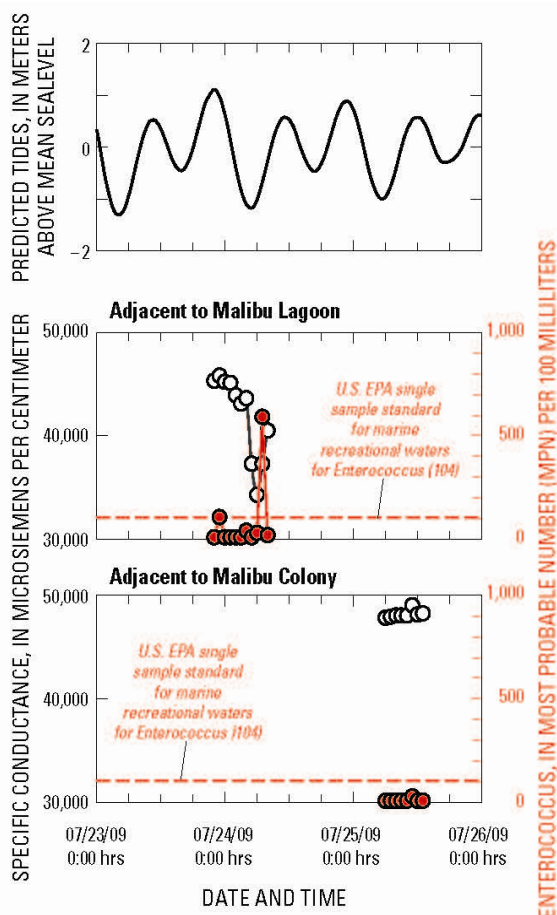


Figure 27 Tides, specific conductivity, and enterococci data for the near-shore ocean adjacent to Malibu Lagoon and Malibu Colony, Malibu, Calif., July 23-24, 2009.

As a consequence, sample collection for field parameters, FIB and radon-222 was repeated during a falling monthly tidal sequence in November 2009, prior to the breaching of the berm during the rainy season (Figure 28). Seawater did not overtop the berm at high tide during the November sample period. Water levels and FIB concentrations in Malibu Lagoon were lower in November than during the July sample period (the median of ten hourly samples for total coliform, *E. coli*, and enterococci were 1,700, 200, and 230 MPN per 100 mL, respectively), consistent with a decrease in FIB inputs to the Lagoon or with cooler air and water temperatures limiting the regrowth of bacteria in the Lagoon.

However, despite lower gradients and lower FIB concentrations in the Lagoon, increases in FIB concentrations were measured in the near-shore ocean

adjacent to Malibu Lagoon during low tide as specific conductance decreased and radon-222 concentrations increased coincident with movement of water through the sand berm to the ocean (Figure 28). Temporary piezometers and seepage samplers were not installed in the sand berm adjacent to Malibu Lagoon as part of the November sample collection.

During the April 2010 sample period, the berm of the Lagoon was open and discharge from the Lagoon was primarily as surface flow at low tide. The gradient driving groundwater movement through the berm was less than when the berm was closed.

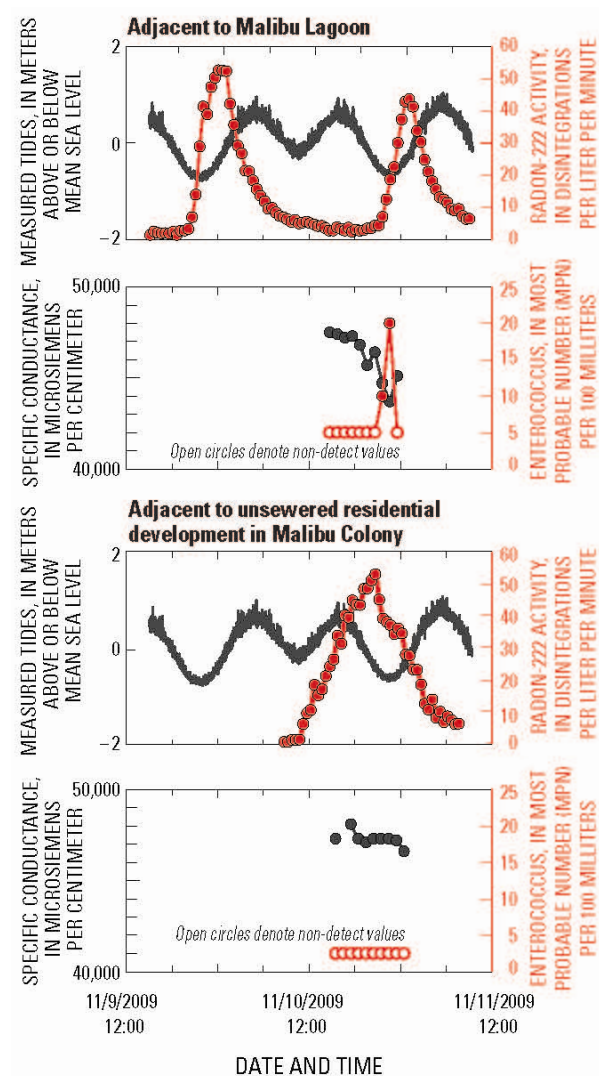


Figure 28 Tides specific conductance, radon-222 and enterococci data for the near-shore ocean adjacent to Malibu Lagoon and Malibu Colony, Malibu Calif., November 9-11, 2009.

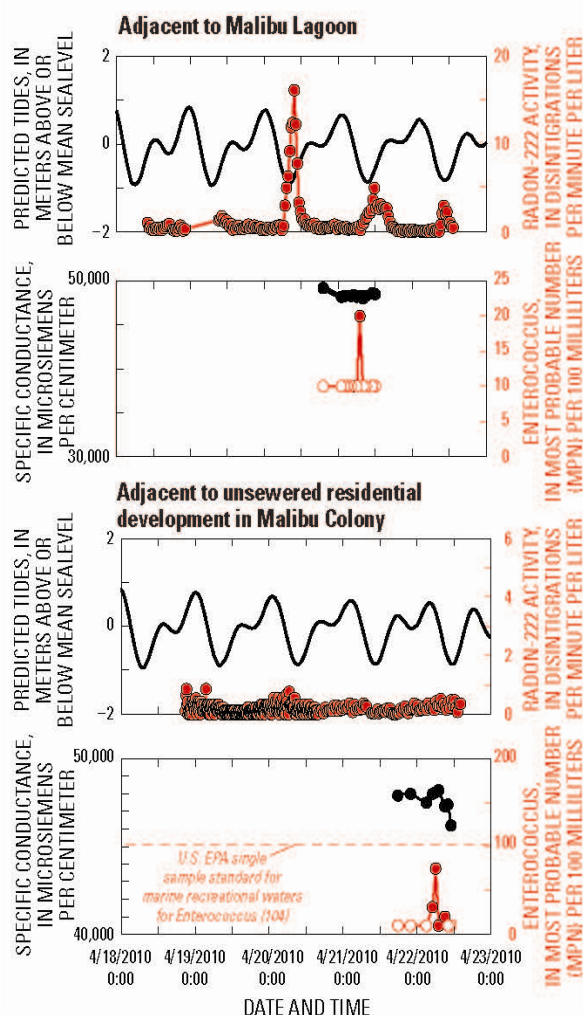


Figure 29 Tides specific conductance, radon-222 and enterococci data for the near-shore ocean adjacent to Malibu Lagoon and Malibu Colony, Malibu Calif., April 17-22, 2010.

Radon-222 activities confirm the lower rates of groundwater discharge through the berm of the Lagoon during April 2010 compared to November 2009 (Figure 29). The lower radon-222 activities on April 21, 2010 (when FIB samples were collected) are probably the result of dilution by stormflow from Malibu Creek.

Unlike the July 2009 sample period, specific conductance in the near-shore ocean during the April 2010 sample period did not decrease at low tide and FIB concentrations in piezometers, seepage samples and the near-shore ocean were low. Total coliform, *E. coli*, and enterococci concentrations were generally

below the detection limit of 10 MPN per 100 mL in piezometers and seepage samplers. Total coliform concentrations did not exceed 70 MPN per 100 mL, and *E. coli* and enterococci concentrations in the near-shore ocean were generally less than the detection limit of 10 MPN per 100 mL in the near-shore ocean (Figure 29). The highest enterococcus concentration, measured in the near-shore ocean during April 2010 was 20 MPN per 100 mL. This value did not occur at low tide, occurred prior to measured decreases in specific conductance or increases in radon activities, and presumably was from a source other than discharge of water through the berm of the Lagoon, such as surface water flowing out of the mouth of the Lagoon.

Comparison of the July 2009, November 2009, and April 2010 datasets shows that movement of water from Malibu Lagoon through the sand berm to the near-shore ocean can result in increased FIB concentrations in the near-shore ocean. When the FIB concentrations in the Lagoon are high and the flux to the ocean is sufficiently large (as in July 2009), FIB concentrations in the near-shore ocean at low tide can exceed the U.S. Environmental Protection Agency single sample standard for enterococci of 104 MPN per 100 mL [9].

Adjacent to Malibu Colony. The beach adjacent to Malibu Colony is narrow and the inland side of the beach ends at a seawall protecting the residential development. OWTS are located behind the seawall (Figure 5). The rate and timing of groundwater discharge to the ocean varies with the tides and varies seasonally with the opening and closing of Malibu Lagoon as a result of the changing groundwater levels in the alluvial aquifer (Figure 2).

During the July 2009 sample period, large waves made nighttime sample collection during the falling tide at this site unsafe. As a consequence, water-quality data were collected the following morning after the waves subsided, during the rising tide (from just before low tide to mid-high tide). DC-resistivity data collected on the beach parallel to the shore at low tide showed a conductive wedge of seawater extending from the water table to a depth of about 4 m. This wedge was underlain by a thin lens of resistive material, presumably sand containing fresher water discharging to the near-shore ocean (Figure 30). A thick layer of conductive and resistive materials was present at greater depths.

Total coliform concentrations in two temporary piezometers driven to a depth of about 2 m were as high as 2,000 MPN per 100 mL at low tide.

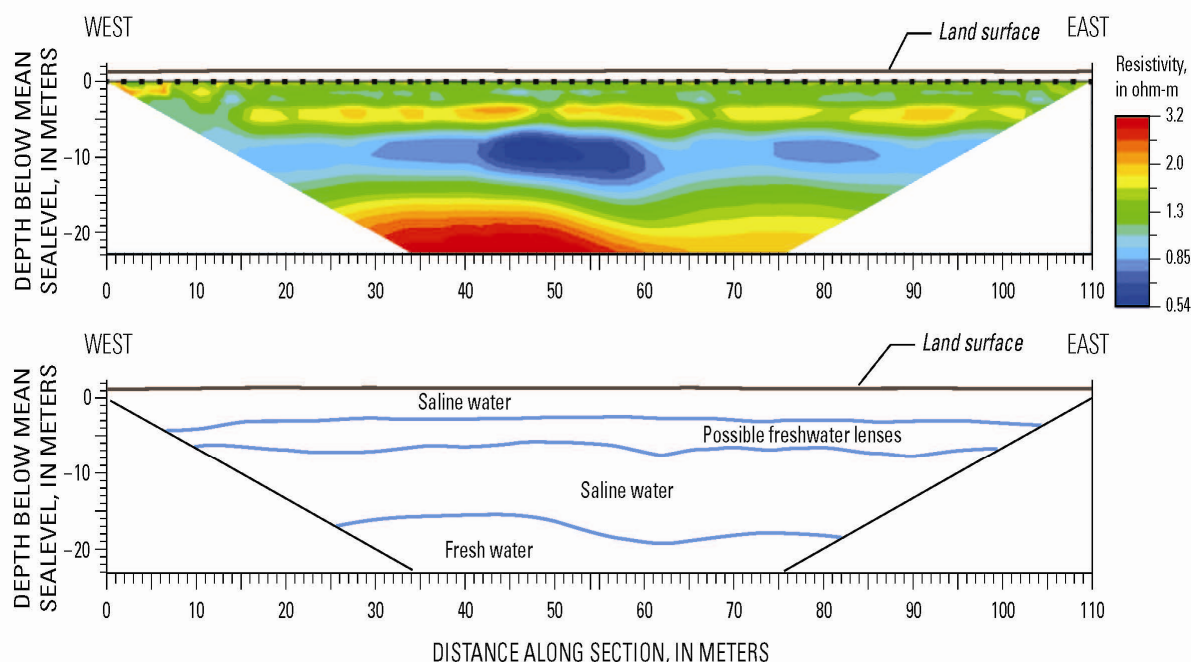


Figure 30 Direct-current (DC) resistivity data collected during low tide along a section parallel to the beach adjacent to unsewered residential development in Malibu Colony, Malibu California, July 24, 2009.

Total coliform concentrations decreased to near the detection limit of 10 MPN per 100 mL by two hours after low tide. *E. coli* and enterococcus concentrations were generally near the detection limit of 10 MPN per 100 mL during the entire period (Figure 27). Specific conductance of water from the piezometers ranged from 47,500 to 48,500 $\mu\text{S}/\text{cm}$ (Figure 27), consistent with seawater.

Given site conditions, it was not possible to drive a piezometer to a depth of 6 m where DC-resistivity data suggested fresher water was present, or to install seepage samplers on the seepage face along the beach. Total coliform concentrations in the near-shore ocean were as high as 600 MPN per 100 mL, and median total coliform concentration was 70 MPN per 100 mL. However, maximum *E. coli* and enterococci concentrations in the near-shore ocean were 10 and 30 MPN per 100 mL, respectively. (Figure 27). The high surf conditions that prevented radon-222 sample collection at the berm adjacent to Malibu Lagoon during the July sample period also prevented collection of radon-222 data on the beach adjacent to Malibu Colony.

Additional sample collection for field parameters, FIB and radon-222 was made adjacent to Malibu

Colony in November 2009 (Figure 28). Radon-222 data collected at that time showed groundwater discharge occurring to the near-shore ocean on the falling daily tidal cycle between high and low tide (Figure 28). The timing of this discharge is unusual in that the maximum radon-222 activity and groundwater discharge was occurring in advance of the low tide. FIB were not detected in any of 11 hourly samples collected from a temporary piezometer driven to a depth of 4 m (near the depth of the resistive (fresh) layer identified on the basis of DC resistivity data collected in July 2009). The specific conductance of water from this piezometer ranged from 24,200 to 30,400 $\mu\text{S}/\text{cm}$, and it presumably contained a mixture of seawater and fresh groundwater discharging to the ocean. Wastewater indicator data show numerous wastewater compounds present in this sample consistent with discharge from OWTS to the near-shore ocean despite the absence of FIB. Total coliform, *E. coli*, and enterococci concentrations in the near-shore ocean were consistently at or below the detection limit of 10 MPN per 100 mL (Figure 28).

During the April sample period, DC resistivity data were similar to data collected in July 2009 with a lens of resistive fresher water present at a depth of

about 4 m. However, radon-222 data show almost no groundwater discharge to the near-shore ocean at this time (Figure 29). This is consistent with water-level data [83] and groundwater flow model results [88] that show increased groundwater discharge to Malibu Lagoon and decreased groundwater discharge to the near-shore ocean near Malibu Colony during the wet season when the berm of the Lagoon is open. Although total coliform were present at the detection limit of 10 MPN per 100 mL, *E. coli* and enterococci were not detected in a temporary piezometer driven to a depth of 5.5 m. The specific conductance of water from this piezometer ranged from 36,100 to 39,200 $\mu\text{S}/\text{cm}$ and it presumably contained a mixture of seawater and fresh groundwater discharging to the ocean. In contrast to July 2009, wastewater compounds were largely absent in water from the piezometer at this time. FIB concentrations in the near-shore ocean adjacent to Malibu Colony were low during the April 2010 sample period (Figure 29). Total coliform concentrations ranged from 10 to 50 MPN per 100 mL, and *E. coli* and enterococci concentrations were generally less than the detection limit, although the enterococci concentrations in one sample were as high as 75 MPN per 100 mL.

Comparison of the July 2009, November 2009, and April 2010 datasets shows that groundwater discharge from the beach adjacent to Malibu Colony occurred in July and November 2009, although this discharge did not result in increased FIB concentrations in the near-shore ocean at low tide. On the basis of radon-222 data, groundwater discharge in this area was small in April 2010 and low concentrations of FIB in the near-shore ocean at this time must have resulted from sources other than groundwater discharge.

4. DISCUSSION AND CONCLUSIONS

The occurrence of FIB at concentrations above standards for recreational water has been a long-standing concern in Malibu Lagoon and nearby ocean beaches. It has been suggested by regulatory agencies that OWTS discharges may be the source of high FIB concentrations in the Lagoon and nearby recreational beaches, leading to a ban on the onsite disposal of human waste in the Malibu Civic Center area [93].

For OWTS discharges to be a source of FIB to Malibu Lagoon or the near-shore ocean, bacteria must move with groundwater to discharge in these areas. FIB concentrations in water-table wells sampled as part of this study were low and commonly below the

detection limit. In addition, human-associated *Bacteroidales* (HF183 SYBR) were not detected in water from wells. Although OWTS discharges compose 28% of the groundwater recharge to the aquifer [88], individual OWTS, especially residential OWTS, are small and the possibility that sampled wells were not suitably located to measure OWTS discharges was considered. On the basis of $\delta^{18}\text{O}$ and δD data, water from some wells contained more than 70% imported water presumably having a wastewater history. Water having a high percentage of wastewater also had a high percentage of wastewater indicator compounds, including fecal sterols. The absence of FIB in groundwater in the Civic Center area suggests that, at least during the period of this study, FIB were removed by OWTS or by the aquifer in close proximity to the OWTS, and not transported with groundwater to the Lagoon or the near-shore ocean. Total coliform present in some wells having low percentages of wastewater were associated with microbial communities and recharge from Malibu Creek.

Although FIB concentrations in groundwater were low, FIB concentrations in Malibu Lagoon were high and 55% of the samples exceeded the U.S. Environmental Protection Agency single sample standard for enterococci in marine recreational water. Similarly, 12% of the FIB samples at nearby beaches exceeded the marine recreational water standard. If groundwater containing OWTS discharges does not contain high FIB concentrations, there must be other sources of FIB to the Lagoon and the near-shore ocean.

Malibu Lagoon is nutrient-rich and eutrophic. During the summer, the temperature of water in the Lagoon approaches 31°C. Although FIB concentrations are high, human-associated *Bacteroidales* (HF183 SYBR) are absent. The ratio of fecal sterols in OWTS and groundwater are similar but different from the ratio in the Lagoon, consistent with a non-human fecal source to Malibu Lagoon. Although not specifically addressed by this study, it is possible that the Lagoon has been inoculated by non-human fecal sources such as birds, and extended survival or regrowth of FIB in the warm nutrient-rich water and sediments of the Lagoon is responsible for high FIB concentrations. This process would be consistent with rapid increases in FIB concentrations measured as part of this study in seawater overtopping the berm of the Lagoon and entering the Lagoon during summer high tides and with results from similar studies in southern California and elsewhere [16-20,33,34]. Additional data collection would be required to confirm extended

survival or regrowth of FIB in Malibu Lagoon.

Malibu Lagoon is a source of FIB to the near-shore ocean when the berm of the Lagoon is open during the wet season and seawater circulates into and out of the Lagoon with the daily tidal cycle. Malibu Lagoon also is a source of FIB to the near-shore ocean when the berm of the Lagoon is closed during the dry season. During low tide, water levels in the Lagoon are higher than in the ocean, and water moves through the berm and discharges at the base of the seepage face that is exposed at low tide. Although attenuation of FIB occurs within the berm, FIB concentrations in the near-shore ocean resulting from movement of water from the Lagoon through the berm to the ocean sometimes exceeded the single sample standard for marine recreational water during the July 2009 sampling period.

In contrast to discharge through the berm of Malibu Lagoon, groundwater discharge to the ocean adjacent to unsewered residential development in Malibu Colony did not contain FIB or human associated *Bacteroidales*. However, wastewater indicator compounds were present. Radon-222 data showed that groundwater discharge to the ocean in this area occurred at low-tide during the summer when the berm of the Lagoon was closed. Groundwater discharge to the ocean in the area was less when the berm of the Lagoon is open and groundwater movement toward the Lagoon is greater.

FIB concentrations at sampled beaches in the study area varied with tides, ocean swells, waves and other factors. With the exception of beaches near Malibu Lagoon, FIB concentrations were commonly higher at high tide, especially during the summer. Groundwater discharge occurs at low tide and therefore could not be the source of FIB at high tide. Results of this and other studies show high FIB concentrations associated with kelp and other debris accumulated near the high-tide line [29,35,36]. FIB washed from this material may be a source of FIB at high tide. Ocean swells and waves disturbed the general pattern of higher FIB concentrations at high tide and lower concentrations at low tide. Predictive models that use hydrologic and meteorological processes to estimate FIB occurrence at recreational beaches [80] may increase understanding of FIB occurrence and help protect the health of swimmers at beaches in the Malibu area.

Microbial communities are a direct manifestation of environmental conditions, such as inputs of nutrient-rich fecal material [51]. The community characterization approach and Principal Component Analysis used to interpret T-RFLP and PLFA data is

based on changes in the occurrence of the abundant members of the microbial community and does not track the less abundant fecal microorganisms directly. Results of PCA of T-RFLP data explained about 20% of the variability within the data. Results of PCA of PLFA data explained about 40% of the variability within the data. PCA results show that T-RFLP data were poor indicators of the presence of OWTS discharges in groundwater. The low cumulative percentage of the data explained by T-RFLP, the distribution of PCA scores from sampled wells and the poor relation between PCA scores and the presence of wastewater do not support a cause and effect relationship linking microbial communities in OWTS with those in Malibu Lagoon or the near-shore ocean. Instead, PCA results suggest that the similarity between samples collected from within OWTS and the samples from Malibu Lagoon reflect microorganisms and microbial communities that have developed independently in response to similar environments.

The higher percentage of variability explained by PCA of PLFA data compared to T-RFLP data is consistent with response of the microbial community to nutrient rich inputs from OWTS and other sources. The PCA results for T-RFLP and PLFA data are consistent with changes in microbial communities, even those that contain a high fraction of wastewater, as a result of filtration, sorption, death, and predation. These processes also result in removal of FIB discharged to groundwater and help to explain the low concentrations of FIB in water from wells.

In contrast, PCA of wastewater indicator data explain 45% of the variability in the data. Even though most wastewater compounds are not transported conservatively in groundwater, wastewater indicator data were interpretable in terms of water movement and were effective tracers of the presence of water discharged from OWTS. Although FIB and human-associated *Bacteroidales* were absent, wastewater indicators were present in groundwater discharging to the near-shore ocean adjacent to unsewered residential development.

In contrast to the microbial community characterization approach, which does not look at specific microorganisms, microarray (PhyloChip) data analyzed as part of this study provided data on the presence of more than 7,400 microbial Operational Taxonomic Units (OTUs) within the study area. *Bacteroidales* and *Fimicutes* OTUs are the dominant microorganisms in fecal material and were the predominant microorganisms in sampled OWTS. However, *Bacteroidales* and *Fimicutes* OTUs were largely absent in groundwater, consistent with the low

levels of FIB and the absence of human-associated *Bacteroidales* in water from sampled wells. More than 90% of the *Bacteroidales* and *Fimicutes* OTUs present in Malibu Creek also were present in OWTS, suggesting contributions from fecal sources in upstream discharges to the Creek. *Bacteroidales* were absent in the near-shore ocean, and *Fimicutes* OTUs present were associated with OTUs in composite samples of gull feces rather than OWTS.

These data suggest that FIB accumulated in beach wrack near the high tide line may be associated with fecal material from gulls and other birds.

This study used a wide range of hydrologic, geophysical, isotopic, and microbiological techniques. Results of this study would not have been possible using any of these techniques alone. Given the complexity of coastal systems and the high temporal variability in tidal and hydrologic processes, it is not reasonable to expect to identify variations in FIB concentrations or the source of FIB solely on the basis of regulatory data collected on a daily basis at a fixed time for public health purposes.

This study is one of many studies done in the Malibu area since work in the early 1990's identified human enterovirus in Malibu Lagoon. Each study prompted regulatory and stakeholder action to improve water quality in groundwater, Malibu Lagoon and the near-shore ocean. Results of this study show low FIB concentrations in groundwater underlying the Malibu area and may indicate that regulatory and stakeholder responses to FIB contamination in the study area were effective in reducing human-fecal contamination of groundwater.

Although a wide range of hydrologic conditions were sampled, the study was done near the end of an extended dry period. It is possible that specific conditions which lead to the failure of OWTS, such as high groundwater levels, were not present during this study. However, high FIB concentrations in Malibu Lagoon and occasional high FIB concentrations in the near-shore ocean were present during this study. This suggests that high FIB concentrations in Malibu Lagoon and occasional exceedances of standards for marine recreational water are at least partly caused by sources other than OWTS discharges, and that these exceedances may occur within the Lagoon and at ocean beaches in the Malibu area even if discharges from OWTS were eliminated. Viruses and other pathogenic microorganisms were not measured as part of this study, and results presented in this paper do not discuss possible health risks associated with OWTS discharges, water from Malibu Lagoon or the adjacent ocean. Epidemiological studies designed to address

public health issues at Surfrider Beach (Southern California Coastal Waters Research Project, <http://www.sccwrp.org/ResearchAreas/BeachWaterQuality/EpidemiologyStudies.aspx>, accessed June 4, 2012) were performed concurrently with this study.

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