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Title: Sources of Fecal Indicator Bacteria and Nutrients to Malibu Lagoon and Near-Shore Ocean Water, Malibu, California
Cooperating Agency: City of Malibu
Project Chief: John A. Izbicki
Period of Project: 2010-2011

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

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

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

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

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

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

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

By: John A. Izbicki

PROBLEM AND STUDY AREA

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

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

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

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

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

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

PURPOSE AND SCOPE

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

RELEVANCE AND BENEFITS

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

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

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

APPROACH

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

Preliminary Data Collection

Groundwater level, radon-222 (^{222}Rn), direct-current resistivity, FIB, and bacterial-source tracking data were collected during a falling monthly tidal cycle in the dry summer season from July 21-27, 2009 near Malibu, California. Data collection was coordinated with an epidemiological study of FIB occurrence and human health coordinated by the Southern California Coastal Waters Research Program (SCCWRP) and University of California Berkeley. Additional data were collected from septic systems and the near-shore ocean in the fall of 2009 to provide end-members for interpretation of the July data, and to verify the ability to collect ^{222}Rn data in the high-energy surf of the near-shore ocean near Malibu. Preliminary data provided a snap-shot in time of the occurrence, distribution and movement of FIB, nutrients, and other constituents in shallow groundwater, Malibu Lagoon (including its tributary Malibu Creek) and near-shore ocean water. In addition, preliminary data collection provided an opportunity to asses: 1) which data collection strategies (including the spatial and temporal distribution of samples) would be successful, and 2) which ancillary data would be useful in this complex hydrologic setting. Data collection issues were of special concern given the high surf conditions commonly present in the near-shore ocean near Malibu. Groundwater and surface water samples sites sampled are shown in figures 3 and 4, respectively.

Preliminary data collection showed:

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

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

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

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

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

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

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

Proposed data collection

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

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

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

Shallow groundwater—Five selected wells will be instrumented with pressure transducers to determine changes in water levels several weeks prior to, during, and for several weeks after the sample period. Water level data from these wells will be compared to and contrasted with tidal data, ocean swell data, and water-level data from Malibu Lagoon (fig. 5) to determine the effects of tides, ocean swells, and changes in lagoon water levels on the discharge of shallow groundwater to Malibu Lagoon and the near-shore ocean. Fifteen wells will be sampled. This represents and increased in the number of wells compared to the July 2009 synoptic sample collection. The increase is intended to allow additional wells to be sampled in the commercial area near Malibu Lagoon and in the Sierra Retreat area. Wells will be sampled for field parameters (pH, specific conductance, and dissolved oxygen), FIB (total coliform, *E. coli*, and enterococcus), major-ion and nutrient concentrations (Table 2), the stable isotopes of oxygen and hydrogen (oxygen-18 and deuterium), ^{222}Rn activity, and dissolved organic carbon and optical property data (including full-spectrum ultraviolet absorbance and excitation emission spectroscopy). Ten sampled wells will be analyzed for a more complete list of constituents including δ^{15}N and δ^{18}O of nitrate (or the δ^{15}N of ammonia where appropriate), and bacterial source-tracking data including genetic (Terminal-Restriction Fragment Length Polymorphism, and human-specific *Bacteoidales*), molecular (Phospholipid fatty Acids), and wastewater indicator (Table 3) data. Observation wells will be purged and sampled using peristaltic pumps. As many as four peristaltic pumps will be used to purge observation wells thereby minimizing purge times. Tubing and hoses used during purging and sample collection will be discarded after each use to prevent cross-contamination between sampled wells.

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

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

Near-Shore Ocean Water—Near-shore ocean water will be sampled at high and low tidal stands (approximately four times each day) at three beach locations to determine how field parameters and FIB concentrations change with tidal fluctuations. The three sample sites (OF-A, AF-B, and OF-C) are located to the west of the unsewered residential development in Malibu Colony, near the berm of Malibu Lagoon, and the east of Malibu Lagoon, respectively (fig. 4). The data will be compared and contrasted with tidal and swell data and with similar data collected from Malibu Lagoon (fig. 10).

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

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

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

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

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

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

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

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

Water-quality sample handling and analysis

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

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

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

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

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

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

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

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

Quality assurance data for the two non-USGS laboratories used as part of this study will be compared with quality assurance data collected as part of previous work (Izbicki and others, 2009). Internal laboratory practices within those laboratories will be assessed on the basis of standard operating protocols provided by the laboratories.

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

Tracer Data

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

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

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

Oxygen-18 and deuterium—Oxygen-18 and deuterium are naturally occurring stable (non-radioactive) isotopes of oxygen and hydrogen, respectively. Oxygen-18 and deuterium abundances are reported as ratios of the heavier isotope (oxygen-18 or deuterium) to the more common lighter isotope (oxygen-16 or hydrogen) using delta (δ) notation in per mil (parts per thousand) differences relative to the standard known as Vienna Standard Mean Ocean Water (VSMOW) (Gonfiantini, 1978). By convention the value of VSMOW is 0 per mil. δ^{18}O and δD ratios can be measured more accurately than absolute abundances, with precisions of about ± 1 per mil and ± 0.1 per mil, respectively. When using this notation samples having less-negative δ values contain more of the heavier isotope than samples having more-negative δ values.

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

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

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

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

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

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

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

The biological reactivity and the wide range of oxidation states in nitrogen compounds results in a wide range in δ^{15}N isotopic compositions spanning more than 200 per mil relative to standard atmospheric nitrogen gas (Coplen et al., 2002). Despite this wide range, the δ^{15}N isotopic composition of ammonia in septage is relatively constant averaging 4.9 ± 0.4 per mil (Hinkle and others, 2008). The δ^{15}N isotopic composition of nitrate derived from septage varies more widely, averaging 7.2 ± 2.6 per mil (Hinkle and others, 2008). The range in δ^{15}N composition of nitrate derived from septic tank discharges is not random, instead higher δ^{15}N values of nitrate result from more extensive loss of nitrogen through biological processes during the conversion of ammonia in septic waste to nitrate and lower δ^{15}N values result from less loss of nitrogen. In the environment, the isotopic composition of nitrate from septic discharges can be further altered as a result of processes such as denitrification. As nitrate is converted to nitrogen gas during denitrification, nitrate is lost from the system and the δ^{15}N composition of residual nitrate increases as nitrate concentrations decrease. Interpretation of nitrogen isotope data from septic discharges is complicated by loss on nitrogen through the volatilization of ammonia, addition of nitrogen from other sources including fertilizer and animal wastes, and by the number and complexity of biological reactions that can alter the $\delta^{15}N$ isotopic composition of nitrate and ammonia. The $\delta^{18}O$ composition of oxygen in the nitrate molecule can be used to understand some of the complexity and in some cases to distinguish nitrate from septic and fertilizer sources (Kendall, 1998). The combination of chemical and isotopic data will be used to understand environmental processes controlling ammonia and nitrate concentrations in groundwater, Malibu Lagoon and near-shore ocean water.

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

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

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

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

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

Comparison of T-RFLP amplicons from Malibu Lagoon (ML-Berm), a peiziometer driven into the berm of the lagoon to a depth of 5 feet (ML-Berm-Pz5), and the near-shore ocean at low tide shows a number of different amplicons representing the microbial communities in each of these samples (fig. 16). Malibu Lagoon is the least diverse of the three communities and the microbial population is dominated by 2 amplicons having lengths of 520 and 690 base pairs (M-spl and H-ha1 restriction enzymes, respectively). These amplicons also are present in water sampled from the pieziometer and from the near-shore ocean at low tide. Common amplicons indicate the potential presence of the same organisms in each sample. This result is consistent with bacterial transport from the lagoon to in the near-shore ocean as seepage through the berm at low tide. In contrast, little similarity was observed in the amplicons representing microbial communities in septic tanks, shallow groundwater and the near-shore ocean at high and low tide near Malibu Colony. Those data suggest large differences in the microbial community between these samples. Although water may flow from the septic tanks to discharge at the near-shore ocean the microbial community is greatly altered and transport of individual bacteria is probably limited from septic tanks to the near-shore ocean is probably.

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

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

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

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

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

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

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

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

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

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

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

REPORTS

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

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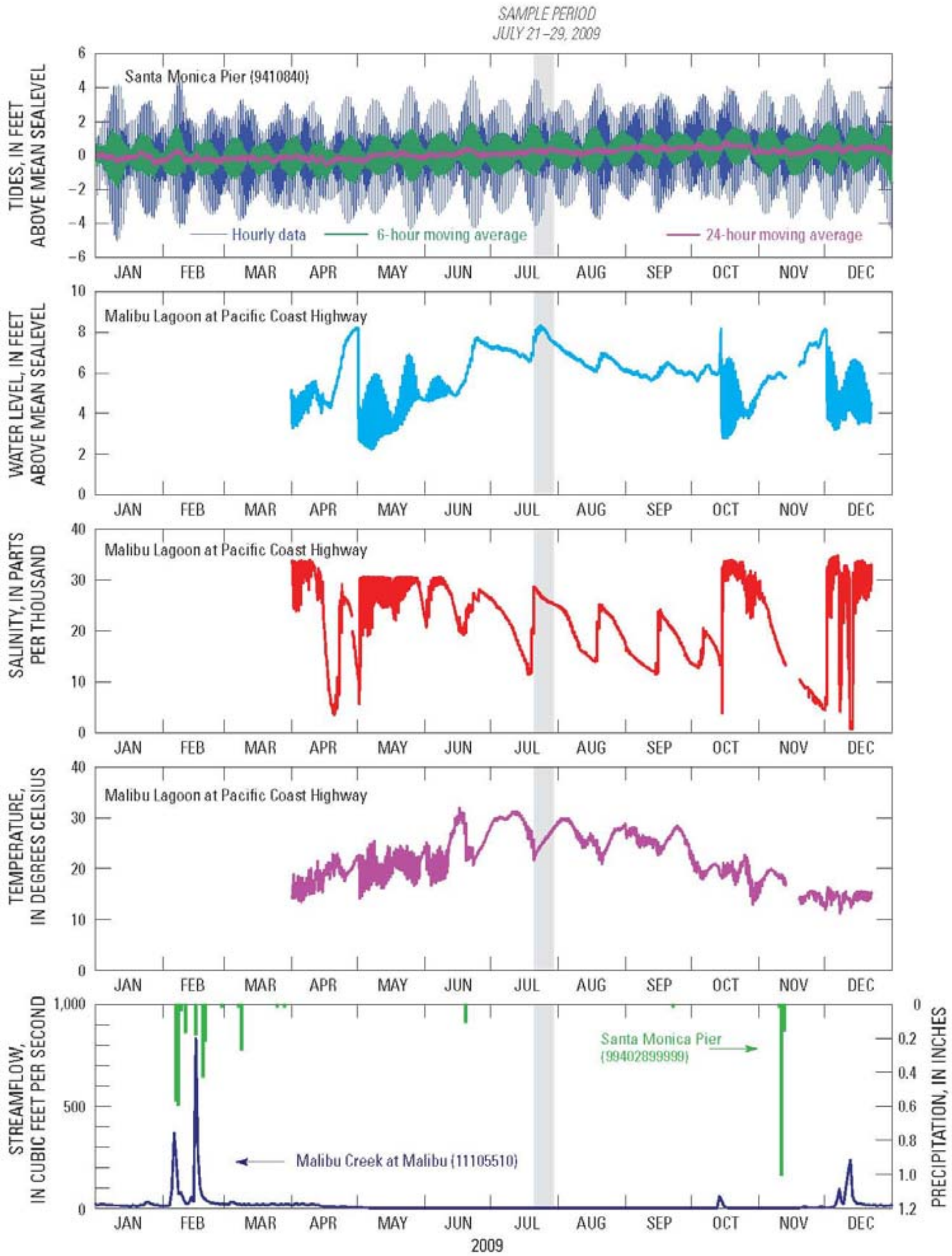

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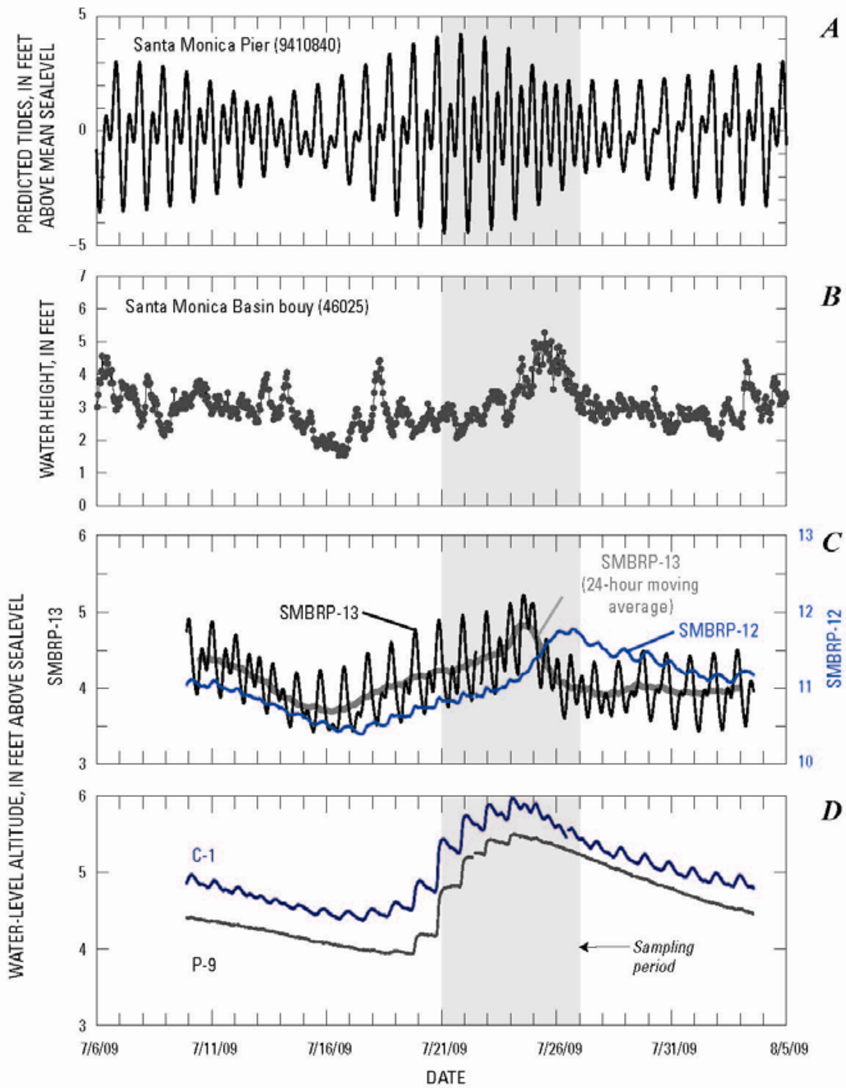

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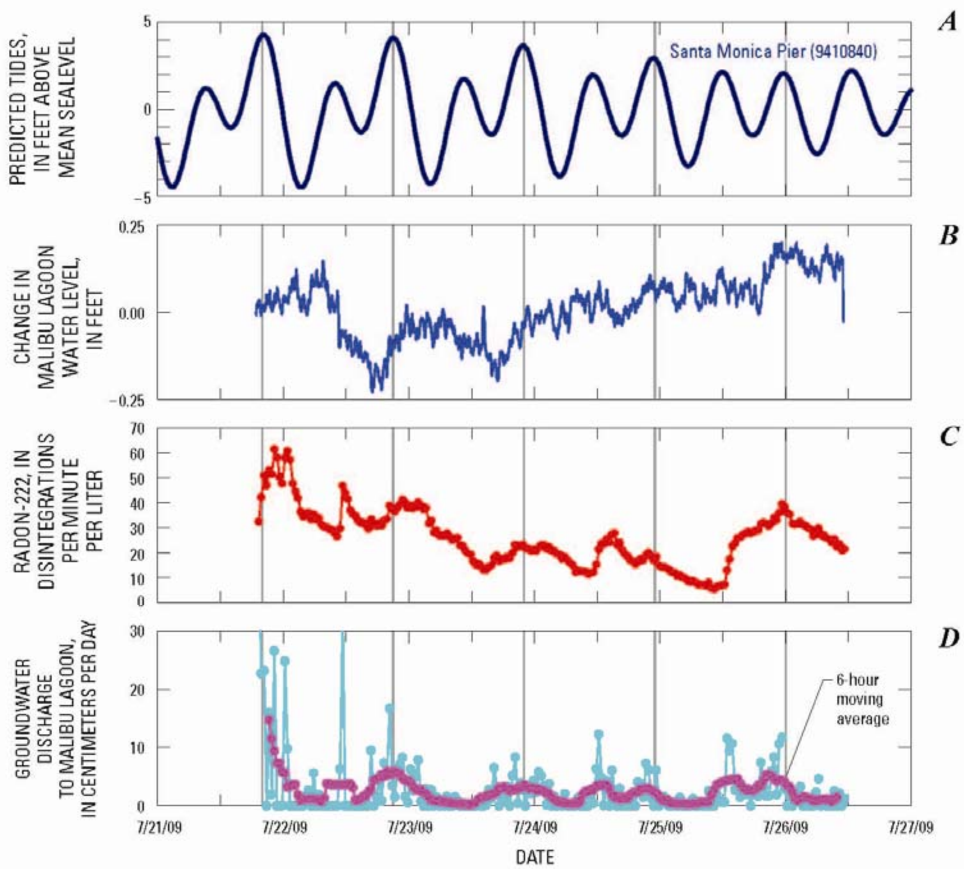

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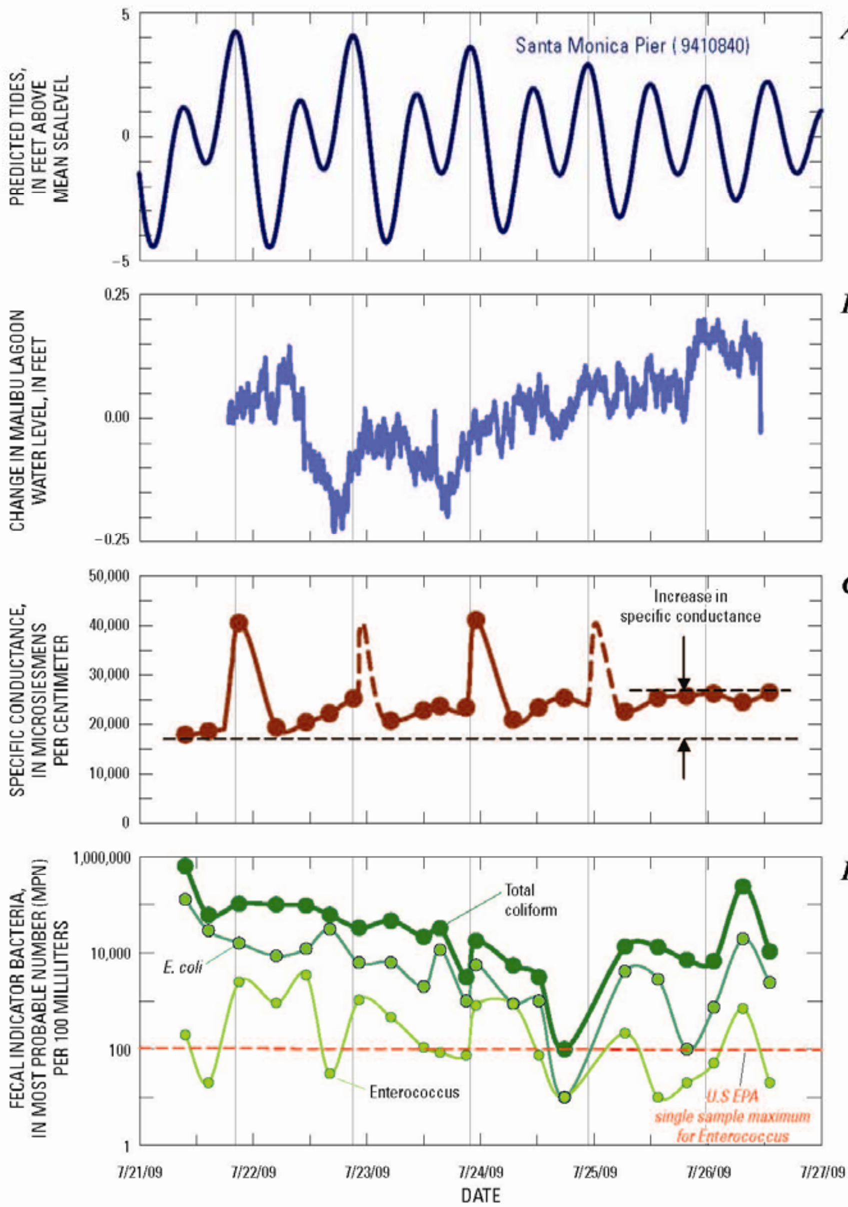

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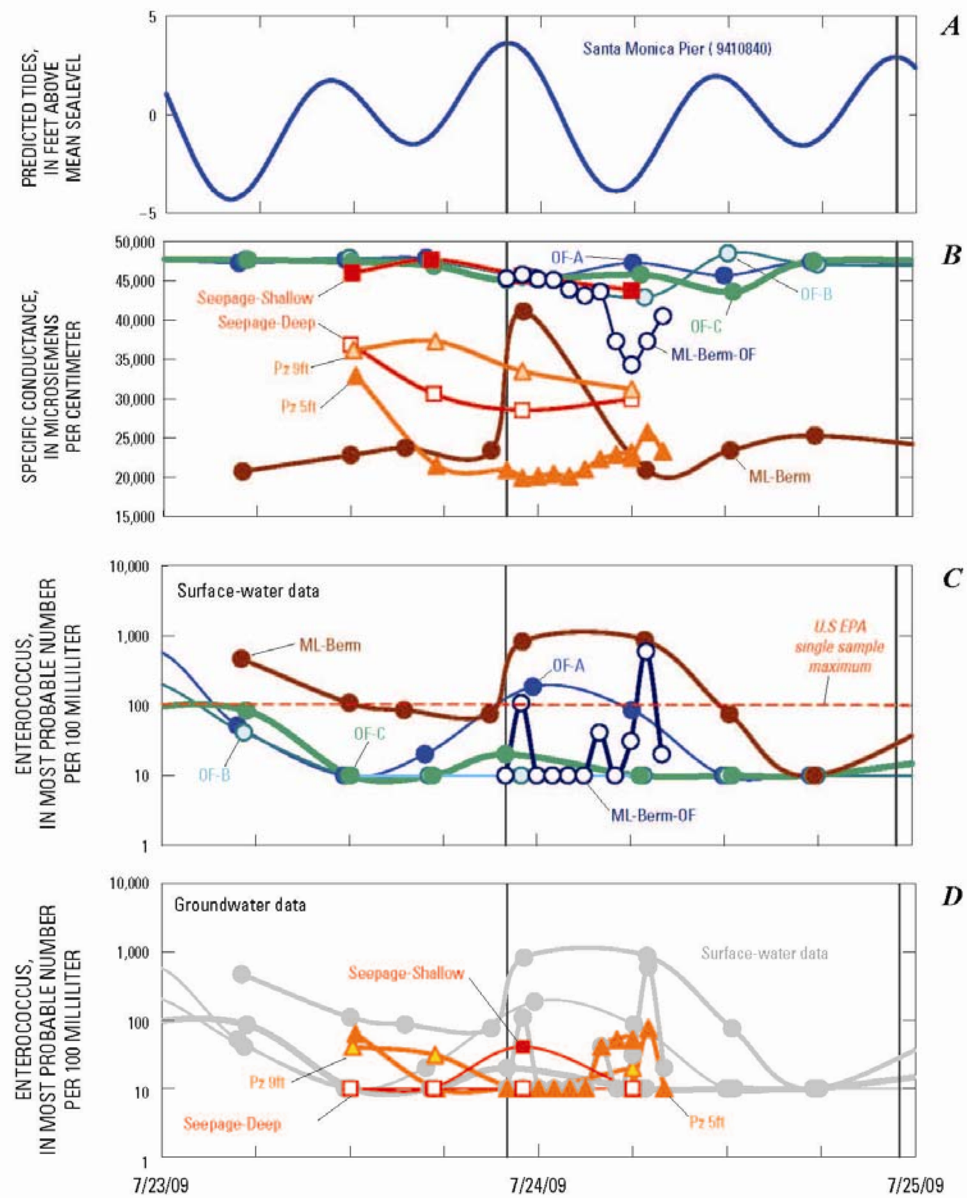

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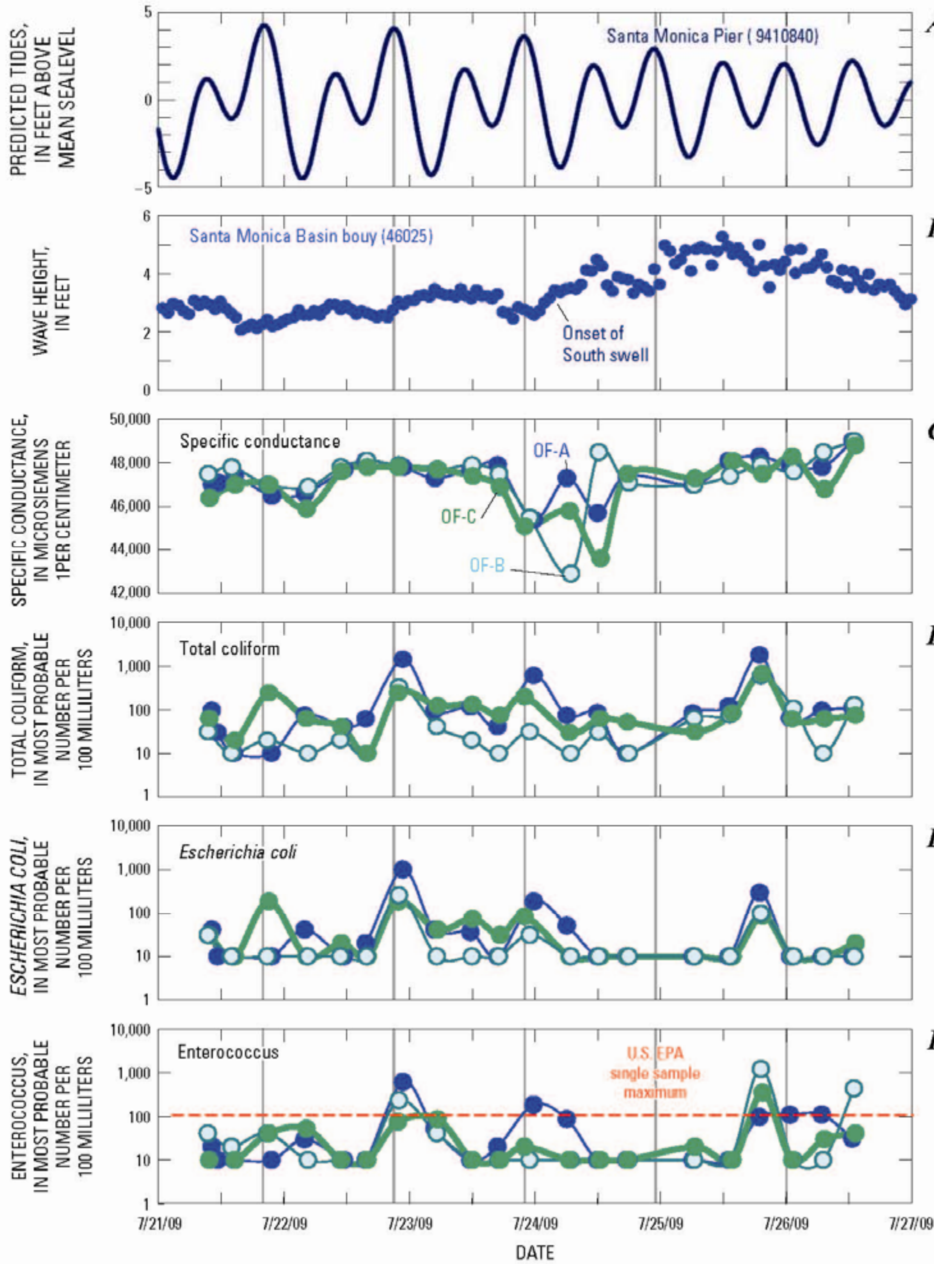

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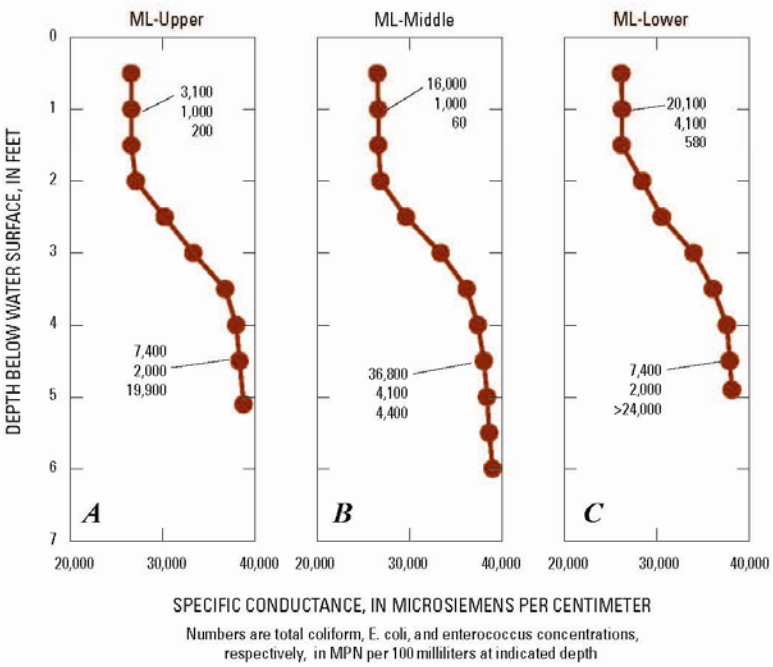

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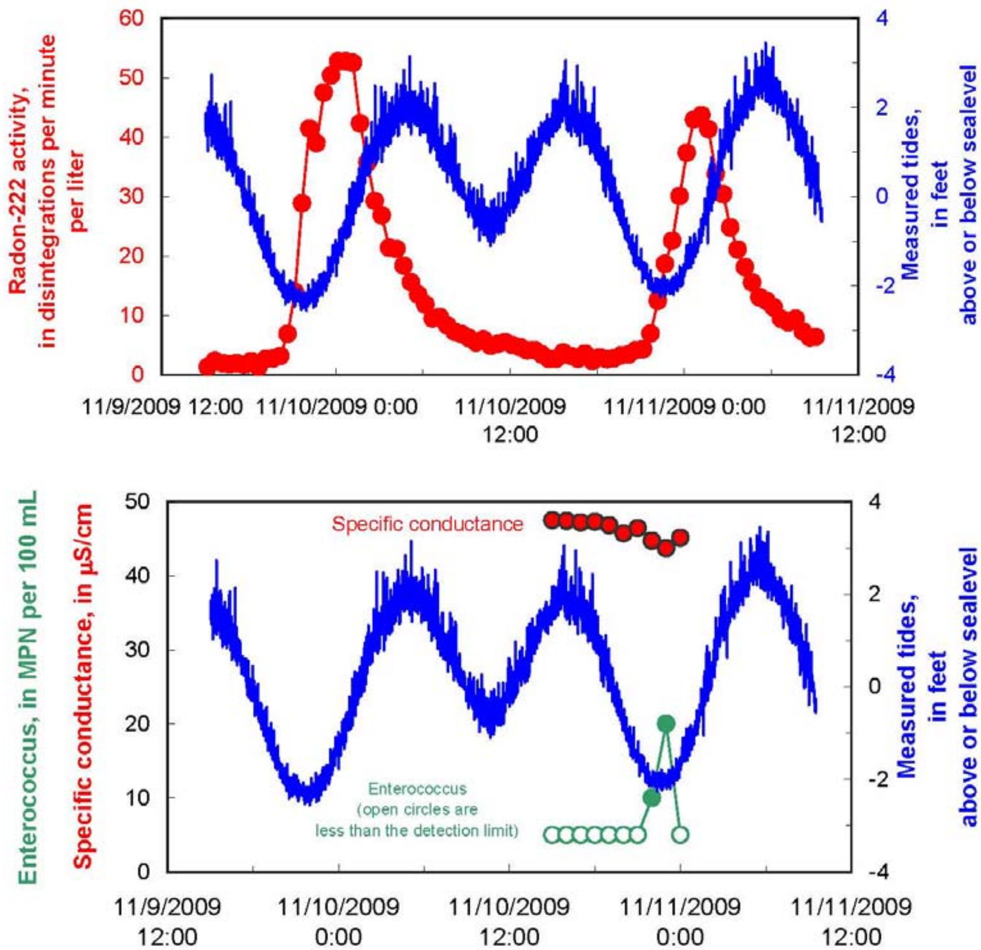

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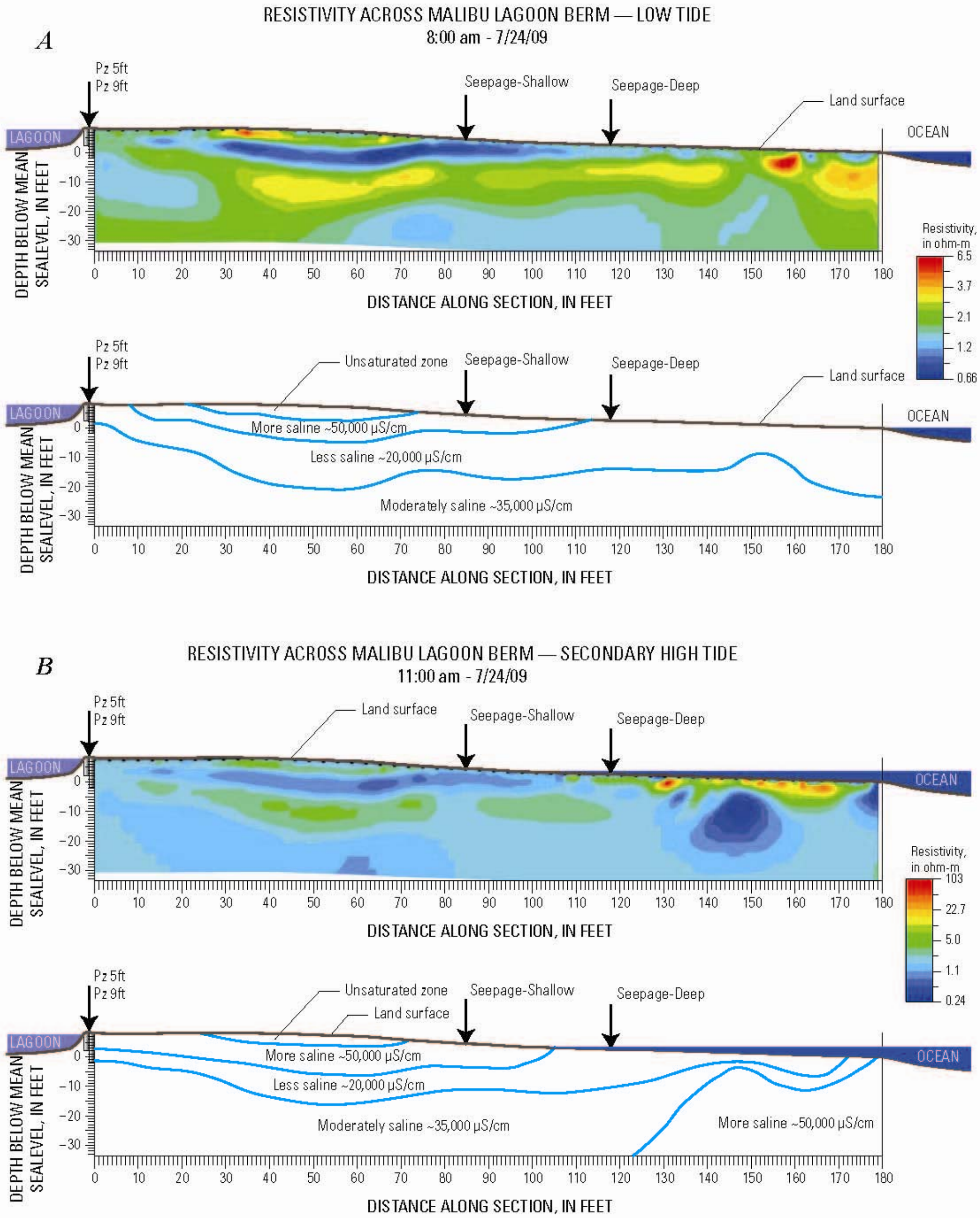

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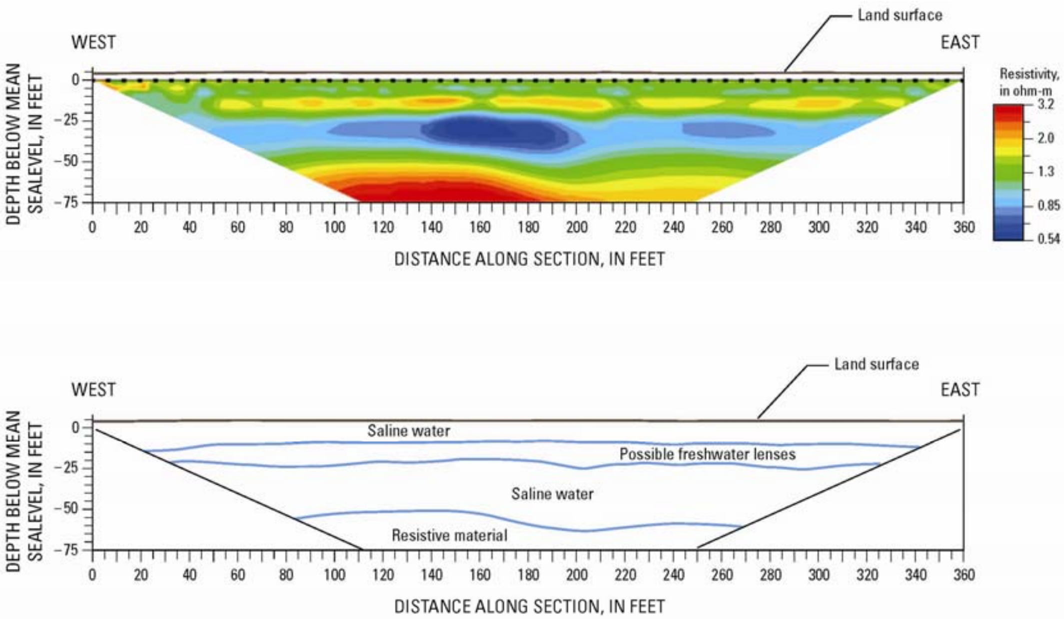

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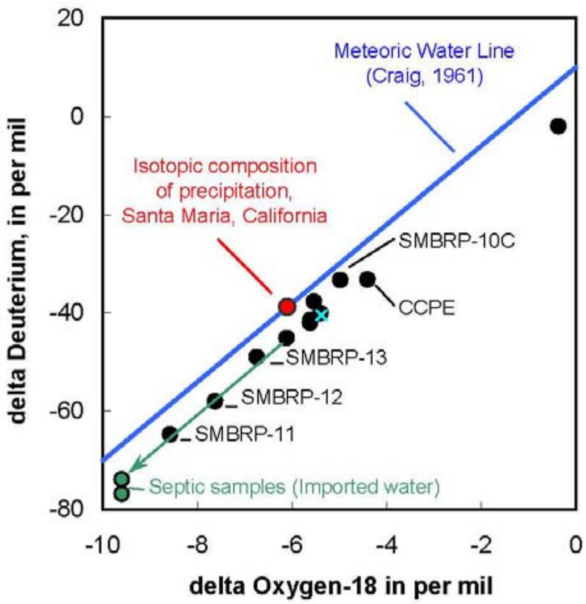

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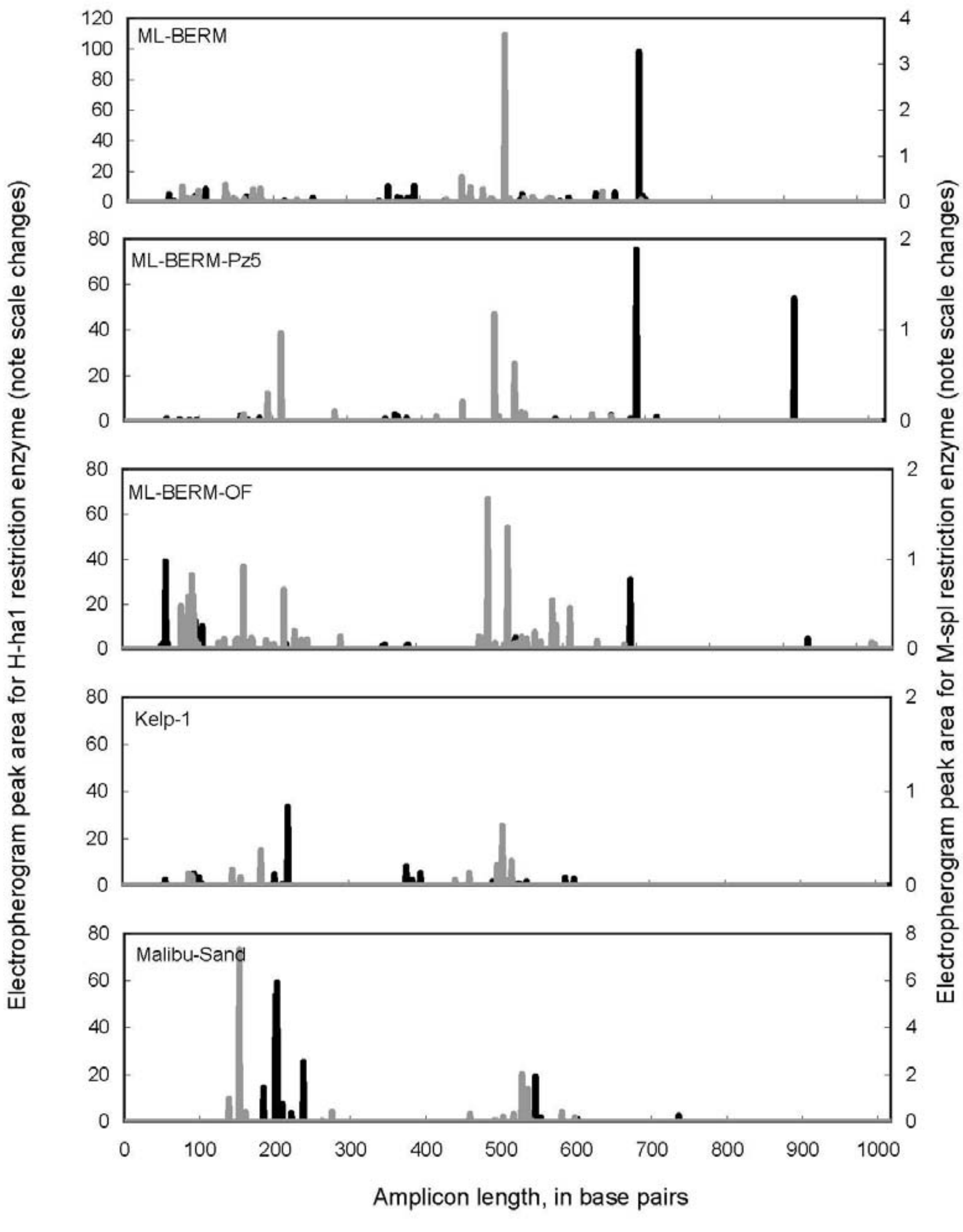

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Tables

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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