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February 24, 2010
File No. 31-370.40.4A

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Final Report and Abatement Plan for the Redondo Beach Pier Pilot Project
Supplemental Environmental Project

On December 14, 2006, the California Regional Water Quality Control Board, Los Angeles Region (Regional Board) approved Order No. R4-2006-0040, which required the Sanitation Districts that are party to the November 13, 2006 Settlement Agreement[1] (Sanitation Districts) to implement the Model Program for Bacterial Source Identification and Abatement Plan – Redondo Beach Pier Pilot Project Supplemental Environmental Project (Redondo Beach SEP). The Work Plan for the Redondo Beach SEP specified that a Final Report and Abatement Plan for the project would be released to stakeholders by March 1, 2010. Pursuant to the Work Plan, the Final Report and Abatement Plan is attached. If you have any questions regarding this report, please contact the undersigned at (562) 908-4288, extension 2803, Theresa Slifko at extension 2805, or Kathy Walker at extension 5614.

Very truly yours,

Stephen R. Maguin

Ann Heil
Supervising Engineer
Technical Services Department

ATH:TS:lmb
Enclosure

cc: Hugh Marley, Russ Colby, Kristie Chung, and Renee Purdy – LA Regional Board

[1] The Sanitation Districts that are party to the settlement agreement are: County Sanitation Districts Nos. 1, 2, 3, 5, 8, 15, 16, 17, 18, 19, 21, 22, 23, 28, 29, and 34 of Los Angeles County, South Bay Cities Sanitation District of Los Angeles County, and Santa Clarita Valley Sanitation District of Los Angeles County.

DOC #1504621

Model Program for Bacterial Source Identification and Abatement Plan – Redondo Beach Pier Pilot Project

Final Report and Abatement Plan

February 24, 2010

Supplemental Environmental Project
in Compliance with Order No. R4-2006-0040

County Sanitation Districts Nos. 1, 2, 3, 5, 8, 15, 16, 17, 18, 19, 21, 22, 23, 28, 29, and 34 of Los Angeles County, South Bay Cities Sanitation District of Los Angeles County, and Santa Clarita Valley Sanitation District of Los Angeles County

DOC# 1362569

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ACKNOWLEDGEMENTS

Technical Advisory Workgroup

The Sanitation Districts would sincerely like to thank the members of the Technical Advisory Workgroup: Renee Purdy (California Regional Water Quality Control Board, Los Angeles Region), Mark Gold and Polly Barrowman (Heal the Bay), Steve Weisberg (Southern California Coastal Water Research Project), Charlie McGee (Orange County Sanitation District), and Mike Shay (City of Redondo Beach), for closely watching the activities of the Redondo Beach Supplemental Environmental Project, reviewing materials, attending the Technical Advisory Workgroup meetings, and providing valuable input for the project.

Project Support

We wish to thank the City of Redondo Beach for accommodating our requests for access to the sampling locations, providing data, and especially for the support for the ground well sampling event. We also thank the staff from SEA Lab for conducting the daily field observations and providing the valuable data.

Key Personnel Involved With the Project

Theresa R. Slifko, Ph.D., an Associate Environmental Scientist with the Monitoring Section at the Joint Administration Office managed the project. Kathy Walker, a Supervisor at the JWPCP Water Quality Laboratory, served as the technical lead. The primary researchers were Ryan Reinke, Ph.D., a Research Scientist at the Sanitation Districts' San Jose Creek Water Quality Laboratory, Michele Padilla, a Microbiologist, and Jason Gregory, a Senior Microbiologist at the JWPCP Water Quality Laboratory.

Project Stakeholders

Project stakeholders included the California Regional Water Quality Control Board, Los Angeles Region, Santa Monica Baykeeper, Heal the Bay, the City of Redondo Beach, and the Los Angeles County Department of Public Health.

EXECUTIVE SUMMARY

On December 14, 2006, the California Regional Water Quality Control Board, Los Angeles Region (Regional Board) adopted Resolution No. R4-2006-0040, approving a settlement of an administrative civil liability complaint for a number of Sanitation Districts' wastewater overflows. The settlement required the Sanitation Districts to contribute toward the development of the Model Program for Bacterial Source Identification and Abatement Plan – Redondo Beach Pier Pilot Project (Redondo Beach Pier SEP). The primary objective of the Redondo Beach Pier SEP was to develop a reliable approach for microbial source identification and utilize the approach to identify the source or sources of bacteria contributing to the dry weather exceedances of bacterial water quality objectives at the monitoring locations near the Redondo Beach Pier in the City of Redondo Beach.

Project Overview

The project consisted of four elements: a literature survey and historical data review, a spatial and temporal fecal indicator bacteria (FIB) investigation (i.e., a sanitary survey), method development, and a microbial source tracking research effort. The literature survey reviewed the status of bacterial source tracking techniques and methods that were available at the commencement of the project, to determine the most promising methods to use in the project. The historical data review assessed available monitoring data in the vicinity of the Redondo Beach Pier for patterns and/or trends that could potentially indicate a source of the dry weather exceedances. Through the literature survey and historical data review, it was determined that the best strategy to identify the source of FIB exceedances was a multi-tiered toolbox approach. The strategy included four tiers capable of examining the study location with increasingly advanced analytical methods (the tools) to detect the populations of FIB and viral pathogens that would be most likely associated with either human or other animal feces. Tier 1 utilized a sanitary survey with routine FIB water quality monitoring and land observations. Accelerated monitoring was conducted in Tier 2 by expanding the geographic region and monitoring frequency to determine the spatial and temporal distributions of FIB and potential source(s). Tier 3 included the use of molecular and biochemical methods to conduct the advanced source identification efforts. A weight of evidence approach was used to examine the combined results to evaluate if the FIB causing exceedances were from a human or non-human source.

The sanitary survey portion of the project utilized a two-pronged approach, combining an extensive land-based survey of potential FIB sources with a water quality sampling effort. The land-based survey involved documenting numerous categories of activities and observations at the pier and the beach south of the pier. The water quality sampling effort employed standard culture based bacteriological methods at ten sites, encompassing both shoreline and offshore locations. When sustained exceedence events occurred, accelerated monitoring efforts were triggered with increased monitoring frequencies.

Concurrent with the sanitary survey, the Sanitation Districts' San Jose Creek Water Quality Laboratory in Whittier, CA developed bacterial and viral quantitative polymerase chain reaction (qPCR) methods for use with seawater samples. Once the qPCR methods were developed, the Sanitation Districts' Joint Water Pollution Control Plant (JWPCP) Water Quality Laboratory in

Carson, CA developed the capability to utilize the qPCR methods to analyze water samples for the microbial source tracking portion of the work. Once the laboratories had developed and validated the qPCR methods, they were deployed as part of a microbial source tracking effort at the Redondo Beach Pier.

The following bacteria and viruses were analyzed in the Redondo Beach Pier SEP:

- Total coliforms by standard culture methods
- Fecal coliforms by standard culture methods
- *E. coli* by standard culture methods
- *Enterococcus* spp. by standard culture methods
- Vitek© 2 for species discrimination
- *Bacteroidales* [total population (Bac_{TOT})] by qPCR
- *Bacteroidales* [human associated population (Bac_{HU})] by qPCR
- Human enteroviruses by RT-qPCR
- Human adenoviruses by qPCR.

Results and Conclusions

The initial water quality sampling effort expanded routine water quality monitoring (Tier 1) to include six shoreline and four offshore monitoring locations in the vicinity of the Redondo Beach Pier (Tier 2). There was a pronounced decline in the number of dry weather exceedances at the shoreline monitoring locations at the Redondo Beach Pier in 2007 and 2008, during the SEP study period. The offshore monitoring locations were consistently below or near the culture method detection limits for the efforts. Accelerated monitoring revealed that the highest FIB counts and exceedance rates occurred at the shoreline locations immediately adjacent to the Redondo Beach Pier, suggesting that the potential source of FIB exceedances was most likely associated with the beach at the Redondo Beach Pier. Therefore, the intense microbial source tracking effort (Tier 3) focused on the shoreline locations at the pier, a storm drain under the pier, and any ponded water in front of the storm drain.

The results from Tier 3 were collectively evaluated using the weight of evidence approach to determine the source(s) of dry weather FIB exceedances. At the shoreline monitoring location immediately adjacent to the pier (Site S-16), FIB standards were exceeded on four of 24 days during the 11-week effort, including three fecal coliform (*E. coli*) exceedances on three consecutive days during an incoming spring tide. Lack of detectable human viruses and the de minimus quantities detection of human-associated *Bacteroidales* in the ocean water strongly implied that a human source was not present. Other sources of FIB may include bacterial persistence in the sand and sea wrack, as well as endogenous sea life and birds. Tide, wave action, wind, and other natural fluctuations may be affecting FIB levels at the shoreline monitoring locations next to the pier.

The study also indicated that the storm drain under the pier and the pond that forms at the storm drain outlet are probably impacted by human fecal pollution but are not contributing to microbial contamination of the ocean water during the dry season. This conclusion is most strongly supported by the differences between the FIB concentrations and *Bacteroidales* populations at the shoreline sites compared to the pond and storm drain samples, particularly with respect to human-associated *Bacteroidales*. However, since the storm drain is a source of FIB and potential pathogens, the City of Redondo Beach has taken steps to address the storm drain.

Mitigation recommendations include completion of the City of Redondo Beach's storm drain investigation and expanding beach maintenance activities to include regular trash and kelp removal from under the pier. Additionally, it is recommended that the warning sign next to pier be updated. This sign currently prohibits swimming under the pier due to dangerous currents, and it is recommended that the sign be updated to include a warning about the storm drain under the pier.

BACKGROUND

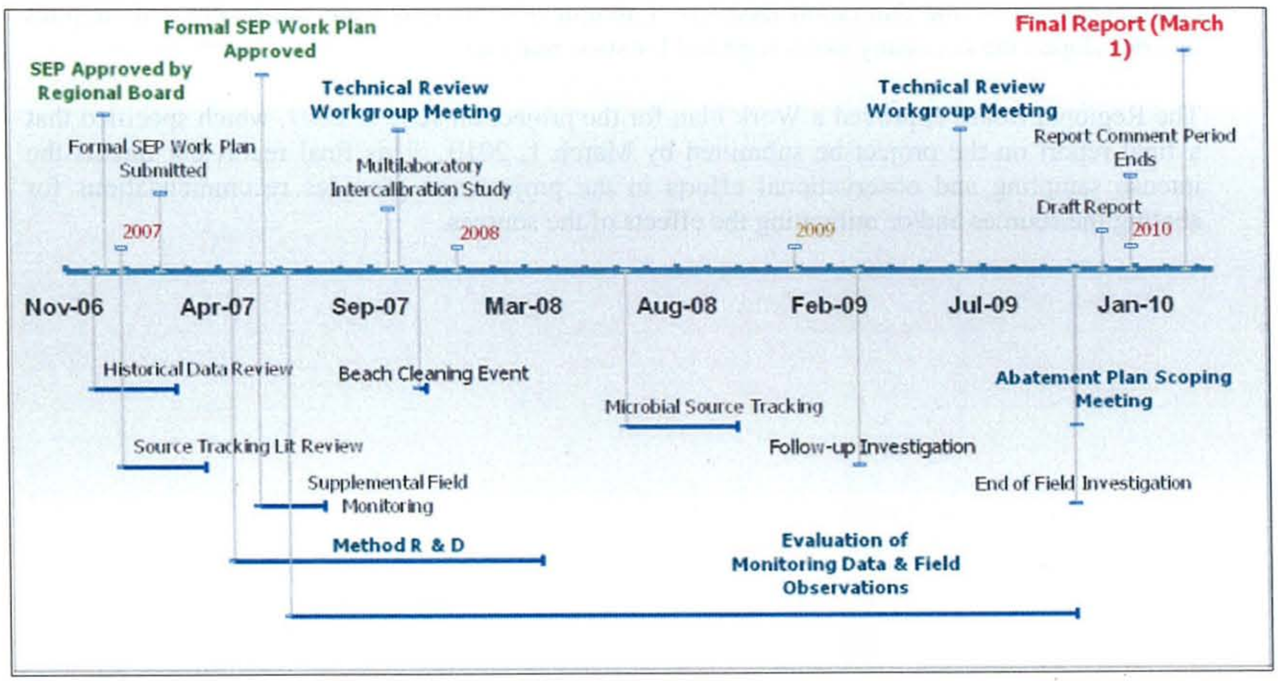
On December 14, 2006, the California Regional Water Quality Control Board, Los Angeles Region (Regional Board) adopted Order No. R4-2006-0040 approving a settlement of an administrative civil liability complaint for spills from the sewerage systems operated by County Sanitation Districts Nos. 1, 2, 3, 5, 8, 15, 16, 17, 18, 19, 21, 22, 23, 28, 29, and 34 of Los Angeles County, South Bay Cities Sanitation District of Los Angeles County, and Santa Clarita Valley Sanitation District of Los Angeles County (Sanitation Districts). The Order required that the Sanitation Districts implement a Supplemental Environmental Project (SEP) entitled the Model Program for Bacterial Source Identification and Abatement Plan – Redondo Beach Pier Pilot Project (Redondo Beach Pier SEP). The primary objective of the project was to develop reliable source identification techniques and utilize the methods to identify the source or sources of bacteria contributing to the dry weather exceedances of bacterial water quality objectives at the monitoring locations near the Redondo Beach Pier in the City of Redondo Beach. To conduct this work, the Sanitation Districts' Laboratories purchased the equipment and supplies and developed the necessary skills required for such analyses.

The Regional Board approved a Work Plan for the project on June 5, 2007, which specified that a final report on the project be submitted by March 1, 2010. This final report documents the intense sampling and observational efforts in the project and provides recommendations for abating the sources and/or mitigating the effects of the sources.

STUDY ACTIVITIES

Activities for this project included Work Plan development; historical data review; a source tracking literature review; microbial source tracking method research, development, and implementation; a study site sanitary survey, including water quality monitoring and analysis and a land survey; a multi-laboratory intercalibration study; technical review workgroup meetings; Redondo Beach Pier cleaning events; accelerated monitoring and analysis; a summer 2008 microbial source tracking (MST) study; follow-up investigation; Sanitation Districts' project team meetings; progress reports to stakeholders; and abatement plan development. Figure 1 provides a summary of project activities and milestones from the time the Regional Board approved the project on December 14, 2006 through December 1, 2009, the end of the study period.

Figure 1. Redondo Beach Pier Pilot Study Timeline (SEP R4-2006-0040).



Work Plan Development

Immediately after the Redondo Beach Pier SEP was approved on December 14, 2006, the project team began reviewing historical water quality data at the sites surrounding the Redondo Beach Pier as well as the available source tracking literature. A Work Plan for the project was developed based on the findings from these efforts and stakeholder input at a scoping meeting held at Redondo Beach City Hall on December 12, 2006. On January 26, 2007, the Sanitation Districts circulated a draft Work Plan among stakeholders from the Cities of Redondo Beach and Los Angeles, the Los Angeles County Department of Health Services, the Southern California Coastal Water Research Project (SCCWRP), the Los Angeles County Department of Beaches and Harbors, the Regional Board, and Heal the Bay. Timely comments were received from the

City of Redondo Beach and from SCCWRP. The Sanitation Districts made appropriate changes and formally submitted the Work Plan to the Regional Board Executive Officer on February 13, 2007, in compliance with February 22, 2007 deadline in Order R4-2006-0040. On March 5, the Sanitation Districts received comments on the submitted Work Plan from Heal the Bay. On March 8, the Sanitation Districts proposed modifications to the Work Plan to Heal the Bay and the Regional Board based on Heal the Bay's comments. On May 15, 2007, Regional Board staff transmitted their comments to the Sanitation Districts. On May 29, 2007, the Sanitation Districts provided responses to the Regional Board's comments. On June 5, 2007, the Work Plan was approved with modifications based on the Sanitation Districts' responses to comments.

Historical Data Review

Historical fecal indicator bacteria (FIB) monitoring data were examined as part of a data review effort to provide background information about the shoreline water quality at Redondo Beach and to identify potential locations for study during this project. Prior to the project, several monitoring programs were in place at the beach near Redondo Beach Pier. Compliance samples for the Santa Monica Bay Beaches Dry Weather Bacteria TMDL are collected each Monday by Michelson Laboratories, Inc. (Commerce, CA) for the City of Redondo Beach from a location directly in front of the lifeguard tower approximately 350 feet south of the Redondo Pier. This location is identified as SMB-6-2 in the Santa Monica Bay Beaches Bacterial TMDL Coordinated Shoreline Monitoring Plan (CSMP). Additionally, the City of Los Angeles Hyperion Laboratory (Los Angeles, CA) collects water quality samples at a site immediately adjacent to the southern edge of the Redondo Beach Pier on Tuesday through Saturday to fulfill the Los Angeles County Municipal Storm Water Permit (MS4 Permit). This location is identified in the MS4 Permit as S-16.

During discussions with the City of Redondo Beach and Hyperion staff, it became apparent that S-16 had been relocated since the alignment of the CMSP and MS4 Permit monitoring program in November 2004. The MS4 Permit monitoring site, S-16, was relocated from its original location in front of the lifeguard tower, approximately 350 feet south of the pier, to a position immediately adjacent to the southern edge of the pier. The TMDL monitoring site, SMB-6-2, continues to be sampled at the original location of S-16 (in front of the lifeguard tower), as identified in the TMDL plan. The locations of S-16 and SMB-6-2 are indicated in Figure 2.

The Beach Report Card issued by Heal the Bay includes the area in vicinity of the Redondo Beach Pier. The Report Card has been modified to include an additional station titled "Redondo Municipal Pier-south side", reflecting conditions adjacent to the pier structure (current S-16 location). The former monitoring location is entitled "Redondo Municipal Pier-100 yards south" is currently based on the data collected at SMB-6-2. Additionally, two sites up-coast and down-coast from the Redondo Beach Pier are monitored each Monday by the Los Angeles County Department of Public Health, DHS-115 and DHS-116.

Figure 2. Monitoring Locations for the Santa Monica Bay Beaches Bacteria TMDL and Hyperion Laboratory NPDES Permit.

It is noteworthy that the methods used by the Hyperion Laboratory to evaluate FIB were changed from the membrane filtration procedure to the IDEXX MPN procedure in 2004. The laboratory began to use IDEXX Colilert for the analysis of total and fecal coliforms (*E. coli*) in May 2004. Enterolert was adopted for enterococci analysis in November 2004. As part of the method changes, new detection limits were also adopted. Prior to the use of the MPN method, the detection limits were 1 CFU/100ml. After adopting the IDEXX methods, the detection limits increased to 67 MPN/100ml for total and fecal coliforms and 10 MPN/100ml for enterococci.

In marine waters designated for water contact recreation (REC-1), the following bacteria standards apply:

1. Geometric Mean Limits
   Total coliform density shall not exceed 1,000/100ml

Fecal coliform density shall not exceed 200/100ml
Enterococci density shall not exceed 35/100ml

2. Single Sample Limits
   Total coliform density shall not exceed 10,000/100ml
   Fecal coliform density shall not exceed 400/100ml
   Enterococci density shall not exceed 104/100ml
   Total coliform density shall not exceed 1,000/100ml, if the ratio of fecal-to-total coliform exceeds 0.1.

The geometric mean values are based on a rolling 30-day average of at least five samples. When the density of any bacteria exceeds the respective standards, the occasion is considered an 'exceedance.'

Analysis of the previous ten years of historical monitoring data (1996 through 2006) showed that the frequency of exceedances during the dry weather season (April to October) was lower than the frequency of exceedances considered on a year-round basis, particularly for Site DHS-116 (Table 1). This finding is consistent with numerous other studies that indicate that FIB exceedances occur predominantly during wet weather. The location with the most exceedances was S-16, the location immediately adjacent to the Redondo Beach Pier. The most frequent parameter exceeded at all sites was the enterococci 30-day geometric mean. Total coliform exceedances were relatively rare, with single sample standard exceedances occurring less than 2% of the time. Fecal coliform single sample standard exceedances occurred up to 12% of the time.

Table 1. Ten-year Summary (1996-2006) of Redondo Beach Pier Fecal Indicator Bacteria Monitoring Data Collected by the City of Los Angeles and the Los Angeles County Department of Public Health.

Parameter Evaluated	All data DHS-115	All data S-16	All data DHS-116	Dry only DHS-115	Dry only S-16	Dry only DHS-116
Total No. Samples	763	3987	596	648	3390	512
% Total Coliforms >10,000	4	2	0.2	1	2	0
% Fecal Coliforms >400	5	12	1	2	11	1
% Enterococcus >104	12	13	8	7	10	6
% of 30-day geo mean Enterococcus >35	19	26	15	13	22	13
% of 30-day geo mean FC >200	2	11	0	0.2	12	0.2
Average Total Coliform (No./100 ml)	1142	862	229	496	813	134
Average Fecal Coliform (No./100 ml)	246	214	46	69	210	37
Average Enterococcus (No./100 ml)	288	70	46	107	52	34

When exceedance rates before and after the S-16 sample site was relocated from the lifeguard tower to the southern edge of the pier in November 2004 were compared, significant increases in fecal coliform and enterococci exceedances were observed following the move (Table 2). Fecal coliform single sample standard exceedances increased from 10% to 25% with corresponding 30-day geometric mean increasing from 6% to 40%. The incidence of enterococci single sample standard exceedances increased from 10% to 15%, but there was no increase in the incidence of enterococci geometric mean exceedances.

A comparison was also made of the data collected at Site S-16 by the Hyperion Laboratory before it was relocated in November 2004 with the data collected and analyzed by Michelson Laboratory at the same location and reported as Site SMB-6-2 since November 2004. Because both sets of data were collected in front of the lifeguard tower 350 feet south of the Redondo Beach Pier, the FIB trends would be expected to be comparable. However, when the data in Table 2 were compared, there were fewer enterococci exceedances in both the single sample and 30-day geometric mean parameters after November 2004. Geometric mean exceedances decreased from 26% to 2% for enterococci and from 6% to 3% for fecal coliforms. It is relevant that two different laboratories analyzed the data and the size of the pre-November 2004 data set is much larger. These discrepancies warranted an interlaboratory calibration exercise, which was subsequently conducted as part of the Redondo Beach Pier SEP efforts.

Table 2. Comparison of Monitoring Results Before and After the Sampling Location for Site S-16 was Relocated in November 2004.

	All data			Dry weather only		
Parameter Evaluated	**S-16 Before Nov. 2004**	**S-16 After Nov. 2004**	**SMB-6-2 After 2004**	**S-16 Before Nov. 2004**	**S-16 After Nov. 2004**	**SMB-6-2 After 2004**
Total Samples	3447	539	105	2940	450	88
% Total Coliforms >10,000	2	3	1	2	2	1
% Fecal Coliforms >400	10	25	3	9	24	3
% *Enterococcus* >104	10	15	4	8	11	2
% of 30-day geo mean FC >200	6	40	3	8	38	4
% of 30-day geo mean *Enterococcus* >35	26	27	2	22	21	4
Average Total Coliform (No./100 ml)	839	1007	637	807	849	563
Average Fecal Coliform (No./100 ml)	191	365	117	185	375	111
Average *Enterococcus* (No./100 ml)	66	75	25	49	55	22

Historical dry weather (excluding data collected within 72 hours after a rain event) fecal coliform monitoring data from January 1, 1995 through December 31, 2006 at Site S-16 and sites north and south of the Redondo Beach Pier (DHS 115 and DHS 116, respectively) are shown in Figure 3. Figure 3 indicates that a substantial change in fecal coliform results for S-16 occurred in the 2004 time frame. In particular, the method change implemented by the Hyperion Laboratory in 2004 resulted in a higher detection limit. Additionally, there was a higher frequency of elevated fecal coliform results after 2004, potentially as a result of the location change for S-16.

Single sample data collected in 2005-2006 did not demonstrate a discernable pattern of exceedence events or a relationship between different indicator bacteria. Total coliform exceedence events appeared to be sporadic and were not sustained. However, when the running average of dry weather total and fecal coliform data were examined for Sites S-16 and SMB-6-2, periodic increases were observed, particularly for the fecal coliforms. Figure 4 shows the periods of increased levels of fecal coliforms that occurred in the spring between March and April, in the summer between May and August, and between October and November for monitoring conducted in 2004 through 2006. Based on the historical data review and these observations, the summer dry weather swimming season between May and August was chosen to conduct accelerated bacteria monitoring and the microbial source tracking efforts.

Figure 3. Historical Dry Weather Fecal Coliform Monitoring Data at Site S-16 and DHS Monitoring sites North and South of the Redondo Beach Pier.

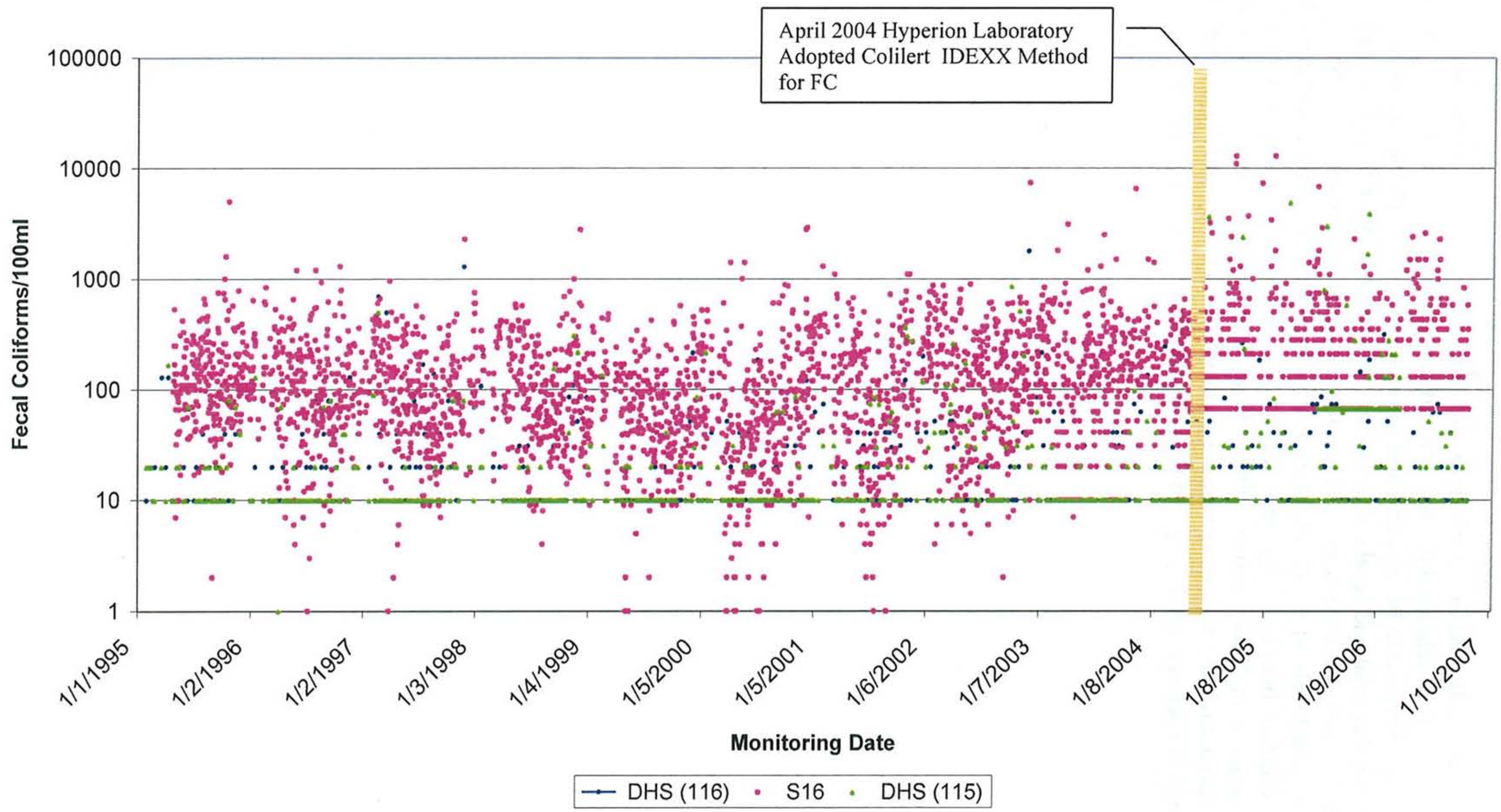
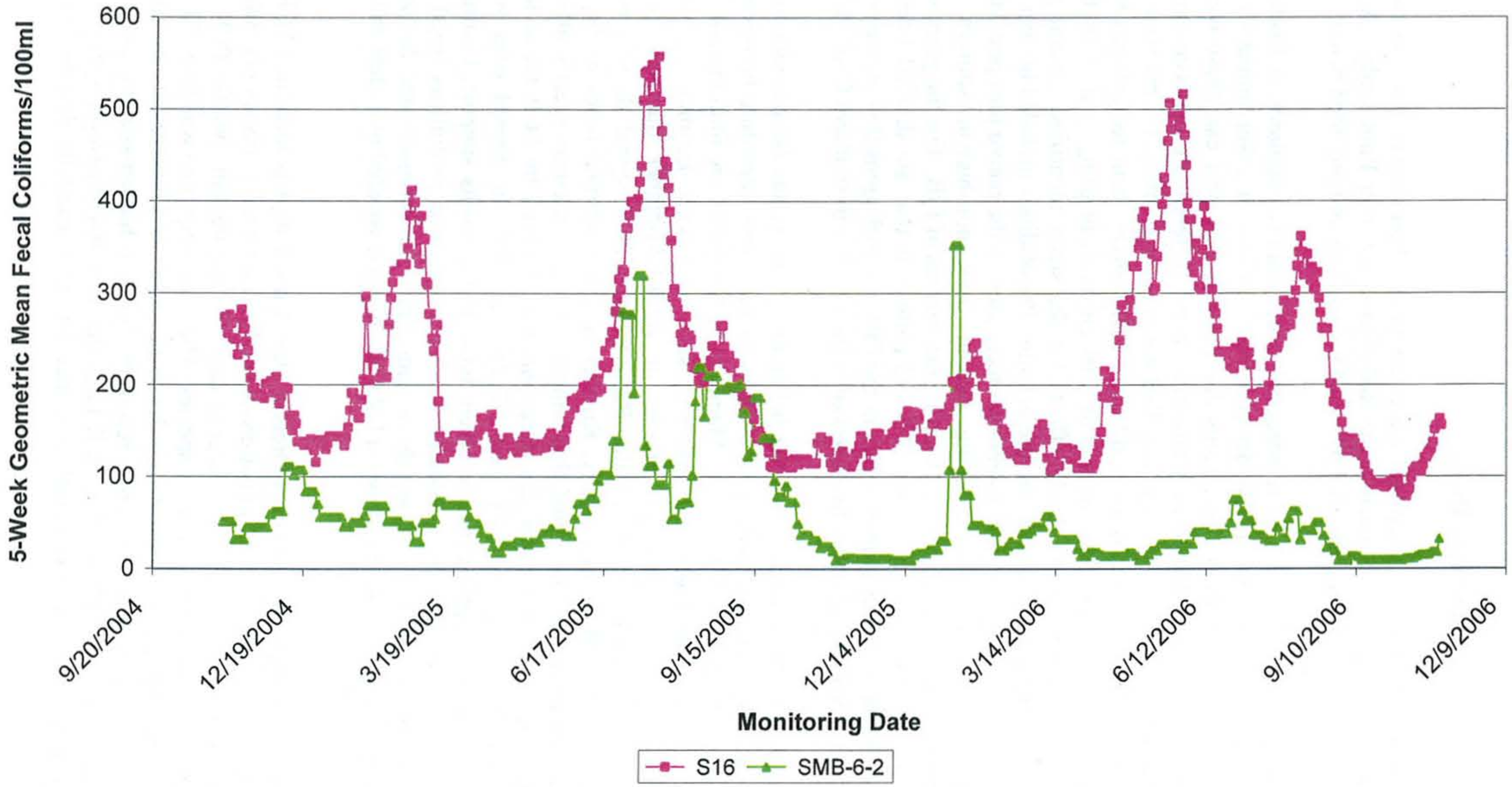


Figure 4. 30-Day Dry Weather Geometric Mean Fecal Coliform Data at S-16 and SMB-6-2 (2004-2005).



Microbial Source Tracking Literature Review

Prior to development of methods for microbial source tracking, a literature review was conducted in order to establish the approach to conduct the Redondo Beach Pier Pilot study. A detailed discussion and rationale for selecting the methods used in this study are provided below.

The current strategy for development of recreational water quality standards is based on the premise that fecal indicator bacteria (FIB) derived from humans or other animals represent human health risks, as the waterborne pathogens that the FIB represent can cause waterborne disease. The REC-1 standards adopted by California do not differentiate between human and non-human sources of FIB, and assume that any FIB are equally as likely to represent a health risk. As such, the health risk of exposure to different FIB sources from recreational waters is largely unknown. There is a tremendous amount of work currently ongoing by the U.S. EPA and other agencies to address this issue. Early efforts for the study identified several potential sources of the dry weather exceedences at the project site. Possibilities included human sources, such as municipal sewage, as well as non-human sources, such as the native bird population and marine mammals. Therefore, differentiating human from non-human sources of FIB exceedences was an important distinction for locating the sources of FIB. For the purpose of this work, the term 'human source' refers to the FIB and pathogens that are derived from human excrement. The term 'non-human source' refers to the FIB and pathogens that are derived from the excrement of any other animal normally observed at the beaches in southern California.

Previous work identified the anaerobic bacteria belonging to the genus *Bacteroides* as a good indicator for recent fecal contamination events. *Bacteroidales* spp. constitute between 20 and 50% of human bacterial fecal flora (13,16,39). Members in this family are found in much higher numbers in human feces than traditional indicators such as *E. coli*. Estimates of culturable *Bacteroidales* demonstrate that they are present at 1000-fold-higher numbers than fecal coliforms (13), although unlike coliforms the *Bacteroidales* PCR signal doesn't persist in environmental waters (28). More specifically, Kreader reported survival times of *Bacteroides distasonis* in Ohio River water. She found that temperature and predation greatly affected the persistence of this species (28). *Bacteroides distasonis* was detected by PCR for at least two weeks at 4°C but only 4-5 days at 14°C and 1-2 days at 24°C. In filtered river water the persistence was extended for approximately seven days. These results supported cultural data from Fiksdal et. al. showing that *Bacteroidales* survival (contained in dialysis bags) in river water was less than eight days (13). Along these same lines, human-associated *Bacteroidales* PCR signal associated with sewage overflows in Lake Michigan dissipated to undetectable signal in anywhere from seven to 23 days (7).

Original work from Katharine Field's lab at Oregon State University has identified PCR primer sets that can detect both total as well as human-associated *Bacteroidales* species (1). Subsequent research demonstrated that the 16S rRNA region used for the human-specific PCR was not exclusive to human-associated *Bacteroidales* species. The same sequence was 97-99% identical to certain cat, dog and gull associated *Bacteroidales* sequences (10). These results are contrary to at least some field reports in which no cross-reactivity between human-specific *Bacteroidales* PCR and gull feces was found (7,14). The PCR targeting total *Bacteroidales* species does not show the same discrepancies, as there is little evidence for cross-reactivity despite detecting 25 to 125-fold more target than for the human-specific PCR (7). The total *Bacteroidales* species PCR products are more ubiquitous in the environment than the species-specific PCR amplicons as would be expected (7,31).

Fogarty and colleagues found that the human-associated *Bacteroidales* PCR described by Bernhard and Field detected only 20% of the human samples tested in their study. On the other hand the PCR did not detect any of the other cow, pig, horse or dog samples tested suggesting good specificity (7). Supporting the argument for human specificity, a higher success rate was described for this PCR in sewage or in relation to combined sewage overflows (7,12). Therefore, independent literature review seems to suggest that PCR primers can be developed to amplify total *Bacteroidales* species and to differentiate between human and non-human sources specifically when sewage contamination is present. However, it should be noted that these PCRs are not perfect and there is likely to be some cross-reactivity with other non-human species. Thus, source identification studies utilizing human-associated *Bacteroidales* PCR should be supported with additional evidence of human fecal contamination.

Viruses show exquisite specificity to specific hosts as well as individual cell types. Human-specific viruses have been described that are excreted in the feces of humans that do not infect other animals, and are therefore excellent for tracking human fecal pollution. This is because enteric viruses are transmitted by the fecal-oral route and are commonly found in sewage and other forms of domestic waste. As such, human enteric viruses have been successfully utilized in microbial source tracking studies to identify possible sources of human fecal contamination (2,8,15,17,18,19,20,22,25,,26,30,32,34,35). Several human enteric viruses have been exploited for this use including adenoviruses (15,20,22,25), enteroviruses (6,15,18,20,26,34), Hepatitis A virus (HAV) (18,20,23), noroviruses (20,25,26) and polyomaviruses (3,22,32). Enteric viruses have been reported to survive longer that traditional bacterial indicators (4,5,39).

Adenoviruses are double stranded DNA viruses and are at least as stable in environmental matrices as enteroviruses. Enteroviruses include polio, coxsackie, and echovirus and are positive-sensesingle stranded RNA viruses that are likely more stable than adenoviruses in certain water-based environments (11). Adenoviruses occur in greater frequency and demonstrate less seasonal variation in sewage than enteroviruses (22,29,30,36) suggesting that they maybe a more consistent indicator of human fecal contamination.

Human enterovirus and adenovirus serotypes are almost exclusively associated with humans (9), again making them ideal targets for identification of the presence of human fecal sources. The biggest disadvantage to using enteric viruses for detection of human fecal matter is that individuals must be infected with the virus to shed the virus in feces. Although the viruses are prevalent in sewage they may not be a good indicator for smaller contamination sources such as outhouses or septic tanks due to the low percentage of infected population.

To date, several microbial source tracking studies have been performed utilizing both *Bacteroidales* and enteric virus specific PCR based methods. Ongoing research is currently focused on use of quantitative PCR based approaches for these targets because they can be valuable attributes to a microbial source tracking study. Recent advances in technology have enabled more frequent and reliable use of these molecular tools for detecting and quantifying microorganisms in environmental water matrices. Therefore, goals of this study included adoption and use of real-time quantitative PCR (qPCR) protocols to quantify *Bacteroidales*, enterovirus, and adenovirus in the affected ocean waters at Redondo Beach.

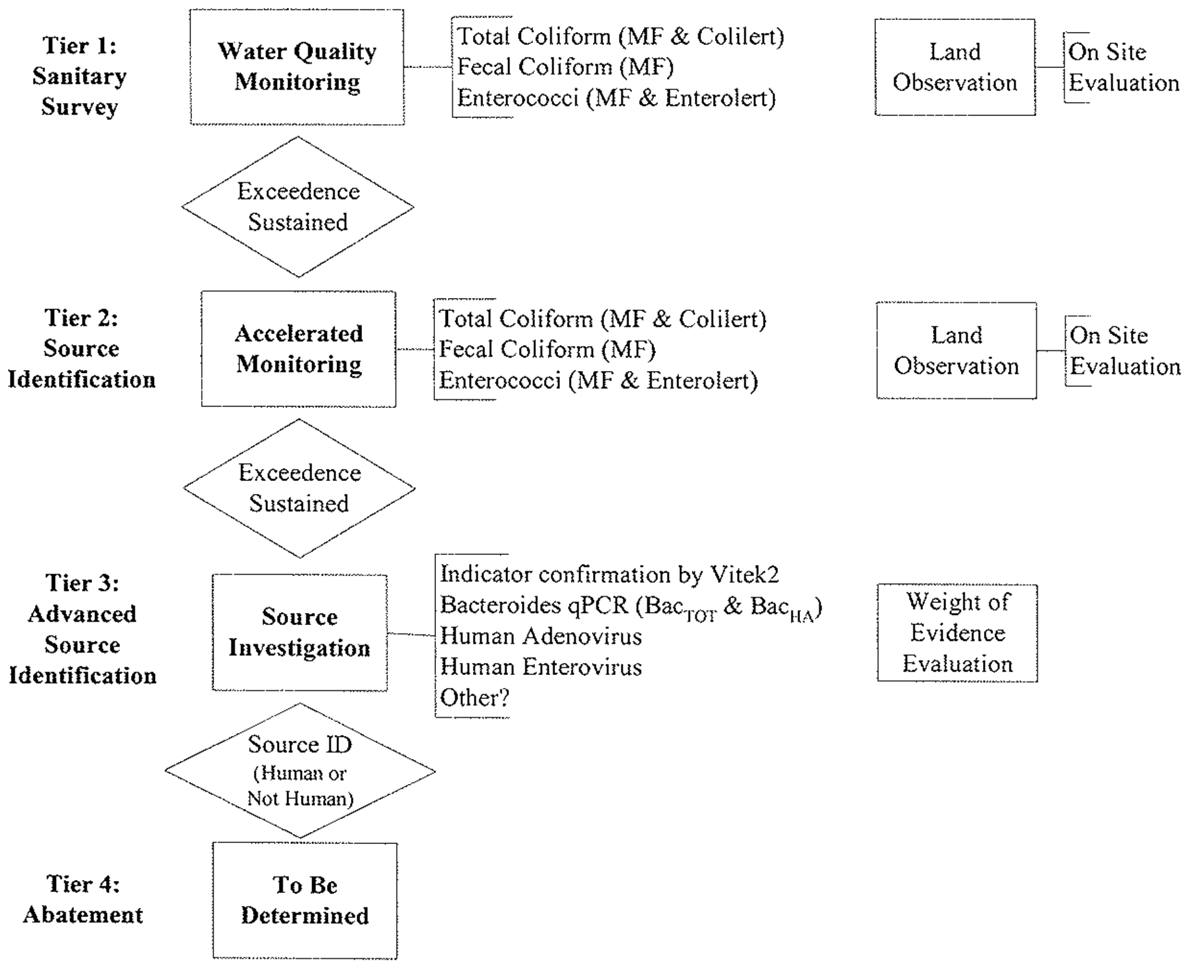
Tiered Toolbox Approach for Microbial Source Tracking

Through the literature survey and historical data review, it was determined that the best strategy to identify the source of fecal pollution was a tiered toolbox approach (2,35). This approach to microbial source tracking was recommended in the U.S. EPA report on the Experts Scientific Workgroup on critical research needs for the development of new or revised recreational water quality criteria (38). Therefore, a multi tiered toolbox framework was developed and used as guidance for determining the source of dry weather FIB exceedances at the Redondo Beach Pier. Figure 5 presents the increasing levels and monitoring efforts developed for the Redondo Beach Pier SEP tiered toolbox for microbial source tracking.

Tier 1 consists of microbial water quality monitoring in conjunction with land-based observations of potential FIB sources. When an episode of elevated FIB is observed, source identification is attempted through expanded spatial and temporal monitoring with accelerated water quality monitoring and additional observations of potential sources (Tier 2). Advanced source identification is initiated in Tier 3 if an exceedance episode is confirmed or a suspected source is identified through land-based observations. A weight of evidence approach using the sophisticated methods for specifically identifying human and non-human microorganisms is used to target and identify the contamination source. The weight of evidence is based on the presence or absence of human-specific pathogens (in this case enteroviruses and adenoviruses), as well as predominance and diversity of the bacteria species detected in each sample monitoring location. Once a source has been confirmed, abatement measures specific to each site and situation are determined in Tier 4.

This tiered toolbox approach is suitable for use in similar environmental studies and watershed assessments to determine if bacteria are predominantly from a human or non-human source of fecal contamination. The qPCR methods used as part of the toolbox were selected because the literature supported their ability to discriminate human feces and sewage from non-human feces. In addition, library-based methods of identifying fecal pollution sources were found to be cost-prohibitive and less effective in environments with multiple fecal inputs.

Figure 5. Tiered Toolbox Approach for Microbial Source Identification



Analytical Methods

The primary goal of the project was to develop reliable source identification techniques and utilize the methods to identify the source or sources of bacteria contributing to the dry weather exceedances of bacterial water quality objectives at the monitoring locations south of the Redondo Beach Pier, in the City of Redondo Beach.

For development of source identification techniques, the following subgoals were created:

1. Develop a method to concentrate human adenoviruses and enteroviruses from ocean water including associated quality control and quality assurance (QA/QC) procedures;
2. Develop a scheme for the collection and isolation of *Bacteroidales* DNA from ocean water including associated quality assurance and quality control (QA/QC) procedures;
3. Develop a quantitative polymerase chain reaction (qPCR) method to detect total *Bacteroidales* as well as human associated *Bacteroidales* in ocean water;
4. Develop qPCR methods to detect the presence of human adenoviruses and human enteroviruses in ocean water;
5. Determine if the presence of human specific microbial markers (adenovirus, enterovirus and human associated *Bacteroidales*) correlates with the temporal and spatial occurrence of fecal indicator bacteria (FIB) exceedances at the Redondo Beach pier.

Overview of Analytical Methods

Monitoring tools used for the assessment and identification of FIB sources at the Redondo Beach Pier study site are listed in Table 3. Three culture methods were used to detect FIB including total coliforms, fecal coliforms, *E. coli*, and enterococci. Four peer-reviewed molecular/biochemical-based methods were used to determine the source of fecal contamination. These methods evaluate the quantity and presence of *Bacteroidales,* two human viruses, and include a microbial identification system used to confirm the FIB genus and species of the isolates obtained from the culture techniques with the Vitek© 2 (Vitek) instrument. The methods are summarized below. The standard operating procedures for the source tracking methods, including Vitek, *Bacteroidales,* adenoviruses qPCR, and enteroviruses RT-qPCR are included in the Appendix on the attached DVD.

Table 3. Redondo Beach Pier SEP Monitoring Tools for the Assessment and Identification of Fecal Pollution.

Analyte	Method	Reporting Units	Comments
Total coliforms	SM 9222B	CFU/100ml	Membrane filtration. Multiple volumes analyzed.
Total coliforms	SM 9223B	MPN/100ml	Colilert – enzyme substrate. Diluted 1:10
E. coli	SM 9223B	MPN/100ml	Colilert – enzyme substrate. Diluted 1:10
Fecal coliforms	SM 9222D	CFU/100ml	Membrane filtration. Multiple volumes analyzed.
Enterococci	EPA 1600	CFU/100ml	Membrane filtration. Multiple volumes analyzed.
Enterococci	Enterolert	MPN/100ml	Enterolert - enzyme substrate. Diluted 1:10
Vitek © 2	Vitek protocol	Genus and species	Confirmation and identification of bacteria/isolates detected in membrane filtration assays. Analyses of species diversity.
Total population *Bacteroidales*	Bac_{TOT} qPCR	Genomic copies/100ml	Total population with qPCR assay by dual probe hybridization.
Human-associated *Bacteroidales*	Bac_{HU} qPCR with melt curve analyses for genotyping	Genomic copies/100ml	Human-associated population with qPCR assay by dual probe hybridization including an IPC. Melt curve analyses for species diversity.
Human Adenoviruses	qPCR	PFU/100ml	Adenovirus primers & probes for qPCR including an IPC. Samples calibrated by culture results. Sample concentrated by HFF.
Human Enteroviruses	RT-qPCR	Genomic copies/100ml	Enterovirus primers and probes for RT- qPCR including an IPC. Sample concentrated by HFF.

CFU = colony forming units; MPN = most probable number; qPCR = Quantitative Polymerase Chain Reaction (detects DNA); RT-qPCR = reverse transcriptase qPCR to detect RNA; IPC = internal positive control; HFF = hollow fiber tangential flow ultrafiltration; PFU = plaque forming units.

Standard Culture Methods

The selected standard FIB methods are quantitative culture methods that use membrane filtration for counting individual colonies or the enzyme substrate methods with the most probable number approach (MPN) to calculate viable bacteria concentrations. Membrane filtration procedures are conducted by filtering a specific volume of water through a semi-permeable filter to collect the bacteria present in the sample. Depending upon the target FIB, the filter is placed onto a selective medium and incubated at an appropriate temperature for 24 hours. Bacteria in the sample capable of growing under selective conditions will multiply and create visible colonies that are subsequently counted. Enzyme substrate approaches are also culture based, except that the selective medium is mixed with the sample and incubated in a tray of individual wells that contain different sample volumes. After incubation, the number of positive wells in each tray, as indicated by color or fluorescence, is counted and numerically translated to a probable concentration of bacteria using a 'most probable number' (MPN/100ml) statistical calculation.

Vitek Identification Methods

The FIB culture methods were supplemented with use of the Vitek Compact by bioMerieux (Hazelwood, Missouri), which is a fully automated instrument used to discriminate the genus and species of FIB colonies initially collected and isolated by membrane filtration. The Vitek system utilizes a colorimetric reagent GPI Card (Gram-Positive) V1305 to identify up to 49 Gram-positive organisms such as *Listeria* and *Corynebacteria*. The GPI card used for this study was also able to determine the following potential *Enterococci* spp. and related bacteria:

- FIB *Enterococcus* (*E. faecalis* & *E. faecium*)
- *E. casseliflavus*
- *E. durans*
- *E. hirae*
- *E. gallinarum*
- *E. columbae*
- Ambiguous – multiple ID W/ low confidence
- *Staphylococcus* spp.
- *Streptococcus* spp.

Following membrane filtration, FIB colonies from approximately 10% of the samples were isolated and analyzed on Vitek cards. The Vitek analysis was conducted on enterococci colonies recovered from the filters incubated on mEI agar by U.S. EPA method 1600. The information provided by the Vitek analysis supported the multiple lines of evidence approach described below to determine the population diversity and bacterial community differences and/or similarities between the monitoring locations in an effort to help determine the source of the FIB. It should be noted that Vitek was not capable of confirming the bird associated organism, *E. avium*, in the analysis.

qPCR Methods

As determined by the literature survey, no standard methods are currently available for microbial source tracking for determining the source of FIB contamination. Such source identification is typically conducted using either a library-based approach or a library-independent approach. With a library-approach, a genetic library composed of thousands of fingerprints or genetic profiles from known fecal sources is compared with unknown isolates to infer their source. Unfortunately, the libraries do not contain the entire genetic pool of bacteria present, as the populations change over time and vary from location to location. These changes make the library-based microbial source tracking approaches difficult to maintain and are generally not reliable. Recently, library-independent approaches that are based on amplification of host-specific genetic markers have been developed that are potentially more reliable, easier to perform and more cost effective than the library-based methods. Thus, a library-independent approach was selected for this project. The approach relied upon a molecular method using quantitative polymerase chain reaction (qPCR).

Bacteroidales, enterovirus, and adenovirus were selected as markers to identify potential human fecal pollution using qPCR. PCR is a powerful tool used to detect specific genetic sequences in target organisms. The process of qPCR is used to selectively measure the quantity of target deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) present in a sample. Specific bacterial or viral targets of DNA or RNA are identified in samples by exponentially amplifying a specific piece of genetic material using temperature-tolerant enzymes. The amplified DNA or RNA is measured using automated instruments and compared to standards to calculate approximate concentrations of the target organism in the sample. Since enterovirus and adenovirus are specific to humans and require a host to grow (i.e., they can not grow independently in a medium such as beach sand), detection of virus genetic material in water samples would infer evidence of a human fecal source.

Although all *Bacteroidales* are not human-specific, the selected qPCR approach provides information on the host specificity. Since qPCR is accepted in the scientific community as an option for detecting and quantifying a variety of microorganisms, published peer-reviewed methods were evaluated. The most sensitive and reproducible *Bacteroidales* qPCR methods were selected and optimized for this project (10,31). To increase specificity, two assays were utilized. One qPCR assay was chosen to detect a broad range of *Bacteroidales* species (Bac_{TOT}) and another assay to discriminate *Bacteroidales* species more commonly associated with human feces, (Bac_{HU}). The assays were shown to be quantitative over a seven-log_{10} range. Internal PCR controls were also incorporated for identification of PCR inhibition as part of the quality assurance and quality control (QA/QC) procedures. To further distinguish results, a novel probe-specific melt analysis was incorporated into the methods to identify different *Bacteroidales* populations by detecting DNA sequence changes or polymorphisms within the PCR amplified sequence. These methods were critical for advanced source identification in this study, as they allowed for a quantitative description of the presence or absence of human-associated fecal bacteria coupled with the ability to further differentiate different populations of human-associated *Bacteroidales* based on melt patterns.

During the course of the study, occasionally a sample result would indicate higher values with the Bac_{HU} assay than the Bac_{TOT} assay. This occurred only in samples that contained high copies of both targets and thus did not affect the overall site evaluation. The discrepancies are likely attributed to two factors: 1) the qPCR assays target different areas of the rDNA and as such should be evaluated as two separate assays, and 2) the Bac_{HU} assay contained an internal control to identify inhibition, which required a subsequent correction to determine the final concentration. The Bac_{TOT} assay did not employ this internal control, because of difficulties in development of the internal control for this particular assay.

Modified Virus Sample Collection

Current water sample collection methods for the detection and enumeration of enteric viruses use expensive filters and have limitations for detecting multiple pathogens and/or indicator organisms. The traditional filter is not ideal for use in the marine matrix, and recovery efficiency is not consistent for the matrix spikes and maximum filtration volumes can vary, depending upon water matrix characteristics. Thus, a modified virus sample collection and concentration method using hollow fiber filters (HFF) was developed.

In HFF, the fluid is pumped tangentially along the surface of the membrane, with a molecular weight cutoff (MWCO) of 50kDa (33). An applied pressure serves to force a portion of the fluid through the center of the hollow fiber and the permeate passes through the fiber wall to the outside of the membrane fiber. The particulates and macromolecules too large to pass through the membrane pores are retained on the inside, thereby making up the retentate. In this case, however, the retained components do not build up at the surface of the membrane as they do with conventional membrane filtration techniques, but instead are carried along by the cross flow.

This feature of the HFF system makes it ideal for processes that require fine separations of particles by size, such as viral concentration.

The advantages of using HFF for viral concentration include minimal manipulation of waters as viral concentration is based on size exclusion and not chemical compositions, ability to concentrate an environmental water sample 10x-1000x, elimination of the elution step, ease of use, long-term cost effectiveness since filters are reusable, increased recovery of viruses, and versatility for extraction of other biomolecules. Disadvantages include viral adsorption to filter creating gel polarization, particle-associated viruses in the waters, pre-filtration of sample waters, potential concentration of PCR inhibitors, and a 10L limit on sample volumes.

Quality Assurance and Quality Control

The Sanitation Districts Laboratories Section is certified through the Environmental Laboratory Accreditation Program (ELAP) administered by the State of California Department of Public Health. All samples handled for this project were analyzed under strict adherence to ELAP standards, as outlined in the Sanitation Districts' Quality Program guidance document, "Quality Assurance Program of the Sanitation Districts of Los Angeles County Laboratories Section, April 2009" (37). Two samples were collected at the same location and time for ten percent of all samples and reported as field duplicates. Ten percent of all collected samples were also analyzed in replicate. All samples were collected and analyzed according to the laboratory QA/QC guidelines already established in the laboratory standard operating procedures (SOPs) for all methods.

The Sanitation Districts' Laboratories developed SOPs for all the qPCR methods used for the study, which are included in the Appendix on the DVD. The Sanitation Districts' Staff validated all the qPCR methods used for this study prior to implementation. Personnel were also required to demonstrate capability for performance of each method before analyzing any samples.

The qPCR methods selected for the microbial source tracking effort of the Redondo Beach Pier SEP are capable of detecting very small amounts of DNA and, like other PCR based methods, are extremely sensitive. However, because of the extreme sensitivity of the methods, they are subject to contamination and require an additional level of QA/QC effort than routine standardized culture based methods. These QA/QC procedures are necessary because the ability of PCR to produce numerous copies of target DNA creates the possibility of contamination by previously amplified products, which can lead to false-positive results. In addition, environmental samples may inhibit the PCR, which can lead to false-negative results. The qPCR methods adopted by the Sanitation Districts were derived from peer-reviewed and published methods but, at the time of this study, none of the adopted methods have been validated by the U.S. EPA or approved by any agency for the analyses of environmental water samples. Because the methods have not been previously validated, the Sanitation Districts Laboratories Section developed QA/QC guidelines that were based on the recommendations outlined in U.S. EPA guidance document: "Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples, October 2004" (38). This included designating separate areas for sample handling, nucleic acid isolation, sample analyses, and post amplification analyses. Dedicated equipment was used for each designated area and stringent cleanliness procedures were followed. Additionally, internal positive nucleic acid controls were used in the qPCR assays to identify and quantify inhibition. The details of the QA/QC procedures specific for each method are in included in the laboratory SOPs in the Appendix on the DVD.

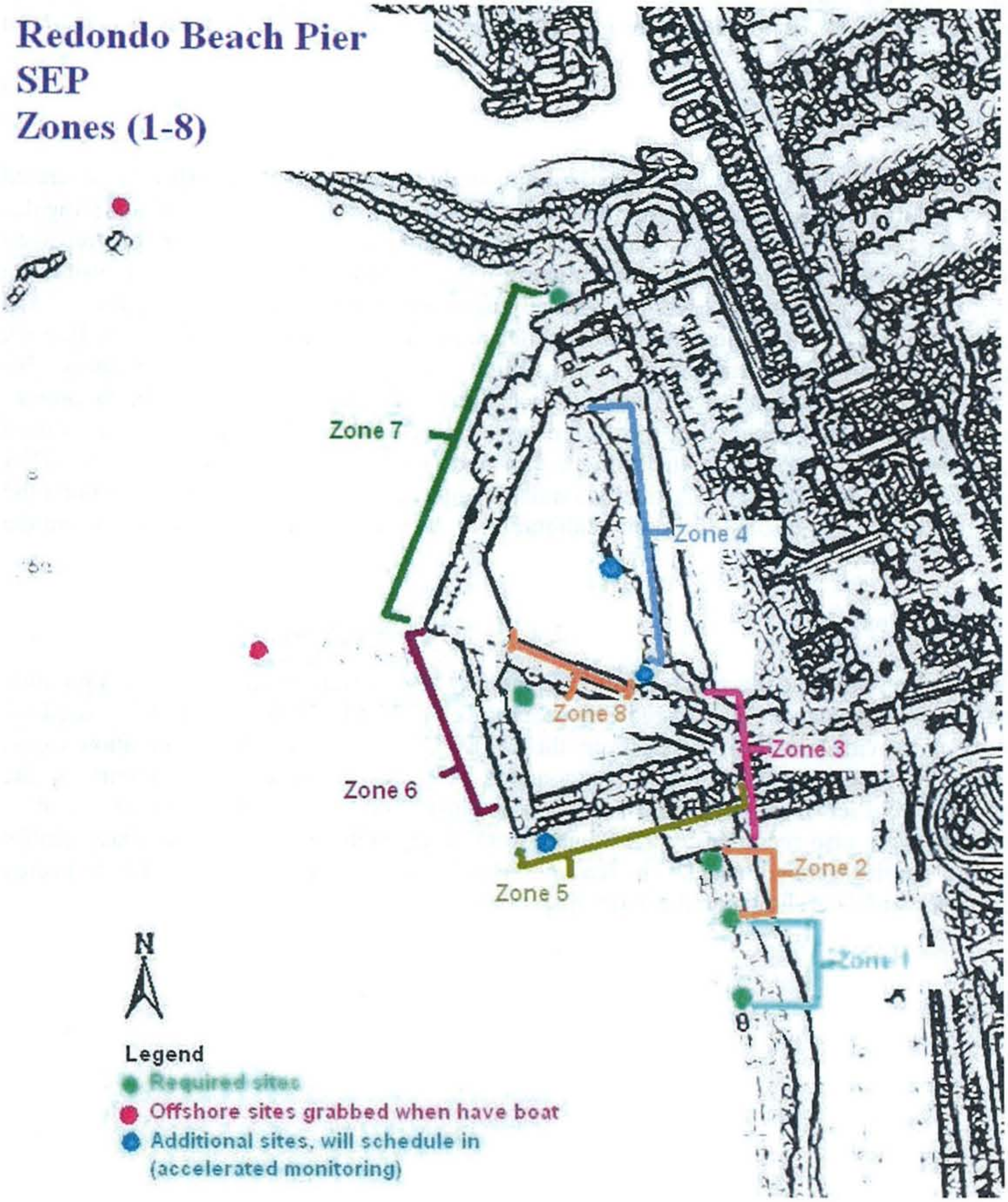
Laboratory Intercalibration Study

Three laboratories participated in an intercalibration study to determine if the FIB data generated by different laboratories were comparable. On October 17, 2007, the City of Los Angeles Hyperion Laboratory, Michelson Laboratory, and the Sanitation Districts' Laboratory received and analyzed one set each of blind spiked marine water samples to analyze for FIB using the laboratory standard operating procedures normally used for analyzing and reporting data. Each laboratory analyzed 13 samples for total and fecal coliforms and enterococci with IDEXX Colilert-18 and Enterolert, respectively. In addition, the Sanitation Districts' laboratory also processed samples with membrane filtration procedures. Data were evaluated for laboratory variability by calculating the precision criterion for each FIB. All results were within the control limits, demonstrating good reproducibility within and between laboratories. Also, ANOVA analysis showed that results were statically similar, suggesting statistical equivalency among the three laboratories. The results of the intercalibration study are included in the Appendix on the DVD.

Field Observations

Land based observations collected as part of the sanitary survey were helpful to use as a possible explanation for exceedances or elevated FIB occurrences. The Field Observation Form used for this study is provided in the Appendix on the DVD. SEA Lab of LA Conservation Corps (Redondo Beach, CA) was contracted to conduct daily observations in the vicinity of the Redondo Beach Pier from June 2007 through August 2008 at various times of the day. Observations were also recorded by the Sanitation Districts each day non-routine water quality monitoring samples were collected. The Redondo Beach Pier study site with the zones inspected for field observations is shown on the aerial map in Figure 6.

Figure 6. Aerial Map of the Redondo Beach Pier Field Observation Zones.



Monitoring Locations and Sampling Frequencies

In addition to the routine sites historically monitored for FIB in the vicinity of the Redondo Beach Pier, a number of additional sites were identified for investigation during the study. These sites originally included six shoreline sites and four offshore sites, depicted in Figure 7. Each site was assigned a number for ease of identification. As the study progressed, a seventh shoreline site was added, SMB-6-2b, directly in front of the storm drain under the pier. Several other sites were also added during the Tier 3 portion of the study, consisting of the storm drain under the pier, the pond formed by the storm drain under the pier, and a ground well. These sites were not assigned site numbers due to their late addition to the study. All of the sites are listed in Table 4.

Table 4. Monitoring Sites for the Accelerated Monitoring Efforts as part of the Redondo Beach Pier SEP.

Site	Site Description	Latitude	Longitude
1	Shoreline: SMB-6-2 in front of the lifeguard tower	N: 33.83796	W: 118.39103
2	Shoreline: In front of storm drain south of pier	N: 33.83833	W: 118.391114
3	Shoreline: S-16 and SMB-6-2a next to the south edge of the pier	N: 33.83872	W: 118.39126
SMB-6-2b	Shoreline: SMB-6-2b In front of storm drain under pier		
4	Shoreline: SMB-6-2c Under south leg, on north side (under Old Tony's)	N: 33.83927	W: 118.39141
5	Shoreline: Between north leg and south leg of pier	N: 33.83974	W: 118.39154
6	Offshore: Southwest edge off the pier corner	N: 33.83870	W: 118.39233
7	Offshore: Inside north corner of triangle	N: 33.83915	W: 118.39246
8	Shoreline: Off north leg of pier	N: 33.84098	W: 118.39204
9	Offshore: Between harbor break water and jetty	N: 33.84143	W: 118.39459
10	Offshore: 200ft off pointed section of pier	N: 33.83943	W: 118.39386
Storm Drain	Onshore: Storm drain under pier		
Pond	Onshore: Pond formed by storm drain under pier		
Ground Well	Onshore: Ground well discharging to storm drain under pier		

N = North; W = West.

Figure 7. Redondo Beach Pier SEP Study Area and Selected Monitoring Sites.

The date ranges and frequencies of monitoring at each location for each tier of the tiered toolbox are presented in Table 5. The Redondo Beach Pier SEP site number is listed with the official routine monitoring designations, when appropriate. Data generated during overlapping monitoring efforts (tiers) were used in both tiers of analyses. Such data included the routine monitoring data generated by the Hyperion and Michelson Laboratories in Tiers 1 and 2.

Table 5. Monitoring Conducted for Each Tier of the Microbial Source Tracking Toolbox.

Tier	Site # (Designation)	Monitoring Dates	Frequency	Analyses Presented
1	3 (S-16)	4/26/1995 – 1/3/2009	5 days/week*	TC, FC, ENT
	1 (SMB-6-2)	12/3/2007 – 12/29/2008	Weekly**	TC, FC, ENT
2	1, 2, 3, 4, 5, 6, 7, 8, 9, 10	5/30/2007 – 8/7/2007	2 days/week***	TC, FC, *E. coli,* ENT, Vitek, Observations
	3 (S-16)	12/4/2007 – 1/3/2009	5 days/week*	TC, FC, ENT, Observations
	1 (SMB-6-2)	12/3/2007 – 1/3/2009	Weekly**	TC, FC, ENT, Observations
	3 (S-16 & SMB-6-2a)	12/3/2007 – 1/3/2009	Weekly**	TC, FC, ENT, Observations
	SMB-6-2b	12/3/2007 – 1/3/2009	Weekly**	TC, FC, ENT, Observations
	4 (SMB-6-2c)	12/3/2007 – 1/3/2009	Weekly**	TC, FC, ENT, Observations
	3 (S-16 & SMB-6-2a)	11/20-21&27-28/2007	Four days*,#	TC, FC, ENT, Observations
	4 (SMB-6-2c)	11/20-21&27-28/2007	Four days*,#	TC, FC, ENT, Observations
3	3 (S-16 & SMB-6-2a)	6/24/2008 – 8/1/2008 & 3/10/2009	4 days/week#	TC, FC, *E. coli,* ENT, Vitek, Bac_{TOT}, Bac_{HU}, Enterovirus qPCR, Adenovirus RT-qPCR, Observations
	SMB-6-2b	6/24/2008 – 8/1/2008 & 3/10/2009	4 days/week#	TC, FC, *E. coli,* ENT, Vitek, Bac_{TOT}, Bac_{HU}, Observations
	Storm Drain	6/24/2008 – 8/1/2008 & 3/10/2009	4 days/week#	TC, FC, *E. coli,* ENT, Vitek, Bac_{TOT}, Bac_{HU}, Observations
	Pond	6/24/2008 – 8/1/2008 & 3/10/2009	4 days/week#	TC, FC, *E. coli,* ENT, Vitek, Bac_{TOT}, Bac_{HU}, Observations
	Ground Well	3/10/2009	Once#	TC, FC, *E. coli,* ENT, Bac_{TOT}, Bac_{HU}

* = Monitoring conducted by Hyperion Laboratory; ** = Monitoring conducted by Michelson Laboratory; *** = Initial sanitary survey monitoring conducted by Sanitation Districts' Staff; # = Monitoring conducted by Sanitation Districts Staff as part of the MST efforts; TC = Total coliforms; FC = Fecal coliforms; ENT = enterococci; qPCR = quantitative polymerase chain reaction used to detect DNA; RT-qPCR = reverse transcriptase qPCR used to detect RNA; Bac_{TOT} = total *Bacteroidales* assessed by qPCR; Bac_{HU} = human associated *Bacteroidales* assessed by qPCR.

Michelson Laboratory collected and analyzed FIB samples for Sites SMB-6-2 (Site #1), SMB-6-2a (Site #3), SMB-6-2b, and SMB-6-2c (Site #4). The Hyperion Laboratory collected and analyzed FIB samples for Site S-16 (Site #3). The Sanitation Districts collected and analyzed the samples for the remainder of the sites. The Sanitation Districts also exclusively collected and analyzed the data for viruses, *Bacteriodales*, and Vitek speciation in Tier 3.

Accelerated Monitoring

As part of the Tier 2 sanitary survey, accelerated monitoring was conducted at the original ten study sites (Sites #1 –10) from May 30 through August 7, 2007 (11 weeks) to expand upon the routine FIB monitoring at the Redondo Beach Pier. The Work Plan indicated that this portion of the water quality survey would continue for six weeks or until sustained exceedances were observed. At that time, monitoring was to be intensified temporally and spatially in the area of the exceedance. However, there were no sustained FIB exceedances at the shoreline locations during the first six weeks of sampling, and most FIB levels at the offshore locations were very low or absent. Therefore, the accelerated monitoring period was extended for an additional five weeks. Despite the extended monitoring period, there were no sustained exceedances observed at any of the locations during the 11-week accelerated monitoring period.

The Sanitation Districts compiled the accelerated monitoring results and presented a summary to the Technical Workgroup for the project on October 30, 2007. The presentation and meeting minutes are included in the Appendix on the attached DVD. Technical Workgroup members reviewed the monitoring data and recommended that the accelerated monitoring efforts be reduced. Instead, it was recommended that monitoring efforts be focused on the locations immediately adjacent to the Redondo Beach Pier. To supplement the routine monitoring at Sites S-16 and SMB-6-2, the City of Redondo Beach expanded its routine monitoring program. The City began weekly sampling at additional locations including SMB-6-2a (at the same location S-16 was collected), SMB-6-2b (at the shoreline in front of the storm drain), and SMB-6-2c (at the shoreline under Old Tony's). The sampling was conducted the same day of the week as routine monitoring at SMB-6-2 (Mondays) from January 2008 through August 2008.

Redondo Beach Pier Cleaning Study

Also during the course of this project, the Sanitation Districts conducted an additional study to evaluate FIB levels before and after debris removal and the spring tide. After the Technical Workgroup Meeting on October 30, 2007, Heal the Bay staff inspected the Redondo Beach Pier study site, including the area under the pier. They reported standing water, a considerable amount of marine debris, strong odor, and dead and swarming kelp flies in front of the storm drain under the Pier. The Technical Workgroup members discussed this observation by email and recommended cleaning the marine debris and standing water from under the Redondo Beach

Pier in coordination with a water quality assessment. The purpose of the suggested cleaning was to evaluate any possible reservoirs of FIB. Since it was apparent that a recent spring tide occurred and the peaking tide height was a likely source of the standing water under the Pier when Heal the Bay staff inspected the site, two cleaning events occurred to coordinate with the November 2008 spring tide.

Samples were collected at study Sites #3 (S-16) and #4 (under Old Tony's) by Sanitation District's staff at approximately 9:45 AM on November 20, 21, 27, and 28, 2007. The effort was deliberately coordinated to occur before and after cleaning and before and after the spring tide that occurred on November 24, 2007. The tide was incoming on November 20 and 21, with the lowest levels observed at 7:00 AM and 8:00 AM, respectively. The tide was outgoing on November 27 and 28 with the highest levels observed at 8:00 AM and 9:00 AM, respectively. Due to the Thanksgiving holiday and weekend, staff was not available to collect samples or clean the beach at the peak of the spring tide on November 24, 2007.

Microbial Source Tracking

The six-week microbial source tracking (MST) sampling effort was conducted between June 24 August 1, 2008. This sampling period was scheduled to capture two or three spring tide events and at least one holiday weekend at the locations immediately adjacent to the Redondo Beach Pier. These locations were identified and selected based on the FIB data and observations conducted during the Tier 2 source identification effort. Also, the sampling period was selected to capture the period when dry weather exceedances were most likely to be observed, as found in the Tier 1 sanitary survey and historical data analyses.

Samples were collected daily at approximately 9 AM by the Sanitation Districts personnel each Tuesday through Friday according to the schedule outlined in Table 6. Specifically, samples were collected and analyzed for FIB and *Bacteroidales* at Site #3 (S-16) and at SMB-6-2b (in front of the storm drain under the pier). Adenoviruses and enteric viruses were only analyzed at Site #3 (S-16). When water was either ponded in front of the storm drain under the RB Pier or flowing from the storm drain, samples were collected and analyzed for FIB and *Bacteroidales*. These sites were selected because the summer 2007 accelerated monitoring showed that the highest levels of FIB were detected at the sampling locations immediately adjacent to the Redondo Beach Pier. Speciation using Vitek assessment was conducted on 10% of all samples each week to speciate fecal coliforms and enterococci. Salinity and turbidity were collected and analyzed each day at Site #3 and SMB-6-2b and, when available, from the pond and the flowing storm drain. Field observations were collected seven days per week at rotating collection times by Sea Lab Staff and also by the Sanitation Districts Staff on sampling days at the same time samples were collected.

Table 6. Sanitation Districts' Microbial Source Tracking Sampling and Analysis Schedule.*

	Site #3 (S-16/ SMB-6-2a)	SMB-6-2b (In front of Storm Drain under Pier)	Pond in Front of Storm Drain	Storm Drain Under Pier
Total Coliforms	Daily	Daily	Daily	Daily
Fecal Coliforms	Daily	Daily	Daily	Daily
E. coli	Daily	Daily	Daily	Daily
Salinity	Daily	Daily	Daily	Daily
Turbidity	Daily	Daily	Daily	Daily
Enterococci	Daily	Daily	Daily	Daily
Vitek	10% of FC & Ent	10% of FC & Ent	10% of FC & Ent	10% of FC & Ent
Bacteroidales qPCR	Daily	Daily	Daily	Daily
Enterovirus RT qPCR	Daily	No sample	No sample	No sample
Adenovirus qPCR	Daily	No sample	No sample	No sample

* Samples were collected between 7:00 and 9:00 AM each Tuesday through Friday. FC = fecal coliforms; Ent = *Enterococcus* spp.

An additional set of samples was collected as a follow-up to the summer 2008 microbial source tracking effort on March 10, 2009, which included a ground well location determined to be a source of the water flowing from the storm drain under the Redondo Beach Pier.

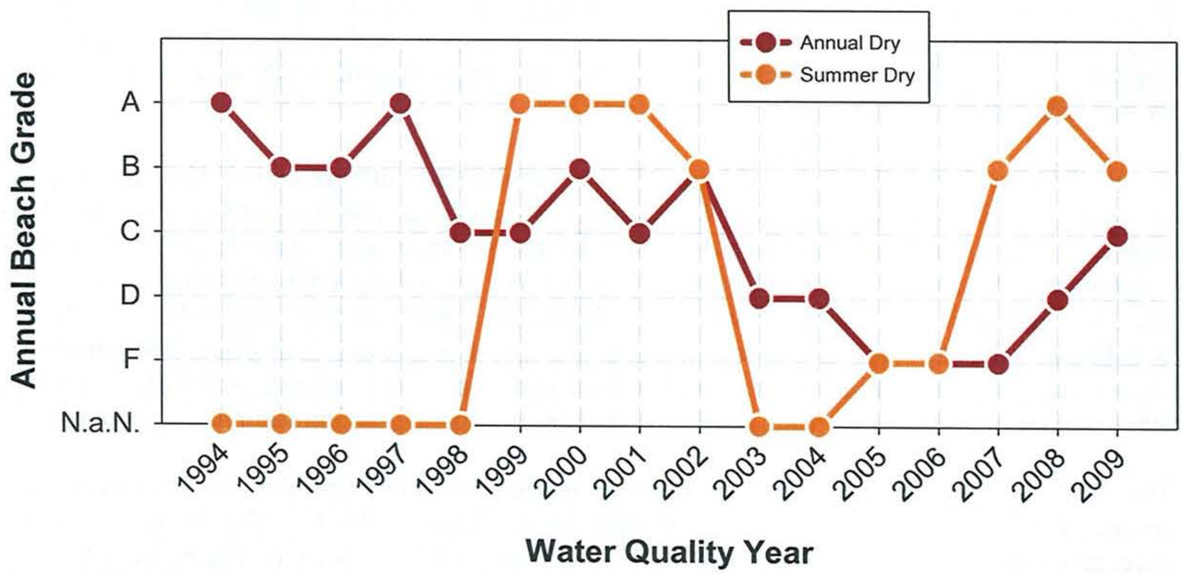
Routine monitoring data were collected for required reporting purposes and watched closely for discrepancies during the microbial source tracking effort. Also during the MST effort, wave height, surface current, wind speed, and rainfall events were monitored and recorded daily.

STUDY FINDINGS

Tier 1. Sanitary Survey

The sanitary survey portion of this study relied primarily upon the routine monitoring associated with existing beach water quality monitoring programs. The review of the historical monitoring data conducted as part of this study revealed that the monitoring location identified as S-16 received poor and failing grades on the Heal the Bay Beach Water Quality Report card between 2003 and 2006, shown in Figure 8. The City of Redondo Beach made significant efforts during this period to mitigate the persistent FIB standards exceedances, but despite their efforts, the beach continued to receive poor and failing grades. Therefore, the areas immediately north and south of the Redondo Beach Pier were identified for the Redondo Beach Pier SEP study and targeted for accelerated monitoring in Tier 2 of the tiered toolbox approach described herein. It should be noted that when the Sanitation Districts conducted efforts in 2007 and 2008 to research the site and search for a source of the dry weather exceedances, the beach water quality substantially improved, particularly during the summer dry weather period.

Figure 8. Heal the Bay Dry Weather Report Card Grades



As noted in the Historical Data Review section of this report, the methods used to analyze FIB and the location of the sampling site for the Hyperion Wastewater Treatment Plant MS4 permit and Heal the Bay report card (Site S-16) changed in 2004. When the initial historical data review for this project was conducted in 2006, there were less than two years worth of historical data available that reflected the new methods and the new sampling site. The Sanitation Districts were concerned that trends would not be apparent during this limited two year period, so the historical data review was expanded to include routine monitoring data generated during the Redondo Beach Pier SEP efforts through December 2008. The recorded rainfall and monthly mean tide height (MTL) were also included in the analyses to compare long-term variability and trends.

The rolling 30-day geometric mean for all three routinely monitored FIB at Site S-16 are presented in Figure 9. The concentrations of all three FIB species appeared to increase in 2004, concurrent with the changes in sampling location and sampling method. However, when the data are analyzed separately before or after the method and location changes in 2004, the mean FIB levels are not substantially different. Additionally, the data before and after the changes in 2004 both show decreasing trends (data not shown). This suggests that data collected before and after the method and location changes should be analyzed as individual data sets and that the long-term FIB levels at Redondo Beach are declining.

Another interesting trend was observed in the data, relating to the monthly tide height pattern and rainfall. Monthly mean tide heights (MTL) and rainfall were compared with the 30-day rolling geometric mean of FIB monitoring data at Site S-16, as presented in Figure 9. Observed tide data were obtained from the National Oceanographic and Atmospheric Association website (http://tidesandcurrents.noaa.gov) for Santa Monica, CA data inventory station ID# 9410840. Rainfall data were obtained from the City of Redondo Beach website (www.redondo.org).

The MTL increased and hit its highest points during the dry weather summer swimming periods between April and October of each year, as indicated in Figure 9. Also during the dry weather summer swimming period, the mean FIB levels decreased and remained low until the rain season began after October of each year. After October, the MTL decreased when the FIB levels increased, along with increases in rainfall. Although not presented in Figure 9, changes in tide height (such as spring tides), MTL, wave activity, wind activity, and storm events all consistently appeared to contribute to increases in mean FIB levels, suggesting the study location is impacted by multiple factors.

In an effort to determine if the FIB levels have significantly changed in the last decade, the historical data presented in Figure 9 were statistically analyzed and examined for trends. This effort also identified the background level of FIB for the study site. Results for the statistical analyses are shown in Table 7. A summary of percent exceedances for the summer periods is presented in Figure 10. Summer each year is defined as April 1 through September 30. Winter is defined as the subsequent October 1 through March 31 period for each year. The percent of exceedances were based on marine water contact recreation single sample standards of 10,000, 400, and 104 total coliforms, fecal coliforms, and enterococci per 100ml sample, respectively.

The total coliforms and enterococci summer exceedance rates (percent exceedances) have remained relatively consistent for each year since summer 2004. The highest summer exceedance rate for total coliforms was 6.6% in the summer 2004 period. The highest summer exceedance rate for enterococci was in summer 2007 with 8.9% of samples exceeding single sample standards. Fecal coliform exceedance rates were higher than those for total coliforms or enterococci. They have varied considerably and peaked in 2006. During this study, the summers of 2007 and 2008 had excellent water quality with exceedance rates well below 10% for all the FIBs. There did not appear to be a relationship between total rainfall during the summer periods and exceedances.

Figure 9. Comparison of Monthly Mean Tide Heights (MTL) and 30-Day Geometric Mean FIB Data at Site #3 (S-16) (April 1995-December 2008).

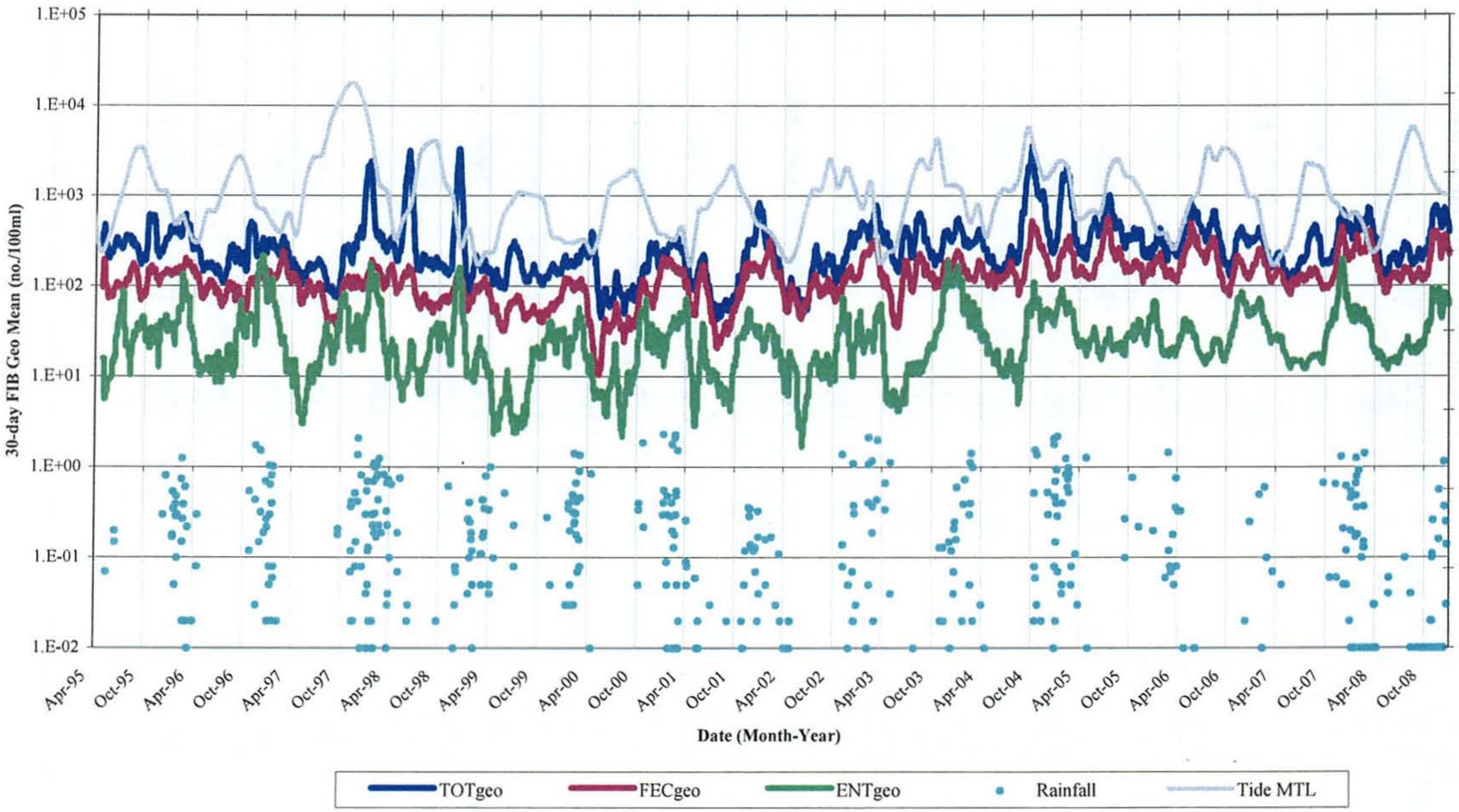


Table 7. Summary Statistics for FIB Data Collected at the Redondo Beach Pier Monitoring Site S-16: April 1995 through December 2008.

Period	*(CFU or MPN/100ml)*	*TOT*	*FEC*	*ENT*		*Rainfall (in.)*
Summer 95	Count	157	157	24		4
	Count > AB411	0	9	1		
	% > AB411	0.00%	5.73%	4.17%		
	Avg	416	148	40		0.11
	SD	530	126	50	*Total Rain*	0.42
	Max	4600	750	250		0.20
Winter 95	Count	183	183	31		24
	Count > AB411	2	22	5		
	% > AB411	1.09%	12.02%	16.13%		
	Avg	870	235	102		0.32
	SD	2019	413	202	*Total Rain*	7.67
	Max	20000	5000	1000		1.26
Summer 96	Count	182	182	32		3
	Count > AB411	0	13	1		
	% > AB411	0.00%	7.14%	3.13%		
	Avg	351	148	61		0.13
	SD	504	179	227	*Total Rain*	0.40
	Max	4000	1200	1300		0.3
Winter 96	Count	188	187	35		31
	Count > AB411	2	19	7		
	% > AB411	1.06%	10.16%	20.00%		
	Avg	787	227	302		0.52
	SD	2745	390	1073	*Total Rain*	16.26
	Max	26000	3600	6300		3.00
Summer 97	Count	183	183	30		2
	Count > AB411	0	8	2		
	% > AB411	0.00%	4.37%	6.67%		
	Avg	202	114	44		0.20
	SD	214	117	96	*Total Rain*	0.39
	Max	1800	570	480		0.21
Winter 97	Count	189	189	37		56
	Count > AB411	12	16	8		
	% > AB411	6.35%	8.47%	21.62%		
	Avg	1830	190	89		0.42
	SD	5147	231	144	*Total Rain*	23.33
	Max	34000	2300	760		2.10
Summer 98	Count	178	178	31		9
	Count > AB411	6	6	1		
	% > AB411	3.37%	3.37%	3.23%		
	Avg	1496	126	22		0.29
	SD	4931	119	25	*Total Rain*	2.57
	Max	35000	590	130		0.76
Winter 98	Count	184	184	35		34
	Count > AB411	8	16	7		
	% > AB411	4.35%	8.70%	20.00%		
	Avg	2518	157	72		0.51
	SD	10955	252	137	*Total Rain*	17.40
	Max	110000	2800	680		9.00

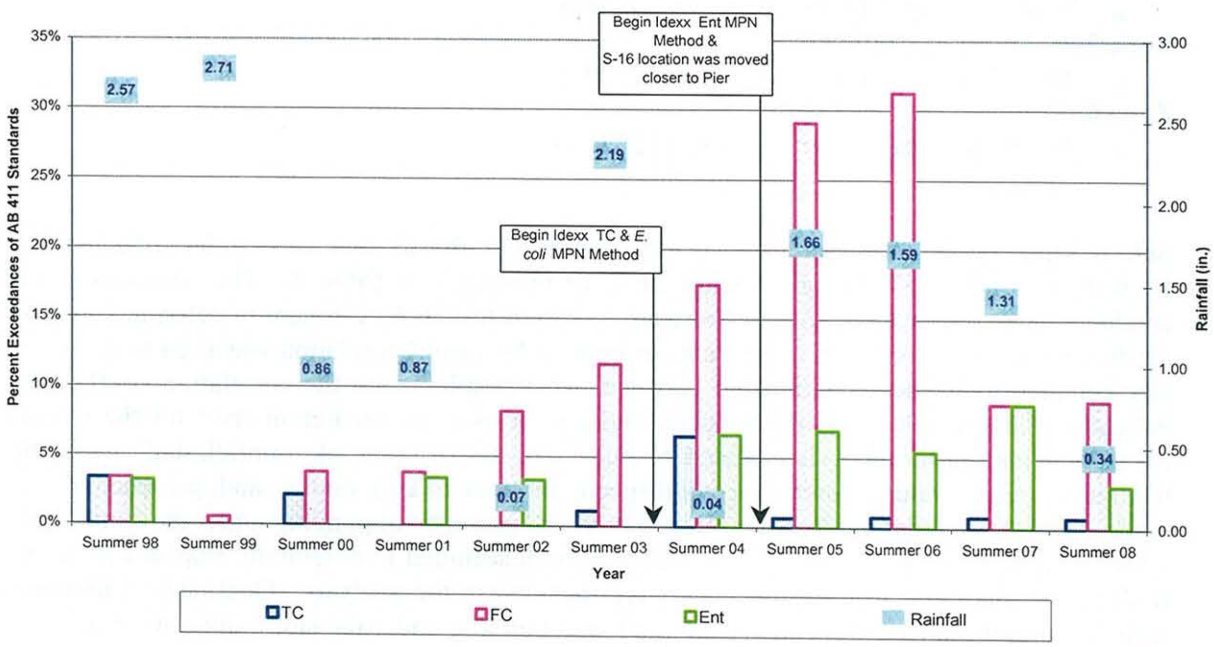
Period	*(CFU or MPN/100ml)*	*TOT*	*FEC*	*ENT*		*Rainfall (in.)*
Summer 99	Count	183	183	30		9
	Count > AB411	0	1	0		
	% > AB411	0.00%	0.55%	0.00%		
	Avg	239	76	11		0.30
	SD	247	68	14	*Total Rain*	2.71
	Max	1400	420	64		1.01
Winter 99	Count	182	182	30		27
	Count > AB411	2	9	4		
	% > AB411	1.10%	4.95%	13.33%		
	Avg	455	133	53		0.37
	SD	1782	232	89	*Total rain*	9.87
	Max	18000	2800	420		1.42
Summer 00	Count	183	183	30		3
	Count > AB411	4	7	0		
	% > AB411	2.19%	3.83%	0.00%		
	Avg	580	90	15		0.29
	SD	2288	192	13	*Total rain*	0.86
	Max	17000	1400	50		0.85
Winter 00	Count	182	182	29		36
	Count > AB411	3	23	5		
	% > AB411	1.65%	12.64%	17.24%		
	Avg	707	226	75		0.56
	SD	2517	349	117	*Total rain*	17.87
	Max	23000	2900	560		2.33
Summer 01	Count	183	183	29		11
	Count > AB411	0	7	1		
	% > AB411	0.00%	3.83%	3.45%		
	Avg	166	110	17		0.08
	SD	212	128	24	*Total rain*	0.87
	Max	1700	720	130		0.26
Winter 01	Count	189	188	36		4
	Count > AB411	3	40	4		
	% > AB411	1.59%	21.28%	11.11%		
	Avg	973	245	41		3.05
	SD	2940	235	47	*Total rain*	12.21
	Max	25000	1100	220		11.00
Summer 02	Count	181	181	30		5
	Count > AB411	0	15	1		
	% > AB411	0.00%	8.29%	3.33%		
	Avg	301	151	23		0.01
	SD	624	165	51	*Total rain*	0.07
	Max	7200	890	280		0.02
Winter 02	Count	182	182	30		24
	Count > AB411	2	40	5		
	% > AB411	1.10%	21.98%	16.67%		
	Avg	976	280	82		0.48
	SD	2473	388	139	*Total rain*	11.54
	Max	24000	3100	620		2.15

Period	(CFU or MPN/100ml)	TOT	FEC	ENT		Rainfall (in.)
Summer 03	Count	179	179	29		5
	Count > AB411	2	21	0		
	% > AB411	1.12%	11.73%	0.00%		
	Avg	732	226	15		0.44
	SD	2110	359	13	Total rain	2.19
	Max	24000	3100	48		1.13
Winter 03	Count	180	180	29		24
	Count > AB411	2	18	8		
	% > AB411	1.11%	10.00%	27.59%		
	Avg	602	239	123		0.31
	SD	1435	507	159	Total rain	7.39
	Max	14000	6500	600		1.43
Summer 04	Count	183	183	30		2
	Count > AB411	12	32	2		
	% > AB411	6.56%	17.49%	6.67%		
	Avg	1566	326	40		0.02
	SD	3375	916	73	Total rain	0.04
	Max	13000	11000	370		0.03
Winter 04	Count	158	158	132		45
	Count > AB411	14	43	34		
	% > AB411	8.86%	27.22%	25.76%		
	Avg	2059	561	121		0.50
	SD	3700	1615	237	Total rain	22.39
	Max	13000	13000	2000		2.20
Summer 05	Count	141	141	143		4
	Count > AB411	1	41	10		
	% > AB411	0.71%	29.08%	6.99%		
	Avg	858	385	60		0.42
	SD	1481	688	199	Total rain	1.66
	Max	13000	6800	2000		1.28
Winter 05	Count	129	129	129		20
	Count > AB411	0	21	22		
	% > AB411	0.00%	16.28%	17.05%		
	Avg	593	248	78		0.32
	SD	1132	328	189	Total rain	6.36
	Max	8700	2300	1700		1.46
Summer 06	Count	128	128	128		9
	Count > AB411	1	40	7		
	% > AB411	0.78%	31.25%	5.47%		
	Avg	731	375	37		0.18
	SD	1346	476	49	Total rain	1.59
	Max	13000	2600	320		0.58
Winter 06	Count	129	129	129		12
	Count > AB411	0	21	36		
	% > AB411	0.00%	16.28%	27.91%		
	Avg	461	243	100		0.18
	SD	478	251	184	Total rain	2.11
	Max	2400	1300	1400		0.60

Period	(CFU or MPN/100ml)	TOT	FEC	ENT		Rainfall (in.)
Summer 07	Count	123	123	123		4
	Count > AB411	1	11	11		
	% > AB411	0.81%	8.94%	8.94%		
	Avg	539	168	33		0.33
	SD	1513	220	55	Total rain	1.31
	Max	13000	1400	320		0.67
Winter 07	Count	127	127	127		38
	Count > AB411	2	42	40		
	% > AB411	1.57%	33.07%	31.50%		
	Avg	873	423	153		0.32
	SD	1791	643	331	Total rain	12.26
	Max	13000	5500	2000		1.42
Summer 08	Count	131	131	131		12
	Count > AB411	1	12	4		
	% > AB411	0.76%	9.16%	3.05%		
	Avg	399	199	29		0.03
	SD	1160	376	41	Total rain	0.34
	Max	13000	3900	320		0.10
Winter 08	Count	65	65	64		26
	Count > AB411	0	19	21		
	% > AB411	0.00%	29.23%	32.81%		
	Avg	990	504	239		0.13
	SD	1423	689	485	Total rain	3.31
	Max	7900	3400	2000		1.15

TOT = total coliforms; FEC = fecal coliforms; ENT = enterococci.

Figure 10. Redondo Beach Pier Site S-16 Summer Exceedance Summary (1998-2008).



Tier 2. Source Identification

Tier 2 of the Redondo Beach Pier SEP included extensive collection and analysis of field observations, as well as FIB monitoring at the shoreline and offshore monitoring sites.

Field Observations

All field observations are included in the Appendix on the DVD. In general, there were no unusual field observations during the study period. The most significant observations for each zone are summarized below:

Zone 2

- Storm drain flow (low) 16% of time;
- Discrepancies of reported storm drain flows with Sanitation Districts' and SEA Lab findings (1 of 5);
- Average 22 birds;
- Bird fecal matter recorded 17% of time;
- Some kelp deposits reported 73% of time;
- Trash consistently reported.

Zone 3

- Storm drain flowed 29% of time;
- Discrepancies of reported storm drain flows with Sanitation Districts' and SEA Lab findings (18 of 28);
- Average 4 birds;
- Trash and kelp deposits frequently reported.

Zone 4

- Average 25 birds;
- Trash and kelp deposits frequently reported.

Zone 5

- Heavy bird fecal matter on August 12, 2008.

Zone 6

- Heavy bird fecal matter on August 12, 2008.

Selected field observations were statistically compared with FIB data for samples collected at S-16 from October 2007 through August 2008, as presented in Table 8. The comparison was conducted using Sigma Stat (Systat Software Inc., San Jose, CA) software to determine Pearson Product Moment Correlations. The Pearson Product Moment Correlation was used to determine whether there is a linear relationship between two variables, with the correlation coefficient r indicating the strength of the relationship, and p indicating the prediction error for the r value. Parameters chosen for analysis included exceedances, day of the week, rainfall, daily maximum tide height range, water temperature, wind speed, presence of kelp on the sand, presence of flow in the storm drain under the Redondo Beach Pier, and presence of ponding under the storm drain. These parameters were selected because they were determined to potentially impact FIB levels. Both dry weather and wet weather data were included in the analysis. Qualitative parameters such as storm drain flowing, kelp on the sand, and ponding under the storm drain were recorded as present or absent and were assigned a '0' or '1', respectively, for the statistical analyses. The rest of the parameters were evaluated with the reported quantified numeric values. FIB results that were below the detectable levels of the analytical methods were included in the data set as values at the method detection limit.

Summaries of the statistical comparison of FIB parameters and selected field observations are presented in Table 8. The most significant field observations that correlated with exceedances were rainfall, kelp on the sand, daily tide height, water temperature, and wind speed. Ponding under the storm drain was marginally correlated with exceedances. It is noted that these correlations should not be interpreted to imply causation, as multiple factors appear to be affecting the correlations. Additional in-depth analyses should be performed to investigate all the field observations and relationships to FIB and exceedances.

Table 8. Significance of Selected Field Observations and FIB at Site #3 (S-16) at the Redondo Beach Pier (October 2007 through August 2008).

Observation	Comparison Parameter	Correlation (Yes or No)	Correlation Coeff. (r)	P Value	N
Rainfall	**Exceedance**	**Yes**	**0.233**	**<<0.05**	**305**
	Total Coliforms	**Yes**	**0.265**	**<<0.05**	**305**
	Fecal Coliforms	**Yes**	**0.247**	**<<0.05**	**305**
	Enterococci	**Yes**	**0.233**	**<<0.05**	**305**
	FC:TC Ratio	No	-0.123	0.31	305
	TC 30-day Geo Mean	**Yes**	**0.139**	**<<0.05**	**434**
	FC 30-day Geo Mean	**Yes**	**0.117**	**<0.05**	**434**
	Ent 30-day Geo Mean	No	0.130	0.304	65
Storm Drain Flowing	Exceedance	No	0.124	0.07	214
	Total Coliforms	No	0.035	0.61	214
	Fecal Coliforms	No	0.029	0.67	214
	Enterococci	**Yes**	**0.162**	**<0.05**	**214**
	FC:TC Ratio	No	-0.083	0.23	214
	TC 30-day Geo Mean	No	0.076	0.19	299
	FC 30-day Geo Mean	No	0.085	0.14	299
	Ent 30-day Geo Mean	**Yes**	**0.223**	**<<0.05**	**299**
Kelp on Sand	**Exceedance**	**Yes**	**-0.281**	**<<0.05**	**209**
	Total Coliforms	No	-0.100	0.15	209
	Fecal Coliforms	**Yes**	**-0.182**	**<<0.05**	**209**
	Enterococci	**Yes**	**-0.242**	**<<0.05**	**209**
	FC:TC Ratio	No	0.087	0.21	209
	TC 30-day Geo Mean	**Yes**	**-0.532**	**<<0.05**	**293**
	FC 30-day Geo Mean	**Yes**	**-0.492**	**<<0.05**	**293**
	Ent 30-day Geo Mean	**Yes**	**-0.437**	**<<0.05**	**293**

Table 8 is continued on the next page.

Table 8 (Continued). Significance of Selected Field Observations and FIB at Site #3 (S-16) at the Redondo Beach Pier (October 2007 through August 2008).

Observation	Comparison Parameter	Correlation (Yes or No)	Correlation Coeff. (r)	P Value	N
Daily Tide Height	**Exceedance**	**Yes**	**0.135**	**<0.05**	**305**
	Total Coliforms	No	-0.096	0.09	305
	Fecal Coliforms	**Yes**	**0.134**	**0.02**	**305**
	Enterococci	**Yes**	**0.138**	**0.02**	**304**
	FC:TC Ratio	**Yes**	**-0.205**	**<<0.05**	**305**
	TC 30-day Geo Mean	No	0.073	0.13	434
	FC 30-day Geo Mean	No	0.049	0.31	434
	Ent 30-day Geo Mean	No	0.083	0.09	434
Water Temperature	**Exceedance**	**Yes**	**-0.264**	**<<0.05**	**293**
	Total Coliforms	**Yes**	**-0.137**	**0.02**	**293**
	Fecal Coliforms	**Yes**	**-0.148**	**0.01**	**293**
	Enterococci	No	-0.058	0.33	292
	FC:TC Ratio	No	0.029	0.62	293
	TC 30-day Geo Mean	**Yes**	**-0.479**	**<<0.05**	**418**
	FC 30-day Geo Mean	**Yes**	**-0.407**	**<<0.05**	**418**
	Ent 30-day Geo Mean	**Yes**	**-0.207**	**<<0.05**	**418**
Wind Speed	**Exceedance**	**Yes**	**-0.110**	**<0.05**	**305**
	Total Coliforms	No	-0.085	0.14	305
	Fecal Coliforms	No	-0.094	0.10	305
	Enterococci	**Yes**	**-0.130**	**<0.05**	**304**
	FC:TC Ratio	No	0.026	0.66	305
	TC 30-day Geo Mean	**Yes**	**-0.380**	**<<0.05**	**433**
	FC 30-day Geo Mean	**Yes**	**-0.341**	**<<0.05**	**433**
	Ent 30-day Geo Mean	**Yes**	**-0.343**	**<<0.05**	**433**
Ponding under Storm Drain	**Exceedance**	**Yes**	**0.142**	**0.05**	**185**
	Total Coliforms	No	0.107	0.15	185
	Fecal Coliforms	**Yes**	**0.155**	**0.04**	**185**
	Enterococci	**Yes**	**0.154**	**0.04**	**184**
	FC:TC Ratio	No	-0.024	0.74	185
	TC 30-day Geo Mean	No	0.065	0.29	271
	FC 30-day Geo Mean	No	0.063	0.30	271
	Ent 30-day Geo Mean	No	-0.006	0.92	271

N = Number of comparisons; FC = Fecal coliform; TC = Total Coliform; Ent = Enterococci; **Bold** font indicates a significant correlation where p<0.05; **Bold red** font in shaded cells indicate significant correlation where p<<0.05; ; < = less than; << = much less than; negative values indicate an inverse relationship.

Rainfall was significantly correlated with all the FIB parameters except the 30-day geometric mean for enterococci. The 30-day enterococci mean data were probably not correlated due to the large number of samples without detectable levels of enterococci during the dry periods. The correlation was significant for number of exceedances, single sample total coliforms, single sample fecal coliforms, single sample enterococci, and the 30-day geometric mean for total coliforms. During this study there were 44 days when rain was recorded. These data show that, as expected, FIB levels increase with increasing rain levels.

Although rainfall was correlated with the storm drain flows (Pearson correlation=0.156, $p<0.05$, n=299, data not shown), there was no correlation between the presence of storm drain flows and most of the FIB concentrations in the shoreline samples collected during this period. The exceptions were single sample and 30-day geometric mean results for enterococci. Ponding under the storm drain was positively correlated with exceedances and single sample results for fecal coliforms and enterococci. The minimal correlation between FIB levels and storm drain flows does not account for the possible intermittent discharges from the storm drain.

Water temperature, wind speed, and kelp on the sand were inversely correlated with exceedances, indicating that increased temperatures, wind speed, and kelp on the sand coincided with a reduced number of exceedances. For kelp, there was a significant inverse correlation observed for each FIB and the presence of kelp on the sand, meaning that more FIB were present in the water column when there was less kelp was on the sand. Although the amount of kelp in the water column was not indicated on the observation forms, it may be possible that the kelp gets re-suspended in the water column, carrying the FIB with it and causing exceedances of FIB standards.

The daily tide height range was positively correlated with exceedances and with the single sample results for fecal coliforms and enterococci. Daily tide height had a significant inverse correlation with the ratio of fecal to total coliforms ($p<<0.05$). These observations support the hypothesis of other recent studies that the FIB concentrations in the water column are being affected by the fluctuations in tide heights. Further, it appears that as the daily tide ranges increase, the ratio of the fecal to total coliforms decreases, suggesting that different compositions of bacteria are present in the water column at different times during the month. The significant positive correlation of exceedances and daily maximum tide height range suggests that exceedances are most likely to occur when the tide has a high fluctuation over a 24-hour period (Pearson correlation = 0.135, $p<0.05$, n= 305).

Water temperature was inversely correlated with exceedances and with all the FIB 30-day geometric means ($p<<0.05$). There was also an inverse correlation with single sample total and fecal coliforms and water temperature, suggesting that as the water temperature decreases, these FIB levels tend to increase. The relationship is likely due to the fact that water temperatures are lower during the winter wet weather period when there are increases in the number of FIB exceedances that occur.

Although surf heights were not quantitatively analyzed in this project, the Sanitation Districts did conduct an additional monitoring event during a high surf period in which no rainfall was recorded to help understand the relationship of FIB and surf. The FIB results for this additional sampling event supported these statistical analyses, as enterococci exceeded standards.

The field observations tracked along with FIB levels helped develop a better understanding about the complex relationship between the FIB and shoreline environmental factors, including wind speed, water temperature, tide height, presence of kelp on the sand, storm drain flows, and ponded water. Although some correlations were found to show statistical significance, the results, as a whole, were inconclusive in helping to identify the potential source of dry weather exceedances at Redondo Beach Pier shoreline locations, except for tide height.

Fecal Indicator Bacteria

Average FIB concentrations and the percent of samples above single sample standards for routine monitoring from December 2007 through December 2008 at the Redondo Beach Pier are presented in Table 9. The highest exceedance rate was observed at Site #3 (S-16), with 29% of samples exceeding single sample standards for this study period. Conversely, SMB-6-2a, which is collected at the S-16 monitoring location, had 8% of samples exceeding standards. The discrepancy between the data sets was observed throughout the study period.

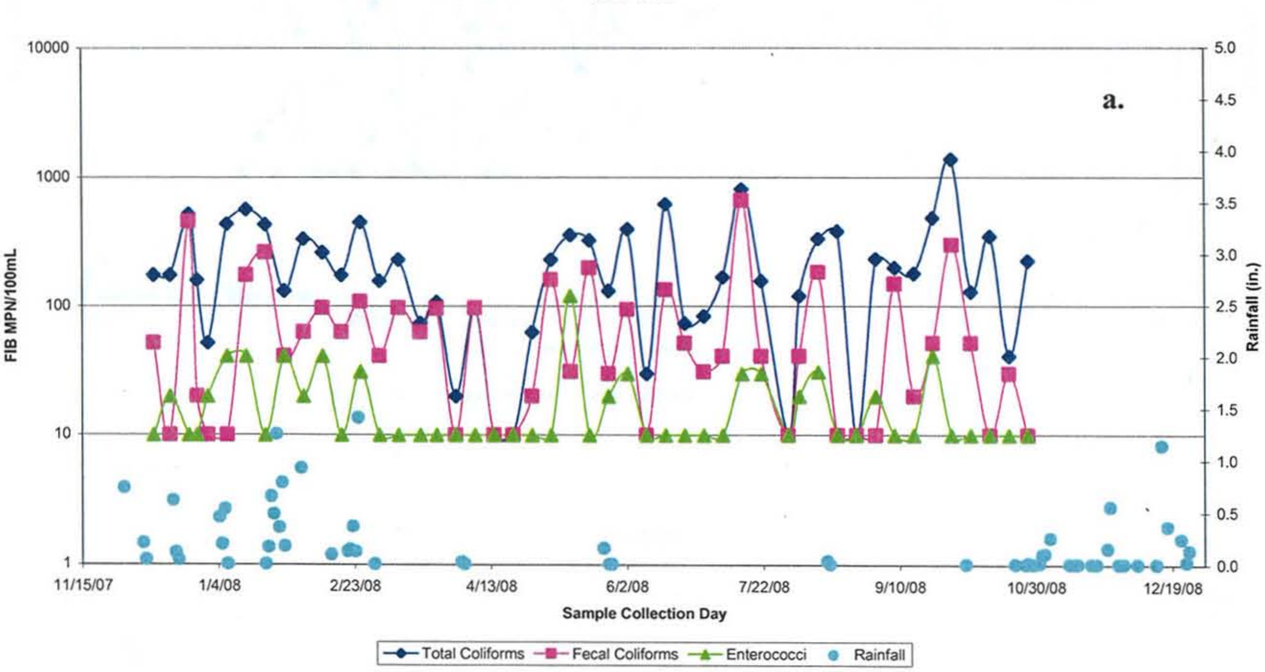
Table 9. Average FIB Concentrations at the Redondo Beach Pier Pilot Project Sampling Locations Collected December 2007 through December 2008 (Project sites are listed from south to north).

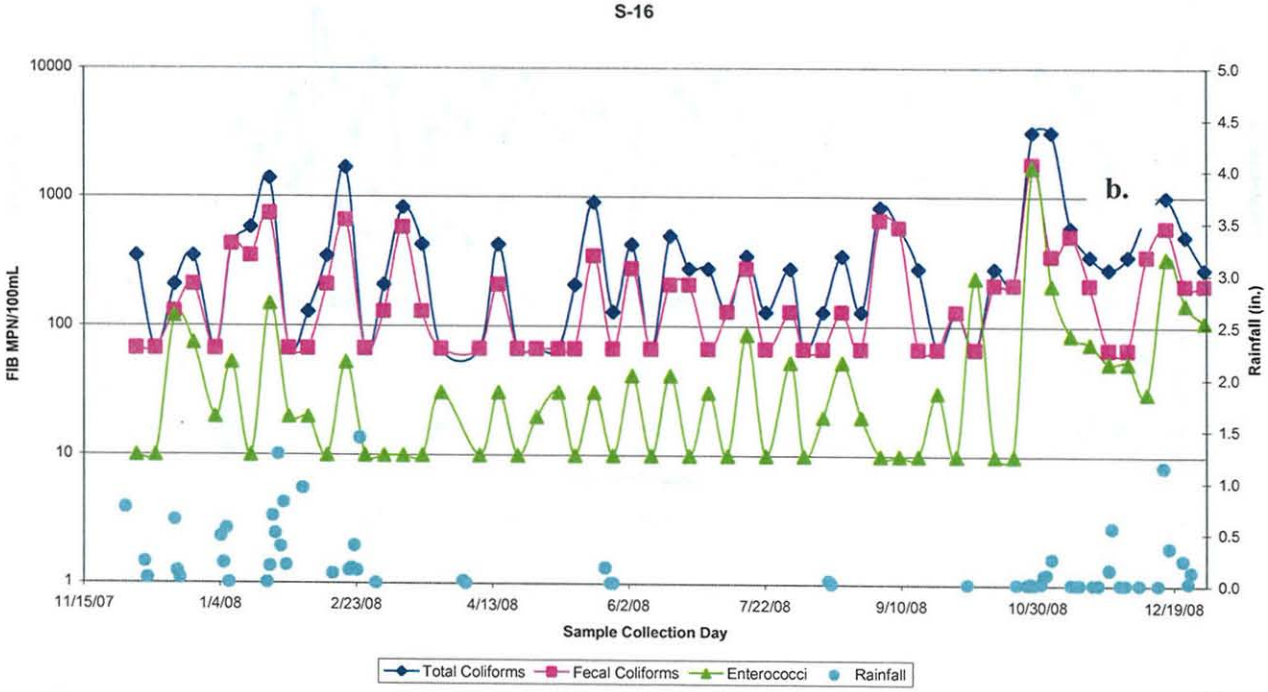
			Average FIB (MPN/100mL)			% Above
Project Site #	**Description**	**n**	**Total Coliform (SD)**	**Fecal Coliform (SD)**	**Enterococci (SD)**	**Single Sample Standards**
#1 (SMB-6-2)	Routine monitoring site in front of lifeguard tower 350 ft. south of Pier	59	162 (291)	34 (45)	33 (94)	7
#3 (S-16)*	Routine monitoring site <50 ft. from Pier on south side	295	741 (1710)	351 (625)	92 (254)	29
SMB-6-2a	<50 ft. from Pier on south side	48	253 (248)	87 (123)	19 (18)	8
SMB-6-2b	Under Pier in front of storm drain	48	526 (1034)	175 (290)	21 (16)	13
#4 SMB-6-2c	Under Old Tony's	49	363 (467)	144 (217)	28 (25)	8
	Single Sample Standard		**10,000****	**400**	**104**	

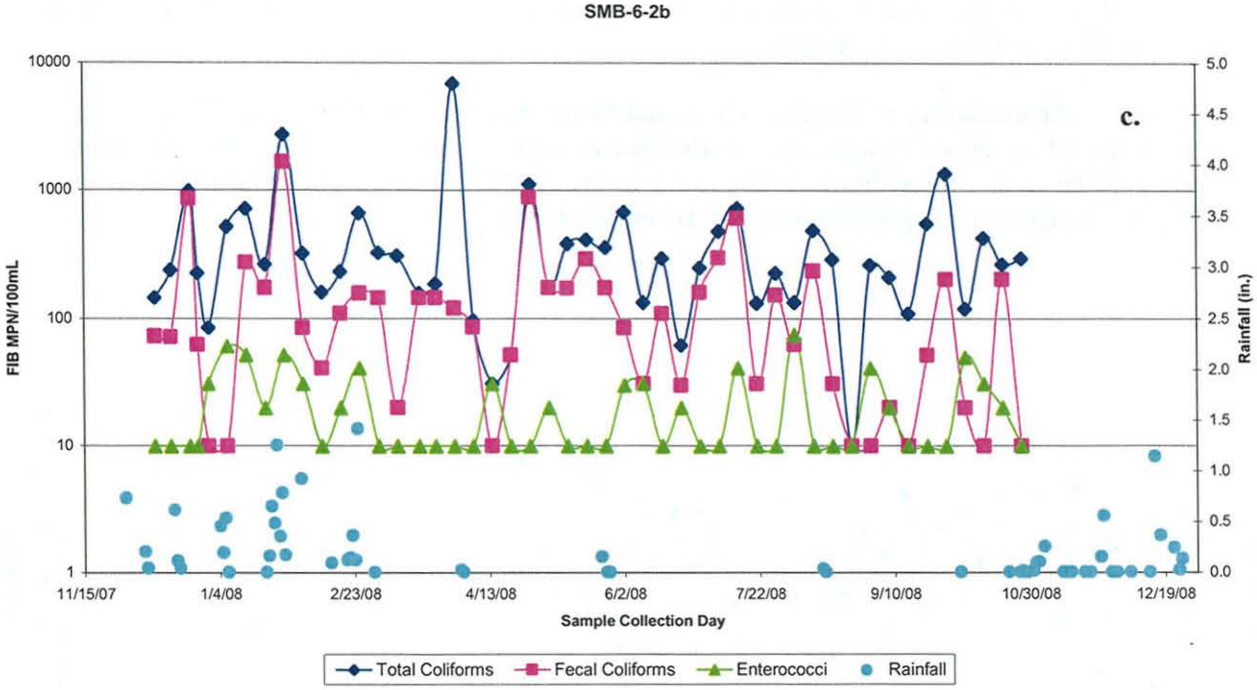
*Samples ware collected and analyzed at Site # S-16 daily on Tuesday through Saturday by the Hyperion Laboratory. Other sample locations ware collected and analyzed weekly on Monday by the Michelson Laboratory. **Total coliform density shall not exceed 1,000/100mL, if the ratio of fecal-to-total coliforms exceeds 0.1.

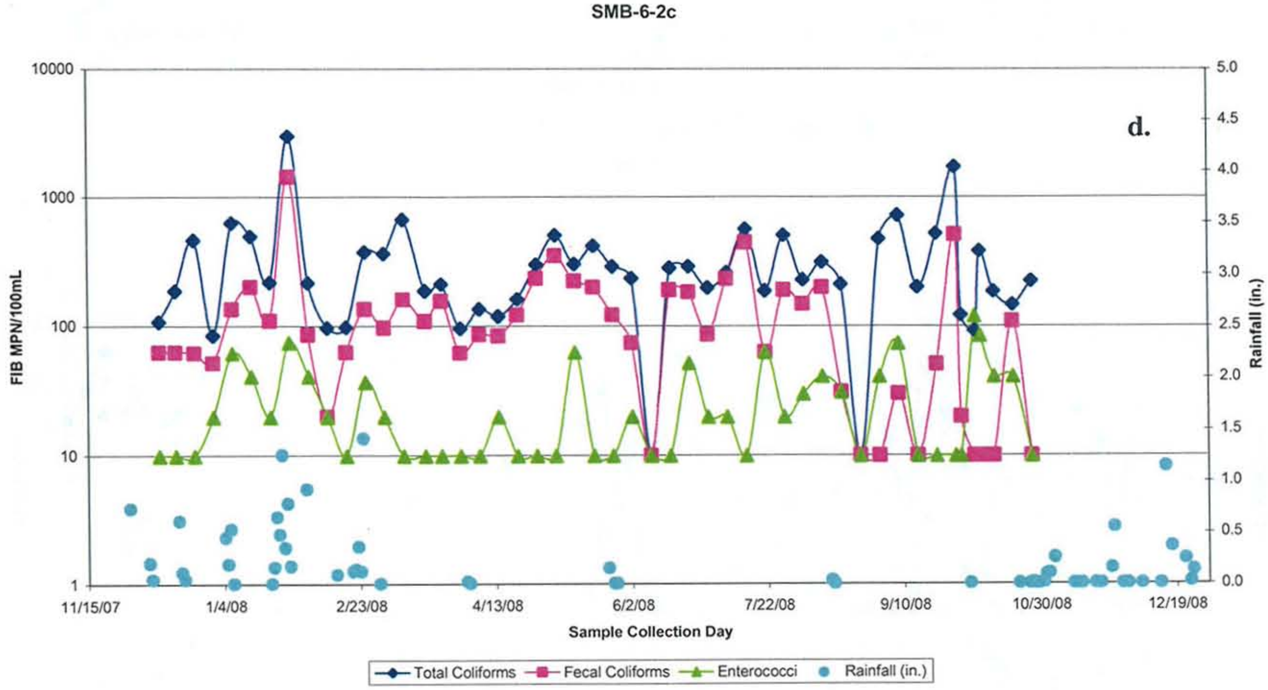
Fecal indicator bacteria data for each sample location for the period October 27, 2007 to December 31, 2008 are summarized in Figures 11a-e, along with rainfall data taken from the City of Redondo Beach website (www.redondo.org). Samples were collected for the SMB sites each week on Monday. Although samples were collected five days per week at S-16, only results from the Tuesday sampling at this site are presented in Figure 11b, to compare how the data trended on a weekly basis. In general, there were no distinct sustained FIB signals at any site during this study, even after rainfall events. There were substantial differences in the FIB averages, exceedance levels, and trends of FIB in samples from Sites S-16 and SMB-6-2a, which were collected at the same location, except at different days by different laboratories. These trends continued throughout the study.

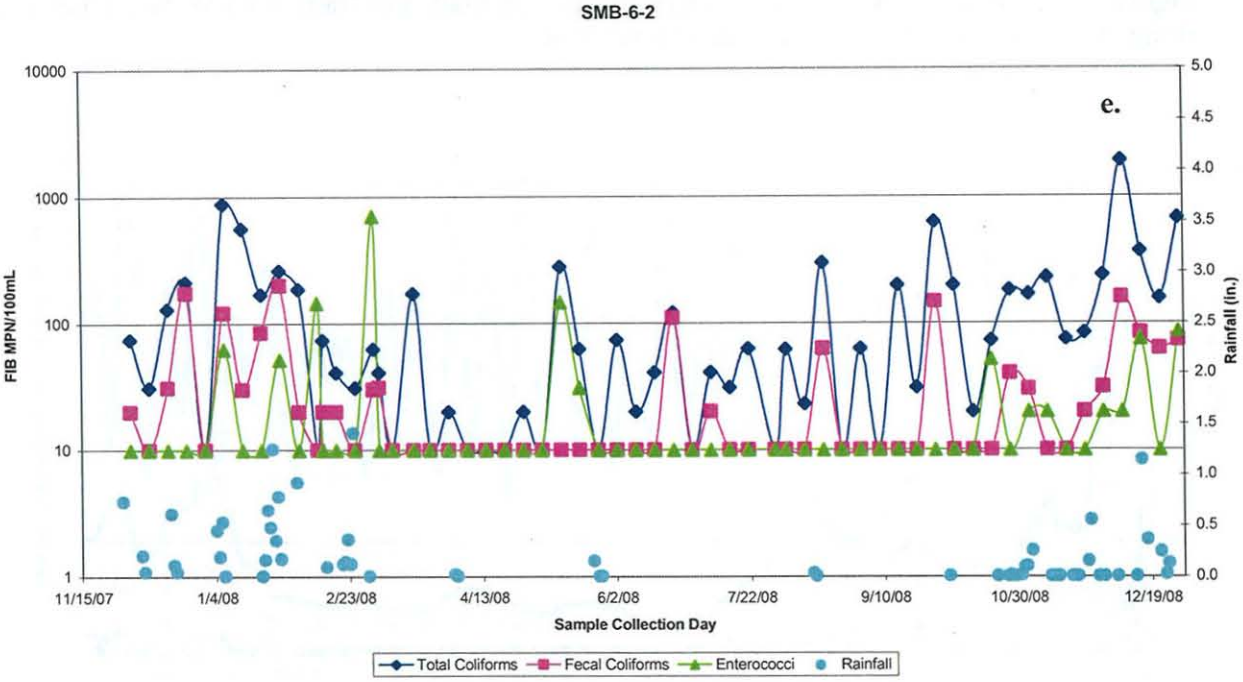
Figure 11. Redondo Beach Routine Microbial Water Quality Monitoring: a) SMB-6-2a <50 ft. from Pier on south side; b) S-16 Routine monitoring site <50 ft. from Pier on south side; c) SMB-6-2b Under Pier in front of storm drain; d) SMB-6-2c Under Old Tony's; e) SMB-6-2 In front of lifeguard tower 350 ft. south of Pier.






SMB-6-2b
c.
10000
1000
100
10
1
FIB MPN/100mL
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Rainfall (in.)
11/15/07
1/4/08
2/23/08
4/13/08
6/2/08
7/22/08
9/10/08
10/30/08
12/19/08
Sample Collection Day
Total Coliforms
Fecal Coliforms
Enterococci
Rainfall

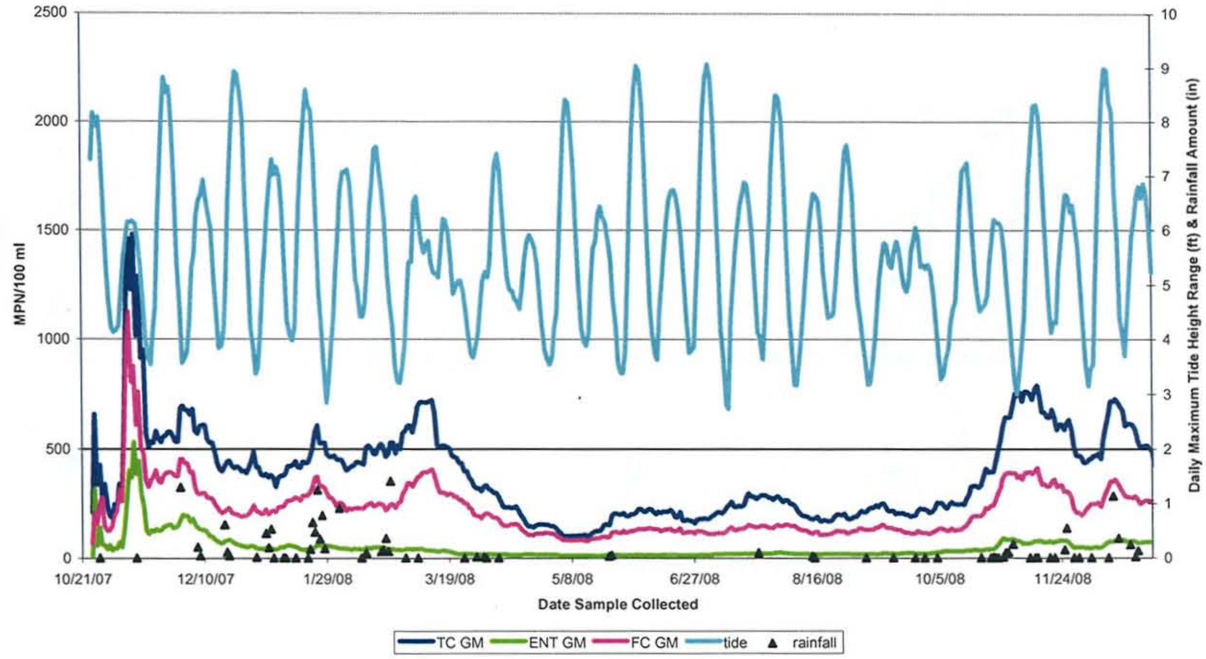

SMB-6-2c
d.
10000
1000
100
10
1
FIB MPN/100mL
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Rainfall (in.)
11/15/07
1/4/08
2/23/08
4/13/08
6/2/08
7/22/08
9/10/08
10/30/08
12/19/08
Sample Collection Day
Total Coliforms
Fecal Coliforms
Enterococci
Rainfall (in.)



As discussed in the Tier 1 section of this report, the FIB monitoring results appear to be related to tidal heights. The correlation of daily tide height and exceedances found during analysis of the field observation further suggests a relationship between tides and FIB. To better understand the relationship between tidal heights and FIB, an in-depth analysis of the routine FIB monitoring results for Site # 3 (S-16) was conducted.

Figure 12 provides a summary of rainfall, mean lower low water (MLLW) tide heights, and 30-day rolling geometric means for FIB reported during the Redondo Beach Pier SEP study period from October 2007 through December 2008. FIB concentrations began decreasing at the beginning of summer 2008 and remained low until the winter rainy season began in October 2008. Simultaneously, the tidal pattern exhibited a steady increase in tide heights that began at the apogee of the cycle that started in March 2008. The peak tide heights occurred during the summer solstice in June 2008 and were followed by a steady decline in tide height until October 2008, when the cycle began to repeat. When the rainy season began in October, FIB levels increased, probably due to increased rainfall. The mean FIB levels consistently followed the rainy season pattern, supporting the notion that exceedance rates during the winter months appear to be impacted by rain. This is significant because it could suggest that there is a normal annual fluctuation of FIB that can be predicted by the tidal cycle and rainfall patterns. A compilation of all the raw data is provided in the Appendix on the DVD, including single sample results, exceedance status, rolling 30-day geometric mean values, rainfall, daily tide changes, and water temperature.

Figure 12. Reported 30-Day FIB Geometric Mean, Rainfall, and Daily MLLW Tide Height Range for Site #3 (S-16) at the Redondo Beach Pier.



Redondo Beach Pier Cleaning Study

The effects of beach cleaning on the changes in FIB levels were found to be inconclusive and are summarized in Table 10. There were considerable improvements after beach cleaning for all the FIB parameters at Site #3 (S-16) when the beach was cleaned before the spring tide. This observation was supported by comparing the Sanitation Districts' and the Hyperion Laboratories results for Site #3 (S-16) samples collected on the same days. However, there were considerable increases in FIB levels after the beach was cleaned after the spring tide at Site #3. There was also an increase in FIB when cleaning was conducted before the spring tide at Site #4 (Under Old Tony's). The beach cleaning under Old Tony's improved FIB levels after the spring tide but all parameters were substantially worse when cleaning was conducted before the spring tide.

Table 10. Redondo Beach Fecal Indicator Bacteria Levels Before and After Beach Cleaning and November 24, 2008 Spring Tide.

	Beach Cleaning Effect				
Sample ID	**TC % Change (MF)**	**TC % Change (Colilert)**	**FC % Change (MF)**	***E. coli* % Change (Colilert)**	**ENT % Change (MF or Enterolert*)**
Site #3 (S-16) Before Spring Tide	**90%**	**88%**	**94%**	**89%**	**82%**
Site #3 (S-16) After Spring Tide	-5%	-92%	-27%	**30%**	-30%
Hyperion S-16 Before Spring Tide	NA	**30%**	NA	**42%**	**3%***
Hyperion S-16 After Spring Tide	NA	**54%**	NA	**>76%**	-335%*
Site #4 (Under Old Tony's) Before Spring Tide	-11%	-45%	-112%	-73%	-378%
Site #4 (Under Old Tony's) After Spring Tide	**63%**	-51%	**63%**	**68%**	**57%**

TC = Total coliform. FC = Fecal coliform. ENT = Enterococci. *Analysis conducted at the Hyperion Laboratory using the enzyme substrate method Enterolert. MF = Membrane filtration. NA = Not applicable. Hyperion Laboratory does not use MF methods. Results shown were routine analysis collected by Hyperion Laboratory. Red text = Increase in FIB level. **Green bold text** = Decrease in FIB level.

Observations at the Pier indicated that there was no standing water present under the Pier at either cleaning event. Marine debris and wrack lines were present and removed from under the Pier, and approximately three bags of marine debris were removed at both cleaning events. Different FIB trends were observed during the two events and at both sites. Since consistent trends were not observed, it appears that the cleaning event had minimal impact on FIB levels and water quality. In summary, the following was observed:

Site #3 (S-16):

- Some improvement after cleaning (15 analyses were better and 11 were worse);
- *E. coli* levels decreased after cleaning when cleaning occurred before and after the spring tide;
- FIB levels reported by the Sanitation Districts Laboratory were different than those reported by the Hyperion Laboratory;
- FIB changes calculated using Sanitation Districts data were different than changes calculated using Hyperion Laboratory Data, possible due to different sampling times;
- Vitek verifications showed that all fecal coliforms were *E. coli* and 33% of enterococci were *Enterococcus* spp.
- Exceedances were observed on November 20 (before cleaning) for all FIB (reported data from Hyperion and Districts' labs);
- Exceedances were observed on November 21 (after cleaning) for *E. coli* (Hyperion Lab only) and enterococci (reported data from Hyperion and Sanitation Districts' labs); and
- Exceedances were observed on November 27 and 28 (before and after cleaning, respectively) for enterococci (Sanitation District's Laboratory data, only).

Site #4 (Under Old Tony's):

- Compared to Site #3, a reverse trend was observed;
- All FIB levels increased after cleaning before the spring tide and some increases were substantial;
- Most FIB levels decreased after cleaning when cleaning occurred after the spring tide; and
- An exceedance was observed for enterococci after cleaning on November 28.

In general, there was no consistent improvement of the FIB parameters after beach cleaning before or after the spring tide at either location but there was some improvement, particularly for *E. coli.*

Tier 3. Advanced Source Identification

Tier 3 of this study used microbial source tracking techniques for advanced source identification. It was primarily conducted in the summer of 2008. The study initially focused on four locations: shoreline Site #3 (S-16, immediately south of the Redondo Beach Pier), shoreline Site SMB-6-2b (in front of the storm drain under the pier), the storm drain under the pier, and the pond formed by the storm drain under the pier. However, an additional sampling event was conducted in March 2009 to characterize the contribution of a ground water well believed to be a source of water for the storm drain.

All the microbial source tracking data from these efforts are included in the Appendix on a DVD.

Turbidity and Salinity Data

To better understand the relationship between the sites studied in the microbial source tracking effort, the turbidity and salinity of each site was characterized. These data are summarized in Table 11. The storm drain flows, pond, and ground water well exhibited high turbidity but low salinity. The shoreline samples consistently exhibited low turbidity and high salinity, which is typical for a marine location. Therefore, the ocean water was not influencing the ground well water, storm drain flows, and pond water during this study.

Table 11. Turbidity and Salinity of Samples Collected for the Summer 2008 Microbial Source Tracking Effort (values in parentheses indicate standard deviations).

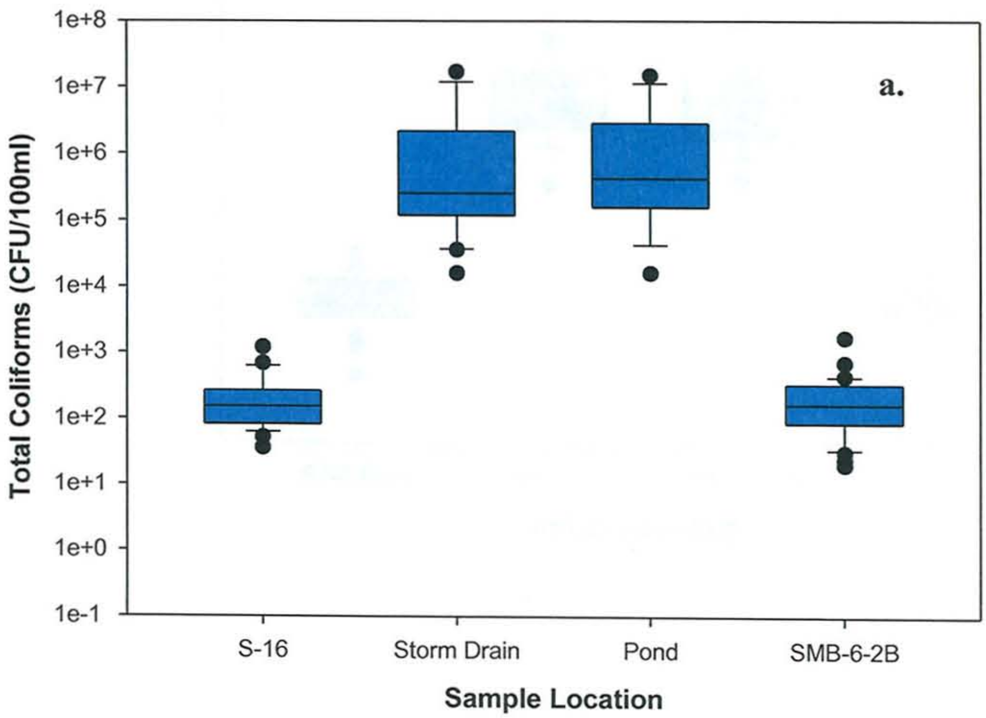
Sample	Turbidity (NTU)	Salinity
Ground Well	4.60	0.30
Pond	9.19 (6.06)	0.48 (0.05)
#3 (S-16)	2.04 (0.92)	31.72 (0.93)
SMB-6-2b	1.60 (0.79)	31.49 (0.82)
Storm Drain	7.71 (5.10)	0.52 (0.085)

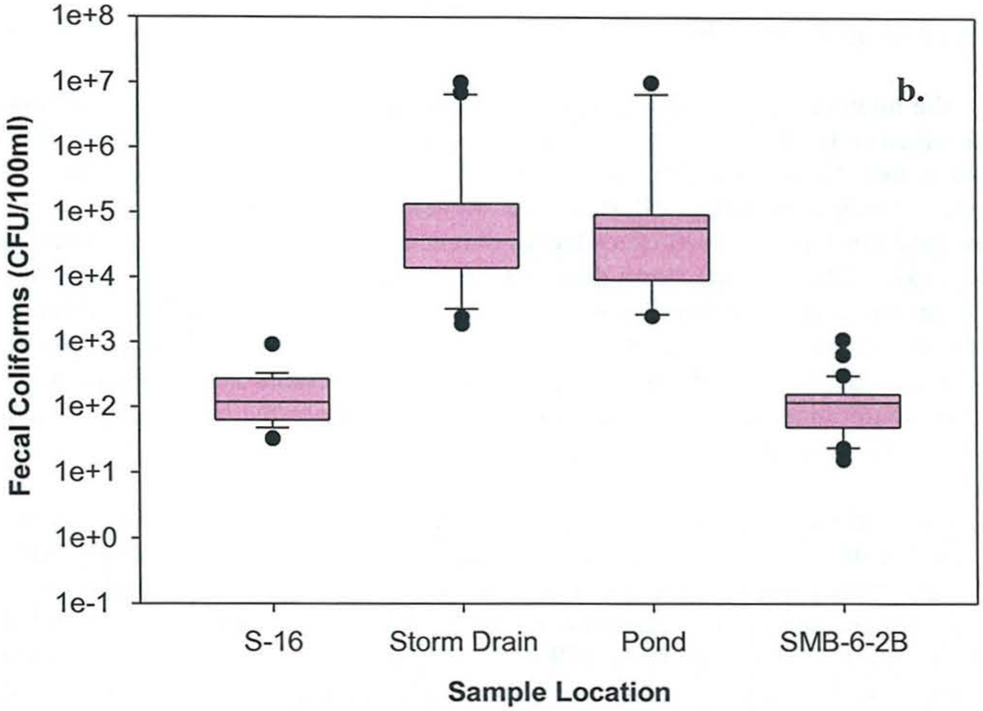
FIB Data Collected During the Microbial Source Tracking Effort

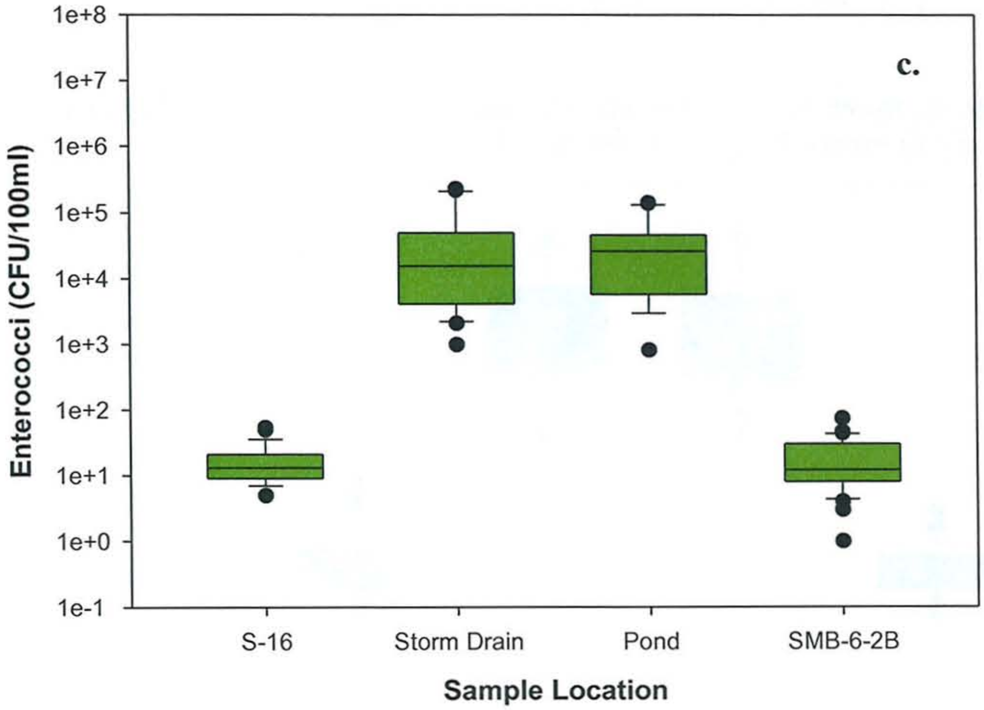
During the study, the microbial water quality of the shoreline samples collected was very good and the beach received a B grade for the summer by Heal the Bay (21). No unusual field observations were noted. There were four days with FIB exceedance during the 24 days of sampling over the 11-week sampling effort at the Redondo Beach Pier. Three of the exceedances were fecal coliform (*E. coli*) exceedances that occurred on consecutive days during an incoming spring tide. Although the storm drain was observed to flow on 18 of 24 sampling days, it was not observed to flow on three of the four days during which exceedances occurred. This suggests that the storm drain is not the primary source of shoreline FIB exceedances. Furthermore, the pond at the outlet of the storm drain did not flow overland into the ocean water during the microbial source tracking study, indicating that neither it nor the storm drain were a source of the shoreline FIB exceedances that occurred.

The FIB results associated with the microbial source tracking effort were compared by site for total coliforms, fecal coliforms, and enterococci, as shown in Figure 13a-c. There were substantially (>2 $\log_{10}$) more FIB present in the storm drain and pond samples compared to S-16 and the site immediately in front of the storm drain under the Pier (SMB-6-2b). Considering the turbidity and salinity results along with these FIB results, it appears that the pond and storm drain were not impacted by the ocean water nor were they impacting the ocean water. Results of the well sampling (data provided in the Appendix on the DVD) were within standards for FIB and had low levels of *Bacteroidales* spp., suggesting that the well was not contributing to the high FIB levels observed in the storm drain flows.

Figure 13. Redondo Beach Pier SEP Microbial Source Tracking Project: FIB Results: a) Total coliforms; b) Fecal coliforms; c) Enterococci.




b.
Fecal Coliforms (CFU/100ml)
1e+8
1e+7
1e+6
1e+5
1e+4
1e+3
1e+2
1e+1
1e+0
1e-1
S-16
Storm Drain
Pond
SMB-6-2B
Sample Location


c.
Enterococci (CFU/100ml)
1e+8
1e+7
1e+6
1e+5
1e+4
1e+3
1e+2
1e+1
1e+0
1e-1
S-16
Storm Drain
Pond
SMB-6-2B
Sample Location

FIB Speciation with Vitek

To evaluate the differences or similarities of the populations of bacteria detected in the culture methods for fecal coliforms and enterococci, a confirmation analysis on at least 10% of the detected colonies in each sample was conducted using Vitek. The majority (>99%) of fecal coliforms were identified as *E. coli* with only a few colonies that were not identifiable. Table 12 summarizes the distribution of the detected *Enterococcus* species for each site during the 2008 microbial source tracking effort. The "FIB *Enterococcus*" are thought to be predominantly associated with human feces and are composed of three species: *E. faecalis, E. faecium,* and *E. gallinarium.* However, these species can also be found in the feces of other animals including fish and crustaceans, as indicated in Table 12. Likewise, other *Enterococcus* species predominantly associated with different types of animal feces can also be found in human feces.

Enterococcus faecalis is the most dominant *Enterococcus* species found in human feces, with 85% of humans expected to harbor these organisms. All the monitoring sites analyzed in this study contained *E. faecalis* with the majority detected at SMB-6-2b. *Enterococcus casseliflavus* is known to be most often associated with plant material and was the dominant population in the pond. The most significant finding from this analysis was that there were different dominant populations of *Enterococcus* spp. detected in every site, suggesting that each site had its own unique bacterial population. More importantly, however, such diversity suggests that the bacteria detected in the storm drain and pond were not the source of the bacteria in the shoreline water samples.

Table 12. Distribution of *Enterococcus* spp. by Vitek Analysis for the Redondo Beach Pier SEP During the Summer 2008 Microbial Source Tracking Effort. (Bold values indicate the most dominant populations in the site.)

Vitek ID	Enterococci Distribution at Each Monitoring Site (%)				Associated Host Feces**
	S-16	SMB-6-2b	Storm Drain	Pond	
FIB *Enterococcus* spp*	43	77	61	45	
E. faecalis	20	**50**	32	27	Humans (85%), cows, dogs, pigs, fish, crustaceans, insects
E. faecium	**20**	18	10	15	Humans (25%), small ruminants, horses, pigs, fish, crustaceans, insects
E. gallinarum	3	9	**19**	3	Humans, birds, fish, crustaceans
E. hirae	**20**	14	3	9	Humans, dogs, cows, small ruminants, horses, pigs, birds.
E. durans	3	6	3	6	Humans, birds, cows, horses
E. casseliflavus	6	5	6	**15**	Humans, birds, cows, snails, insects, predominant on plants

Table 12 is continued on the next page.

Table 12 (Continued). Distribution of *Enterococcus* spp. by Vitek Analysis for the Redondo Beach Pier SEP During the Summer 2008 Microbial Source Tracking Effort. (**Bold** values indicate the most dominant populations in the site.)

Vitek ID	S-16	SMB-6-2b	Storm Drain	Pond	Associated Host Feces
E. columbae	0	0	3	0	Pigeons, dogs, cats
Staphylococcus spp.	0	0	0	3	
Streptococcus spp.	0	0	3	3	
Ambiguous	**23**	0	20	6	
Unidentified	0	0	0	**12**	

* FIB *Enterococcus* spp. include combined results for *E. faecalis, E. faecium*, and *E. gallinarium.*
** = www.bacterio.cict.fr/bacdico/ee/tenterococcusisolement.html (40).

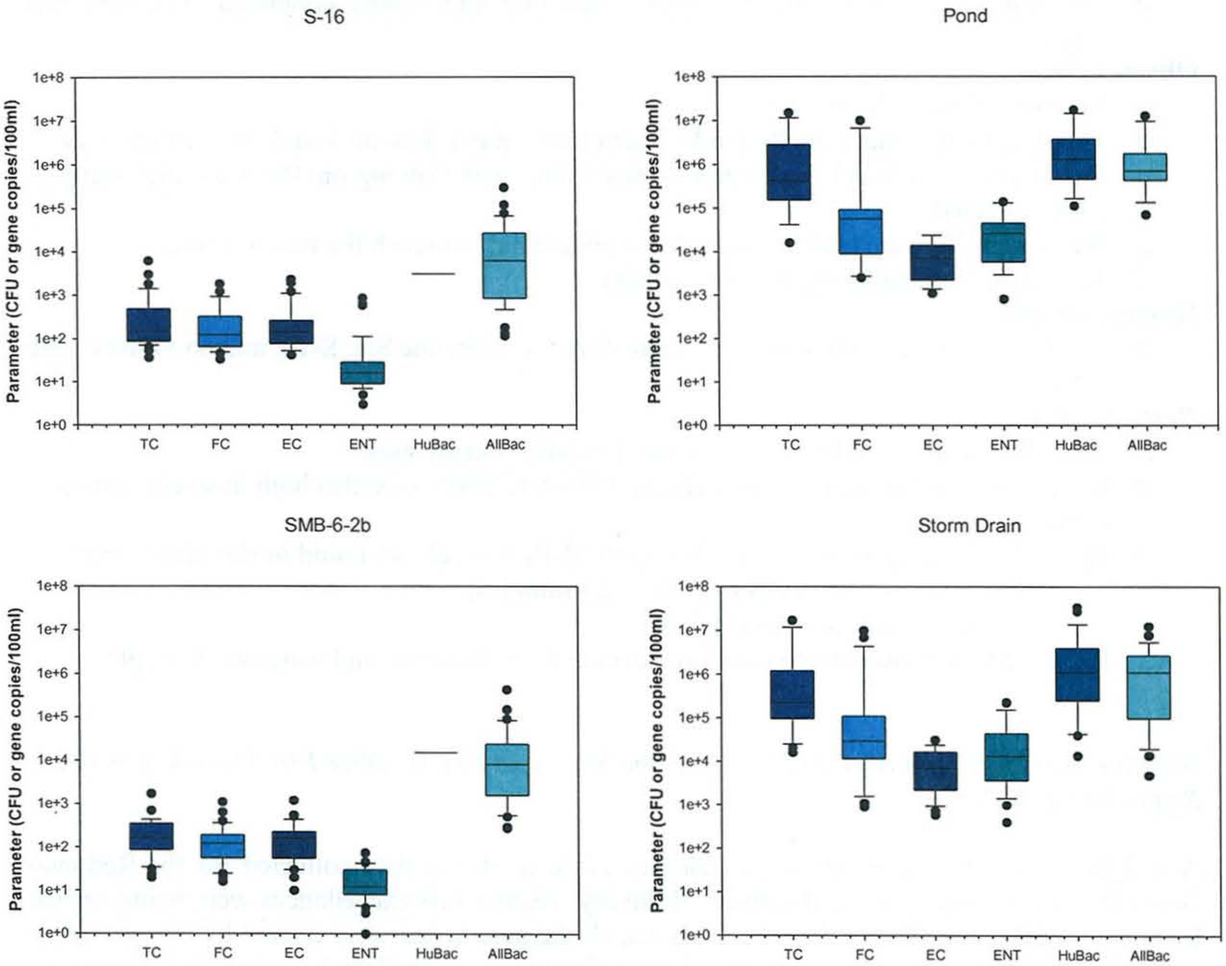
Bacteroidales Sampling Results

The total population of *Bacteroidales* (Bac_{TOT} or AllBac) and human-associated *Bacteroidales* (Bac_{HU} or HuBac) at each site are presented in Figure 14, along with data at each site for total coliform, fecal coliform, and enterococci.

Human-associated *Bacteroidales* were only detected in one of 24 samples, each, in the shoreline monitoring Sites #3 (S-16) and SMB 6-2b. However, they were ubiquitous and present at higher levels in all the samples collected from the pond and storm drain. Total *Bacteroidales* were detected at high levels in all sites. An examination of the melt curve analyses of the human-associated *Bacteroidales* indicated that a different population of human-associated *Bacteroidales* was present in the positive sample at SMB-6-2b than in the pond and storm drain.

The de minimus levels of human-associated *Bacteriodales* present in the shoreline monitoring sites suggest that the FIB present in the ocean water are not from human fecal pollution. The higher levels of human-associated *Bacteriodales* in the storm drain and pond suggest a source of human fecal pollution may contribute to FIB at these locations. The melt curve analysis of the SMB-6-2 human-associated *Bacteroidales* positive sample, as well as the differences in FIB populations from the storm drain and pond relative to the shoreline sampling locations, suggest that the storm drain and pond are not causing the FIB exceedances in the ocean water.

Figure 14. Relative FIB Levels at the Redondo Beach Pier During the Microbial Source Tracking Effort (June 24 through August 1, 2008).



Virus Sampling

The 10L samples collected and concentrated by HFF at Site #3 (S-16) were analyzed by qPCR for the presence of two viruses specific to humans, adenoviruses and enteroviruses. There were no detections of the viruses in any of the samples. This suggests that human sources are not contributing to exceedances of FIB standards at this site.

Microbial Source Tracking Effort Results Summary

The results of the microbial source tracking effort showed the following:

Bacteria

- No sustained exceedances were observed;

- Four FIB exceedance days of 24 days sampled;
- Lowest FIB exceedance rate observed at the Redondo Beach Pier since 1997;
- The storm drain under the Redondo Beach Pier and puddle contained very high FIB levels.

Observations

- No unusual field observations;
- The storm drain under the Redondo Beach Pier did not flow on 3 of 4 exceedance days;
- The storm drain under the Redondo Beach Pier was flowing on 18 of 24 days samples were collected.
- When samples were being collected, the puddle did not reach the beach water;
- The storm drain and pond had low salinity.

Human Viruses

- Enteroviruses and adenoviruses were analyzed at shoreline Site S-16, and no viruses were detected.

Bacteroidales

- Total *Bacteroidales* (Bac_{TOT}) DNA was ubiquitous in all sites;
- Human-associated *Bacteroidales* (Bac_{HU}) DNA (2 SNP) was also high in storm drain and pond;
- Human-associated *Bacteroidales* Bac_{HU} (0 SNP) was seldom found in shoreline samples
  - Detected once at SMB-6-2b (1 of 24 samples)
  - Detected once at S-16 (1 of 24).
- Bac_{HU} (2 SNP) was detected at a low level in the solitary ground water well sample.

Weight of Evidence Approach to Determine the Source of Dry Weather FIB Exceedances at the Redondo Beach Pier

A weight of evidence assessment was used to evaluate all the data collected for the Redondo Beach Pier Pilot Project and to determine if the dry weather FIB exceedances were being caused by human fecal input. To conduct the analyses, all the parameters were ranked by tier according to increasing specificity and likelihood of human fecal input, from Tier 1 routine FIB monitoring and observations to Tier 3, with the human associated bacteria and human virus analyses, as shown in Table 13.

Table 13. Multiple Lines of Evidence Assessment for the Redondo Beach Pier SEP Microbial Source Tracking Study: Summer 2008 Effort

Increasing specificity to human fecal input.

Toolbox Tier	Parameter Analyzed	Location				
		SMB-6-2	S-16	SMB-6-2b	Storm Drain	Pond
T1	Field Observations	-	-	-	++	++
T1	Total Coliforms	-	+	+	+++	+++
T1	Fecal Coliforms	-	+	+	+++	+++
T1	*E. coli*	-	+	+	+++	+++
T1	Enterococci	-	+	+	+++	+++
T2	Vitek speciation*	NA	43%	77%	60%	45%
T3	Bac_{TOT}	NA	++	++	+++	+++
T3	Bac_{HU}	NA	+**	+**	+++	+++
T3	Enterovirus	NA	-	NA	NA	NA
T3	Adenovirus	NA	-	NA	NA	NA
Impact of human fecal input?		Minimal	Minimal	Minimal	Probable	Probable

"-" = Analyzed but not detected.
"+" = Relative concentration or occurrences detected in water sample or observed (+ = low; ++ = medium; +++ = high).
NA = Not assessed.
* = FIB *Enterococcus* spp.
** = Detected in 1 of 24 samples analyzed.
*** = Not enough isolates were detected to perform the analyses.

The microbial source tracking results did not indicate there was a human source of fecal pollution at the shoreline monitoring locations immediately adjacent to the Redondo Beach Pier. The lack of detectable quantities of human viruses supports the conclusion that a human source of fecal pollution is unlikely. The de minimus presence of human-associated bacteria (*Bacteroidales*) in the ocean water, with only two of 24 samples containing detectable quantities, also supports this conclusion, as does the melt curve analyses of *Bacteroidales*, which indicated that the human-associated *Bacteroidales* were not from the same population in the ocean water as in the storm drain and pond. Since the human-associated *Bacteroidales* can also be associated with certain animals, the low frequency of detection in the ocean water, in combination with different populations of FIB and *Bacteroidales* in the ocean water as compared to the storm drain and pond, supports the conclusion that the shoreline dry weather fecal indicator bacteria exceedances were not of human origin.

Results also indicate that the storm drain and pond are probably impacted by human fecal pollution but are not contributing to microbial contamination of the ocean water. For example, the two shoreline samples that were positive for human-associated bacteria (*Bacteroidales*) did not contain the same species that were consistently detected in the storm drain and pond. However, since the storm drain is a source of fecal indicator bacteria and potentially pathogens, the City of Redondo Beach has been investigating it.

An important question not addressed by this study is how long viable *Bacteroidales* spp. and DNA can persist in the sand, kelp, and storm drain. The *Bacteroidales* method for the human-associated species was selected for the MST effort because previous research suggested they were the most closely associated with human feces, and as obligate anaerobic bacteria, would not survive for extended periods in the environment. However, survival of viable bacteria and the DNA in storm drains, biofilms, sand, kelp, sewage, and other reservoirs is not well characterized and should be determined to support the conclusions of probable human fecal input when the $Bac_{(HU)}$ DNA is detected.

The Sanitation Districts compiled and reviewed the data collected in the summer microbial source tracking effort and presented the results to the Technical Workgroup on June 3, 2009. The presentation and draft meeting minutes are included in the Appendix on the DVD. The Technical Workgroup agreed with the conclusions of the data analysis.

Tier 4. Abatement

The Redondo Beach Pier SEP included examination of potential measures to abate bacterial exceedances. Potential abatement measures based on results from the study were discussed with the Technical Workgroup for the project at a meeting on June 3, 2009. Additionally, the Sanitation Districts held an Abatement Plan Scoping Meeting open to all project stakeholders on August 12, 2009. Notes and presentations from both meetings are included in the Appendix to this report (on DVD).

Prior to initiation of this project, the City of Redondo Beach had already implemented a number of mitigation measures to reduce the frequency of FIB standards exceedances. These efforts began in 2003, through the Redondo Beach Clean Waterfront Plan Water Quality Task Force. Measures implemented included installation of a fish cleaning station, replacement of the sewer lines under the pier, purchase of trash enclosures, purchase of new covered trash cans, prohibition of pier washing from dripping off the pier, and installation of a low flow diversion at the Torrance Circle storm drain.

In addition to these efforts already in place, the Technical Workgroup and the project stakeholders discussed several ideas for further abatement. The ideas are listed below. It is recommended that all three of these abatement measures be pursued.

1. **Complete the storm drain investigation.** Although overland flow of the storm drain to the ocean was not observed during the MST portion of the study, the storm drain is a source of FIB and potentially pathogens. The City of Redondo Beach has already begun addressing concerns identified by the Redondo Beach Pier SEP relating to the storm drain under the pier. The City of Redondo Beach conducted an extensive investigation of the storm drain system, including televising the lines. The City is considering several other

options, including further investigation of the operations of the well pump, sampling the CDS unit, and/or hiring a sewer-sniffing dog.

2. **Expand beach maintenance program.** Beaches and Harbors already grooms the beach up to the mean high tide line and next to the pier but it does not conduct beach maintenance under the pier. The City of Redondo Beach plans to expand the maintenance program to include regular trash and kelp removal under the pier.

3. **Update the permanent sign posted next to the Redondo Beach Pier.** The City of Redondo Beach already has a warning sign next to the pier that prohibits swimming under the pier due to the dangerous currents. The City plans to replace the sign with another permanent warning sign that includes a warning about the storm drain under the pier.

CONCLUSIONS

The specific source of the dry weather FIB exceedances at the shoreline monitoring locations south of the Redondo Beach Pier could not be determined during this study. The study did indicate that the highest rate of FIB exceedances occurred in the vicinity of the Redondo Beach Pier, suggesting that there was a potential source of FIB exceedances associated with the pier area. The microbial source tracking segment of the study indicated that it is unlikely that a human source of fecal pollution is causing the exceedances of bacterial standards. Tide, wave action, wind, and other natural fluctuations affect the frequency of dry weather FIB standards exceedances observed near the pier, suggesting that these natural occurrences result in the FIB migrating into the ocean from the sand and kelp. Furthermore, the lack of detectable quantities of human viruses and the de minimus quantities detection of human-associated *Bacteridales* in the ocean water strongly implied that a human source is not present or too low for detection by the qPCR methods. However, considering that the MST efforts were conducted during a period of low FIB exceedences, these conclusions are limited to the period of the study. Results should not be extrapolated to describe sources during periods of high FIB exceedences. In such an event, additional accelerated monitoring should be used to investigate potential sources.

The study also indicated that the storm drain under the pier and the pond that forms at the storm drain outlet are probably impacted by human fecal pollution but are not contributing to microbial contamination of the ocean water during the dry season. This conclusion is most strongly supported by the differences in the FIB concentrations and populations of the shoreline samples compared to the pond and storm drain samples, particularly with respect to human-associated *Bacteroidales*. Furthermore, there was no evidence of intermittent storm drain discharges reaching the ocean during the summer 2008 MST efforts, as a pond at the outlet of the storm drain prevented overland flow.

In summary, the results of this project support the conclusion that the source of dry weather FIB exceedances at the Redondo Beach Pier could be:

- Persistence of FIB in the sand;
- Physical parameters such as wind, wave, tide height, and kelp on the sand; and
- Association with the pier (aka "pier effect").

PROPOSED ABATEMENT PLAN

The following items are recommended to address bacterial exceedances in the vicinity of the Redondo Beach Pier:

1. **Complete the storm drain investigation.** The City of Redondo Beach has already begun addressing concerns identified by the Redondo Beach Pier SEP relating to the storm drain under the pier. The City conducted an extensive investigation of the storm drain system, including televising the lines. The City is considering several other options, including further investigation of the operations of the well pump, sampling the CDS unit, and/or hiring a sewer-sniffing dog. It is recommended that the City complete its investigation.

2. **Expand beach maintenance program.** Beaches and Harbors already grooms the beach up to the mean high tide line and next to the pier but it does not conduct beach maintenance under the pier. It is recommended that the City of Redondo Beach expand the maintenance program to include a regular schedule of trash and kelp removal under the pier.

3. **Update the permanent sign posted next to the Redondo Beach Pier.** The City of Redondo Beach already has a warning sign next to the pier that prohibits swimming under the pier due to the dangerous currents. It is recommended that the City replace the sign with another permanent warning sign that includes a warning about the storm drain under the pier.

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APPENDIX

Contents of DVD include the following:

- Redondo Beach Pier SEP Work Plan
- Field Observations:
  - Observation Forms
  - Observation Form Instructions
  - Observation Data
  - Field Observation Data Summary
- Photos
- Study results:
  - Tier 1 Historical Data Analysis
  - Tier 2 Accelerated Monitoring
  - Tier 3 Microbial Source Tracking
  - March 10, 2009 Follow-up Study
  - Intercalibration Study
  - Beach Clean Up
- Presentations:
  - Technical Workgroup Meeting October 30, 2007
  - Redondo Beach Clean Waterfront Poster October 10, 2008
  - Technical Workgroup Meeting June 4, 2009
  - Abatement Plan Scoping Meeting November 12, 2009
- SOPs:
  - Vitek 2
  - *Bacteroidales* (Bac_{TOT}) qPCR
  - *Bacteroidales* (Bac_{HU}) Qpcr
  - Adenovirus qPCR
  - Enterovirus RT-qPCR

Slifko, Terri

From: Amanda Griesbach [agriesbach@healthebay.org]
Sent: Monday, January 11, 2010 11:39 AM
To: Slifko, Terri; rpurdy@waterboards.ca.gov; bbrand@earthlink.net
Cc: Mark Gold; Kirsten James
Subject: Redondo Beach Abatement Comments (HtB)

Attached are general comments concerning the Redondo Beach Pier abatement project. Overall, Heal the Bay is very impressed with the research and time LA county has put into this project. We want to express concern that beach water is still polluted, and intermittent discharges by the drain during dry weather are unacceptable.

We would like to thank LA County Sanitation District for the opportunity to comment on such an important issue, and their thorough investigation in this project.

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Be a part of Heal the Bay's online community: join us on Facebook or follow us on Twitter

Model Program for Bacterial Source Identification And Abatement Plan— Redondo Beach Pier Pilot Project

Final Report and Abatement Plan

(Heal the Bay's comments)

- Heal the Bay agrees with the recommended items in order to better address bacterial exceedances.
  - Complete storm drain investigation
  - Expand beach maintenance program
  - Update the permanent sign posted next to the Redondo Beach Pier

**Emphasis on investigation of storm drain, due to inconclusive results regarding source of FIB exceedances.

- Further investigation regarding the storm drain underneath the pier is essential.
  - Field observations of storm drain (under the pier) were conducted at various times of the day (p27 & 33). Observations were only taken once a day and not during the night. This leaves a large window of opportunity for intermittent discharge from the drain.
  - On October 30, 2007, Heal the Bay staff inspected the area under the pier and reported standing water, marine debris, and a strong odor coming from the storm drain.
  - Table 8 (p43), shows minimal correlation between FIB (S-16) and the storm drain flowing. This observation and lack of correlation does not account for possible intermittent discharge.
  - An additional investigation surrounding the storm drain (under the pier) including further up the watershed, from which this drain flows, is crucial in ruling out the possibility of contaminated irregular discharge.
- Additional comments
  - Page 34, states an additional set of samples were collected on March 10, 2009, in which ground water was determined to be a source of water flowing from the storm drain (under the pier). These data are not included in the draft report.