Storm Event Shoreline Microbiology

SOUTHERN CALIFORNIA BIGHT
1998 REGIONAL MONITORING
PROGRAM
Vol . III

Southern California Bight 1998 Regional Monitoring Program
Volume III.: Storm Event Shoreline Microbiology

Rachel T. Noble[1,2]
Molly K. Leecaster[1]
Charles D. McGee[3]
Douglas F. Moore[4]
Victoria Orozco-Borbón[5]
Ken Schiff[1]
Patricia Vainik[6]
Stephen B. Weisberg[1]

[1]Southern California Coastal Water Research Project
7171 Fenwick Lane
Westminster, CA 92683

[2]USC Wrigley Institute for Environmental Studies
AHF 107, University Park
Los Angeles, CA 90089-0371

[3]Orange County Sanitation District
10844 Ellis Avenue
Fountain Valley, CA 92708

[4]Orange County Public Health Laboratory
1729 W. 17th Street
Santa Ana, CA 92706

[5]Instituto de Investigaciones Oceanológicas
Universidad Autónoma de Baja California
Km. 103 Carretera Tijuana-Ensenada
Ensenada, México

[6]City of San Diego
Metropolitan Wastewater Department
4918 North Harbor Drive
San Diego, CA 92106

MEMBERS OF THE MICROBIOLOGY COMMITTEE

Amador, Ric	City of San Diego
Anson, Nancy	Encina Wastewater Authority
Branch, Nicki	San Elijo Joint Powers Authority
Cook, Marsha	San Diego County Department of Environmental Health
Cressey, Ron	City of Los Angeles Environmental Monitoring Division
Dorsey, John (Chair)	City of Los Angeles Stormwater Division
Flores-Báez, Bernardo	Instituto de Investigaciones Oceanológicas, UABC, México
Gallagher, Robert	Ventura County Environmental Health Division
Gonzales, Mary	City of Oceanside
Gregorio, Dominic	Southern California Marine Institute
Groves, Susan	City of Santa Barbara
Harley, Ann	Aliso Water Management Agency/South East Regional Reclamation Authority
Jay, Florence	City of Ventura Wastewater Treatment Plant
Jiang, Sunny	University of California at Irvine, Department of Social Ecology
Lehner, Jim	Los Angeles County Sanitation Districts
Lyons, Michael	Los Angeles Regional Water Quality Control Board
Mazur, Monica	Orange County Environmental Health Division
McGee, Charles (Co-Chair)	Orange County Sanitation District
Meehan, John	City of Oxnard
Moore, Charles	Algalita Marine Research Foundation
Moore, Douglas	Orange County Public Health Laboratory
Nakauchi, Steve	City of Long Beach Department of Health & Human Services
Nikaido, Mae	City of Long Beach Department of Health & Human Services
Noble, Rachel	USC Wrigley Institute for Environmental Studies
O'Connell, Linda	State Water Resources Control Board
Orozco-Borbón, Victoria	Instituto de Investigaciones Oceanológicas, UABC, México
Ornelas-Pérez, Arturo	Instituto de Investigaciones Oceanológicas, UABC, México
Peters, Greg	San Diego Regional Water Quality Control Board
Petralia, Jack	Los Angeles County Department of Health Services
Plana, Jennifer	City of Santa Barbara
Ramirez, Phil	Aquatic Bioassay and Consulting
Reid, Dan	Santa Barbara County Health Service
Rico-Mora, Roxana	Facultad de Ciencias Marinas, UABC, México
Schulz, Don	Surfrider Foundation
Vainik, Patty	City of San Diego
Walker, Kathy	Los Angeles County Sanitation Districts
Weisberg, Steve	Southern California Coastal Water Research Project
Werner, Kathleen	Goleta Sanitation District

FOREWORD

This study was coordinated by the Southern California Coastal Water Research Project (SCCWRP) as a complement to the Summer and Winter Shoreline Microbiology studies. This study used the same approach as the previous studies to facilitate comparison among the three shoreline microbiology studies. Copies of this and other shoreline microbiology reports are available for download at www.sccwrp.org.

The proper citation for this report is: Noble, R.T., M.K. Leecaster, C. D. McGee, D.F. Moore, V. Orozco-Borbon, K. Schiff, P.M. Vainik, and S.B. Weisberg. 2000. Southern California Bight Regional Monitoring Program: Storm Event Shoreline Microbiology. Southern California Coastal Water Research Project. Westminster, CA.

ACKNOWLEDGEMENTS

This report is the culmination of many hours of hard work by the dedicated individuals at the 36 organizations that participated in the Storm Event Shoreline Microbiology Study. Although the report was prepared by a subset of the Microbiology Committee, we would like to thank all members of the Committee, who represented their organizations admirably and displayed a spirit of cooperation throughout the planning and implementation of this effort. We would also like to thank Larry Cooper for his creation of the microbiology data entry program and his maintenance of the microbiology database; and Scott King and Idexx Laboratories, Inc., for providing the supplies necessary to perform the method comparison study. We would also like to thank the following organizations for providing volunteers to help with sample collection: the Huntington Beach, Long Beach, and South Bay Chapters of the Surfrider Foundation; the Algalita Marine Research Foundation (AMRF); the Cabrillo Marine Aquarium, Divers Involved in Environmental Rehabilitation & Safety (D.I.V.E.R.S.); and the Los Alamitos High School Marine Science Club.

Definition of Terms

Chromogenic substrate method - Method used for the detection of indicator bacteria that is based upon the release of a fluorogen by metabolism of a nutrient-indicator substrate by members of the indicator bacteria group of interest.
Enterococci - Subgroup of the fecal streptococci that includes *Enterococcus faecalis, Enterococcus faecium, Enterococcus gallinarum,* and *Enterococcus avium.*
Ephemeral freshwater outlet - Outlet that typically only flows for a portion of the year, not year-round.
***Escherichia coli* -** Organism that is the predominant component of the fecal coliform group.
Exceedence - Bacterial indicator level that is above a pre-defined threshold.
Fecal coliforms - Subset of the total coliform group that is of fecal origin. Fecal coliforms have the ability to ferment carbohydrates at 44.5° C within 24 h while the rest of the total coliform group do not.
Freshwater outlet - Natural or constructed freshwater source associated with multiple land use types (urban, rural, agricultural, industrial).
Indicator - Bacterial group used to infer microbiological water quality. The three indicators used in this study were total coliforms, fecal coliforms/*E. coli*, and enterococci. See Table II-4.
Membrane filtration (MF) - Method used to determine the presence of specific bacterial organisms by filtering a water sample through a membrane (usually 0.45 µm) and incubating the membrane on media that selects for the organism of interest. Permits a direct count of colonies, and therefore direct enumeration.
Multiple tube fermentation (MTF) - Method used to determine the presence of specific bacterial organisms based upon fermentation of a sugar and subsequent gas production. Results are reported in the form of the most probable number index.
Objective - Limits or levels of water quality characteristics for ocean waters to ensure the reasonable protection of beneficial uses and the prevention of a nuisance as determined by the California Ocean Plan. Refers to bacteriological indicator levels. See Table II-4.
Perennial freshwater outlet - Natural or constructed freshwater source that typically produces measurable and observable flows year-round.
Point zero sample – For the purposes of this study, a sample that was taken at the mouth of a freshwater outlet, at the location of surfzone-freshwater mixing.
Random sample - In this study, a sample that was taken at a random location within 100 yards of the mouth of a freshwater outlet.
Standard - Water quality measurement (characteristic) for ocean waters set by State of California statute and regulations; e.g., Assembly Bill 411, which refers to bacteriological indicator levels. See Table II-4.
Storm drain – Constructed subset of the freshwater outlets that generally does not have a main source from riverine or creek freshwater inputs, rather its source is primarily stormwater (from storm events) and its runoff is contributed mainly to the coastal environment.
Threshold - Any bacterial indicator level determined by state, local, or federal standards; proposed standards; or ocean water quality objectives. See Table II-4.

Total coliforms - Group of bacteria that is aerobic and facultative anaerobic, gram-negative, nonspore-forming, rod-shaped bacteria that ferment lactose with gas formation within 48 h at 35° C.

Urban runoff - Runoff from a freshwater outlet or storm drain whose watershed is primarily an urban land use area.

Water quality – For the purposes of this report, water quality of a microbiological nature.

Executive Summary

The coastal waters of the Southern California Bight (SCB) are the most intensively monitored in the United States, with as many bacterial samples collected in this area as the rest of the country combined. Despite the large sampling effort conducted in the SCB, the resulting data are difficult to integrate into a regional assessment of bacteriological water quality because most sampling effort is allocated to public-interest areas, such as well-used beaches or sites of concern near sewage and storm drain infrastructure. Additionally, these data are collected by 22 different organizations that use different laboratory methods and maintain independent data management systems.

To address these limitations, all of the organizations that conduct routine monitoring in the SCB pooled their efforts to conduct an integrated survey of overall microbiological water quality along the southern California shoreline in August of 1998. To address the historical bias toward known or suspected "problem areas," the Bight '98 Summer Shoreline Microbiology Study used a stratified random sampling design. A follow-on Winter Study conducted in February and March of 1999 similarly measured bight-wide bacterial water quality during the traditionally wettest months in southern California. While the winter survey successfully presented a region-wide, integrated picture of shoreline water quality, the absence of significant rain events during the sampling period thwarted a primary objective of the survey, to characterize water quality during wet weather.

The organizations that participated in the prior surveys conducted a third survey in the late winter of 2000 to capture beach water quality data during a storm event. The goals of the –Storm Event study were to:

- Determine the percentage of shoreline miles along the SCB that exceeded bacterial standards set forth in Assembly Bill 411 during a storm event.

- Compare the responses among the four bacterial indicator thresholds used to assess beach water quality in California.

- Compare the analytical results from standard methods with those of the new chromogenic substrate method.

Samples were collected from 251 sites between Point Conception, California, and Punta Banda, Mexico. These were the same sites used in the two previous surveys and were selected using a stratified random design, with sandy beaches, rocky shoreline, and areas adjacent to freshwater outlets as the strata. Sampling took place on February 20, 2000, approximately 36 h after a rainstorm that produced at least one inch of rain throughout the SCB. Samples were analyzed for total coliforms, fecal coliforms (or *E. coli*), and enterococci using standard methods.

Fifty-eight percent of the shoreline waters were found to exceed water quality standards during this study, which was almost 10 times that found to exceed water quality standards during the dry summer period. Moreover, the magnitude of the exceedences was much greater during wet-weather conditions. In the summer, more than two-thirds of the water quality exceedences were attributable to a single bacterial indicator, and most of those exceedences were barely above the indicator threshold. During wet–weather conditions, two-thirds of the exceedences were for to multiple indicators, where at least one indicator was measured at a level that was twice the allowable standard. Coastal waters in areas close to freshwater outlets had the worst water quality during all three studies. During dry-weather conditions, the water adjacent to 60% of the freshwater outlets had poor quality; this number rose to 90% following the storm.

A much higher degree of consistency was found among the threshold exceedence by the bacterial indicators following a storm event than during dry-weather studies. During the dry–weather studies, almost 80% of water quality threshold exceedences involved only one indicator, whereas following the storm nearly half of the threshold exceedences involved all of the indicators. In both wet–weather and dry–weather conditions, the number of exceedences attributable to enterococci was much higher than either total coliforms or fecal coliforms. The concentrations of all three bacterial indicators (total coliforms, fecal coliforms, and enterococci) correlated strongly, suggesting that the different levels of sensitivity among indicators reflect their different thresholds, with the enterococci threshold being the most conservative.

The laboratories participating in this study used either membrane filtration (MF) or multiple tube fermentation (MTF) to process the bacterial samples, which are standard analytical methods certified by the State of California and the U.S. Environmental Protection Agency (U.S. EPA). A subset of laboratories also analyzed the samples using a second method, a commercial application of the chromogenic substrate method produced by Idexx Laboratories, Inc. Previous tests conducted in southern California have shown that this method yields results comparable to the conventional methods currently in use in laboratories that perform routine monitoring. This study was the first to compare these methods in side-by-side field tests conducted during wet–weather conditions. The results produced by the new method were comparable to those from both of the conventional methods.

I. INTRODUCTION

The Southern California Bight (SCB) is noted for its shoreline and beaches, which attract an estimated 175 million visitors annually (USLA 1998). The intensity of this beach usage exceeds any other state in the nation, including Florida, New Jersey, or Hawaii. Ocean-related activities generate an estimated $9 billion in revenues each year, the bulk of which come from tourism. As a result of this high usage by the public, local agencies spend considerable effort monitoring the bathing water quality in the SCB. More than $3 million is spent collectively evaluating the water quality of beaches to protect the public health as it relates to water-contact recreation.

Shoreline monitoring has shown that areas in southern California beaches are impacted by human activities, particularly beaches adjacent to densely populated urban centers. A major source of stress to the environment resulting from human activity in these areas is non-point sources of runoff. Runoff enters the recreational waters from many freshwater outlets ranging in size from rivers having year-round flows to small ephemeral drains that flow only during wet–weather. Recreational waters affected by urban runoff have demonstrated elevated levels of indicator bacteria and human enteric virus (Gold *et al.* 1990, 1991, 1992) that can result in increased risks of illness to swimmers (Haile *et al.* 1999).

Although the scope of bacteriological monitoring in southern California is impressive, the data collected cannot easily be integrated to provide a regional assessment of recreational water quality. Most monitoring is spatially focused on a small set of high-use beaches or other areas of concern; as a result, only 7% of the SCB shoreline is routinely monitored. Moreover, the frequency and timing of sampling is inconsistent among agencies, which can bias results. Finally, the data are often stored in uncommon formats that are difficult to collect, collate, and synthesize.

To overcome these technical challenges and provide the public with an integrated assessment of beach water quality, all of the agencies that routinely monitor bacteriological water quality along the southern California shoreline, as well as several university and volunteer organizations, coordinated their efforts for the purpose of conducting regional surveys to assess the overall condition of the SCB and northern Baja California, Mexico. Two Microbiological Regional Monitoring Surveys have been conducted and described thus far; one conducted during summer 1998 (Noble *et al.* 1999) and one conducted during winter 1999 (Noble *et al.* 1999). The two studies found that 94% (summer) and 90% (winter) of the shoreline in the study area met State of California water quality standards. However, not all shoreline types had similar water quality. Areas near freshwater outlets had the poorest water quality, with more than 50% of the shoreline immediately adjacent to freshwater outlets exceeding at least one of the State of California standards during both regional surveys. Another finding of significance during the regional surveys was the applicability of water quality standards for different bacterial indicators. The State of California maintains water quality standards for bacterial indicators, and the probability of exceeding a standard for any of these indicators differed during both previous studies. The enterococci standard was exceeded three times as often as any other standard. In areas distant from freshwater outlets, 78% of the standards exceedences were for enterococci alone. Moreover, no sample distant from a freshwater outlet exceeded all four indicator thresholds.

While both of these regional surveys provided important insights, managers were still uncertain about the effect of wet-weather discharges on regional shoreline water quality. The insights these surveys provided include the following: (1) Rainfall has the potential to increase shoreline contamination as waters from freshwater outlets flow into the sea. (2) More than 90% of the annual runoff volume occurs during rain events (Cross *et al.* 1992). Moreover, rainfall is infrequent in the SCB, allowing bacteria and other contaminants to accumulate in coastal watersheds. (3) Finally, runoff plumes are known to be large oceanographic features that extend for many kilometers (Bay *et al.* 1999).

To address the concern that beach water quality is substantially different during a storm event compared to dry–weather conditions, the same organizations that conducted the Summer 1998 Study and Winter 1999 Study joined forces to conduct a similar regional survey in the winter of 2000. The goals for this regional survey were similar to those of the previous surveys, but were focused toward conditions during a storm event. Their goals included:

- Determine the percentage of shoreline miles along the SCB that met bacterial indicator thresholds following a significant storm event.

  The number of shoreline miles provides a spatial assessment of the likelihood that a beachgoer electing to swim on a southern California beach during a storm event will do so in waters that meet all of the State's water quality standards. While the focus of the effort of this study was on the shoreline in the United States, the project also included a coordinated effort by Mexican scientists to assess water quality along the shoreline from the Mexican-U.S. international border to Ensenada, Mexico. The international participation provided a cross-border comparison of bacteriological water quality using comparable methods.

- Assess the comparability of the responses of the three bacterial indicators measured during storm event conditions.

  California regulations require county health departments to measure three bacterial indicators of fecal contamination--total coliforms, fecal coliforms (of which *E. coli* is the major component), and enterococci--on high-use beaches during summer months, a practice that all southern California county health departments extend to the winter months as well. The fourth commonly used indicator threshold is the ratio of total coliforms to fecal coliforms, whenever total coliforms exceed 1,000 MPN or cfu/100 mL. The three bacterial groups respond differently to the physical and chemical conditions outside of the gut of warm-blooded animals (Hanes and Fragala 1967, Sieracki 1980). Comparing the responses of these indicators can increase understanding of which indicator organisms are most "conservative" at each of several shoreline types, and enable the assessment of potential redundancy among the indicators.

Chapter II describes the scientific methods and quality assurance procedures used to accomplish the above objectives. Chapter III addresses the first study goal by providing an assessment of bacteriological water quality along the shoreline of the SCB. Chapter IV addresses the second goal by comparing responses among the bacterial indicators measured in the study. Conclusions from the study are presented in Chapter V, which summarizes the study conclusions and integrates the results and analyses presented in Chapters III and IV. Chapter VI provides recommendations that follow from the study results. Chapters III and IV are intended for a scientific audience and contain detailed technical information that provides the foundation for our conclusions and recommendations. Chapters V and VI are intended for a wider audience and provide a more general overview of the study findings.

II. METHODS

A. Sampling Design

The Storm Event Shoreline Microbiology Study involved sampling at 254 sites along the coastline of the SCB on February 22, 2000, immediately after a storm event that produced at least one inch of rain over the entire SCB. A date was selected when the entire Bight experienced rainfall in order to allow for the comparison of rain effects along the entire coast. This date followed approximately 36 h after the end of the storm; sampling during the storm would have been dangerous and sampling more than two d after the storm would have significantly reduced the influence of stormwater runoff to the receiving waters.

The study area extended from Point Conception in Santa Barbara County, California, to Punta Banda, Baja California, just south of Ensenada, Mexico. This area includes approximately 690 miles of coastline, although the sampling frame for the study included approximately 270 miles or 39% of the coastline. The remaining shoreline was classified as inaccessible to swimmers due to the presence of ports, private marinas, private land, military property, or steep cliffs.

Sampling sites were selected using a stratified random approach, with the strata corresponding to the four different types of shoreline (Table II-1). To implement this design, a GIS layer of shoreline types was created based upon the participating organizations' knowledge of local shoreline conditions. The high-use and low-use categories that were used in the summer survey were combined to form one sandy beach stratum and one rocky shoreline stratum. A total of 81 freshwater outlets were identified and differentiated as perennial or ephemeral based upon whether water flowed year-round or seasonally, respectively. The freshwater outlets selected were those outlets that are typically responsible for 99% of the total freshwater/stormwater inputs to the SCB.

The number of samples allocated to each stratum was that necessary to achieve a 95% confidence interval of approximately +/- 5% around estimates of areal extent. The site selection process was implemented separately by county, with the number of sites within a stratum and within a county in proportion to the percentage of southern California shoreline of that stratum type within the county. A county-specific selection process was implemented to accommodate the availability of additional effort in some counties, beyond that necessary to achieve the program's precision goals.

Although the basic sample allocation scheme was stratified random, a systematic component was added to minimize clustering of sample sites along the shore. This approach was accomplished using an extension of the National Stream Survey sampling design (Messer *et al.* 1986, Overton 1987), whereby each stratum was divided into a series of linear sections of coastline, with each section identified by a count variable. The sections were joined in a stratum line, which was then partitioned into a number of intervals equal to the desired sample size. The partition was randomly placed over the stratum line by selecting a random starting point for the beginning of the first interval.

Based upon this starting point, the intervals were defined as consecutive sections of equal length. A simple random sample was then chosen from within each interval. Each point was translated back to the shoreline using the section count variable. The resulting sample pattern achieved spatial separation of sites as well as a random component to ensure statistical validity.

Sample sites within the perennial and ephemeral water outlet strata were selected using two methods. First, sites were selected at a random distance within 100 yards from the mouth of the outlet, using the systematic random approach described above. Second, a site was placed at the mouth of the outlet (referred to as the point zero site). Random sites were placed around 39 of the 40 perennial water outlets in southern California. Point zero sites were placed at the mouths of 30 of the 40 systems, which were selected by availability of effort. At the ephemeral outlets, 36 random sites were sampled of the possible 47 systems. Twenty-nine of the 47 ephemeral outlets also received point zero samples.

The approach used to select sample sites in the United States was also used for the Mexican shoreline, but the Mexican component of the study was limited to sandy beaches (19 sites) and point zero outlet sites (10 sites). The Mexican point zero sites were associated with the perennial water outlets with the highest flow rates.

Volunteer Monitoring

Volunteer organizations enhanced the sampling effort with a total of 30 sampling sites, 11 of which ranged in location from the Talbert Marsh area of Huntington Beach northward to the Long Beach Harbor region of San Pedro Bay, and 19 sampling sites in southern Santa Monica Bay (between Ballona Creek and the Palos Verdes Peninsula). Volunteer sites were limited to the sandy beach stratum. Volunteer sites were selected as a supplement, rather than as an integrated part of the program. This supplemental overlay of sites was selected using the same statistical design approach described above in numbers that would not have affected the integrity of the base sample design had the volunteer effort been unsuccessful. Since the volunteers were successful in collecting all of their assigned samples and meeting all of the quality assurance requirements, their results were integrated directly into the base program.

B. Field and Laboratory Methods

Bacteria

Microbiological water quality was determined by testing seawater samples for the presence of certain bacterial indicator microorganisms whose presence indicates that pathogenic microorganisms may also be present. These bacteria can easily be isolated and quantified by simple bacteriological methods. Detection of these bacteria in water typically means that fecal contamination has occurred and suggests that enteric pathogens may also be present. However, these bacterial indicator microorganisms cannot be used independently as an indication of fecal contamination from humans. Therefore, it is difficult to infer public health risk due to their presence alone.

Three groups of bacteria were tested in this study: total coliforms, fecal coliforms (of which *E. coli* is a subset), and enterococci. Total coliforms, which include the member species *Escherichia*, *Citrobacter*, *Enterobacter*, and *Klebsiella,* include all aerobic and facultatively anaerobic, gram-negative, non-spore forming, rod-shaped bacteria that produce gas upon lactose fermentation in prescribed culture media within 48 h at 35° C. Even though the total coliform group has been used as the standard for assessing the water quality of recreational and drinking waters for most of the last century, it should be remembered that many of its member species can be found naturally in freshwater and marine environments. Therefore, the presence of total coliforms is not always a direct indication of fecal contamination. In addition, many members of the total coliform group have been observed to regrow in natural surface and drinking waters.

Although the total coliform group has been the indicator group of choice for many years, as previously mentioned, many of its member species are not limited to fecal sources. Fecal coliforms are a subset of the total coliform group that include *Klebsiella* and *Escherichia*, and are differentiated from their total coliform counterparts by their ability to ferment lactose with the production of gas within 24 h at a much higher temperature (45° C). This test indicates fecal coliforms, but once again does not distinguish between human and animal contamination. *E. coli*, an organism that is often the predominant type of fecal coliform, has been used as an indicator because it can be easily distinguished from other members of the fecal coliform group. The Colilert® test, for example, has the advantage of detecting total coliforms and *E. coli*, the principal fecal coliform, simultaneously within 24 h.

The enterococci group (which specifically includes only the genera *Enterococcus*) includes all streptococci that share certain biochemical properties and have a wide range of tolerance of adverse growth conditions. They are differentiated from other streptococci by their ability to grow in 6.5% sodium chloride, pH 9.6, and at a temperature of 45° C, and include *Enterococcus avium, Enterococcus faecium, Enterococcus durans, Enterococcus faecalis,* and *Enterococcus gallinarum*. Of this list, only *Enterococcus faecalis* and *Enterococcus faecium* are considered to be more specific to the human gut, with *Enterococcus avium* more commonly found in fecal contamination from birds. However, once again the enterococci group cannot be used to distinguish between human and animal fecal contamination.

Samples were collected in sterile sample bottles or Whirl-Pak bags from ankle-deep water on an incoming wave just prior to receding, with the sampler positioned downstream from the bottle and the mouth of the bottle facing into the current. After the sample was taken, the bottle was tipped to decant enough sample to ensure 1 to 2 inches of airspace in the sample bottle. The bottle was then tightly capped and stored on ice in the dark. All samples were returned to the laboratory in time to begin analysis within 6 h of sample collection. Total coliforms, fecal coliforms or *E. coli*, and enterococci were measured for all sites.

Three methods were used to detect and enumerate bacteria: membrane filtration (MF); multiple tube fermentation (MTF); and defined substrate technology tests (Idexx kits). The first method, MF, is a direct plating method used for the detection and enumeration of bacteria in seawater. The second method, MTF, involves inoculating multiple tubes of broth with dilutions of the sample. Organism density is based upon the number of tubes with acid and gas production at the various dilutions and is reported in terms of the most probable number (MPN) as determined by a series of probability formulas. The third method used defined substrate technology tests, Colilert® and Enterolert®, manufactured by Idexx Laboratories, Inc. The Idexx kits use either multiple tubes or multiple wells, with an MPN approach, to detect the presence or absence of total coliforms, *E. coli,* or enterococci. With Colilert®, the detection of coliforms is based upon a color change for total coliforms and the release of a fluorogen by metabolism of a nutrient indicator substrate specifically by *E. coli*. This assay is read within 18-22 h. In this study, *E. coli*, which typically constitute the majority of fecal coliforms, were treated as fecal coliforms for data analysis. However, it should be mentioned that the percentage of *E. coli* that makes up the fecal coliform group varies depending upon the sample matrix and location. With Enterolert®, the detection of enterococci is based upon the release of a fluorogen by metabolism of a nutrient-indicator substrate by members of the enterococci group.

Each participating laboratory used its established analytical methods for sample processing, as outlined in Table II-2. More detailed information on these methods can be found in *Standard Methods for the Examination of Water and Wastewater, 18th Edition, 1995* (APHA 1995), and the EPA-821-R-97-004, May 1997 publication.

A subset of laboratories also performed side-by-side analyses. Total coliforms and *E. coli* were analyzed using Colilert® and enterococci were analyzed using Enterolert®. The methods and number of samples analyzed for the method comparison are outlined in Table II-3. The results of this method comparison are presented in Appendix B.

C. Quality Assurance/Quality Control

Two distinct but related activities, quality assurance (QA) and quality control (QC), were incorporated into the Southern California Bight 1998 Regional Monitoring Program (Bight '98) to ensure that the data were collected using scientifically valid methodologies that were comparable among participating organizations. The QA activities were undertaken prior to sampling and fall into two major categories: (1) methods standardization and (2) intercalibration exercises.

Specific QA activities included the agreement of each laboratory to follow the procedures set forth in *Standard Methods for the Examination of Water and Wastewater, 18th Edition, 1995* (*Standard Methods*), acceptable U.S. EPA-approved test methods or the manufacturer's recommended procedures for Colilert® and Enterolert®. Each laboratory also ascribed to common guidelines regarding culture media, water, equipment and instrumentation, and data handling. Whenever possible, commercially available pre-sterilized media were used. Manufacturers' specifications were followed for all laboratory-prepared media. The water used to prepare culture media and reagents was of distilled or demineralized reagent grade quality, and was stored away from direct sunlight to prevent the growth of algae. Laboratory-specific established protocols for ensuring proper temperatures for ovens, autoclaves, and refrigerators were reviewed and deemed acceptable for this project. Balances were calibrated to provide a sensitivity of at least 0.1 g at a load of 150 g, and pH meters were calibrated to maintain an accuracy of 0.1 pH units.

Positive and negative growth performance and sterility tests were performed on newly prepared batches of media. Broth cultures and plates were read within specified times. Proper functioning of coliform water baths was demonstrated while analyses were in progress using control cultures of *E. coli* and *Enterobacter aerogenes*.

A performance-based approach was employed to ensure data comparability among laboratories; an intercalibration test using a common sample was performed before both the winter and summer surveys. All laboratories involved obtained results within the +/- 0.5 median log count comparability goal.

Quality control measures were defined as the routine practices incorporated into each laboratory's analytical method protocols. Examples of quality control measures included, but were not limited to, maintaining and complying with all aspects of sample collection, sample storage, sample handling, chain of custody, sample preparation, sample analysis, and data reporting. Other measures include quality assurance checks for precision and accuracy at the prescribed frequency including analysis of blanks, duplicate analyses, sterility checks on equipment, satisfactory growth performance, pH and sterility of each batch of media, incubation of positive and negative control cultures, and performance of confirmed and completed tests.

D. Data Analysis

The assessment of shoreline condition focused on estimating the percent of shoreline miles that exceeded a threshold of concern. Data obtained from indicator comparisons (laboratories where multiple methods were performed simultaneously) and the testing of Mexican waters were not used for the overall assessment of shoreline condition. Thresholds derived from the standards established in Assembly Bill 411 legislation were based upon daily measurements (Table II-4).

Estimating the percent of shoreline miles was accomplished for each of the strata and for the shoreline as a whole using a ratio estimator (Thompson 1992):

$$m = \frac{\sum_{i=1}^{n} (p_i \times w_i)}{\sum_{i=1}^{n} w_i}$$

where:

m = Percent of area exceeding the threshold for strata *j*
p_i = Binomial parameter value (e.g., 1 if it exceeded the threshold value and 0 otherwise) for station *i*
w_i = Weighting for station *i*, equal to the inverse of the inclusion probability for the site
n = Number of stations sampled in population *j*

Use of the ratio estimator for the standard error approximates joint inclusion probabilities among samples and assumes a negligible spatial covariance, an assumption that appears to be warranted based upon preliminary examination of the data. This assumption is conservative in that its violation would lead to an overestimation of the range of the confidence interval (Stevens and Kincaid 1997).

The comparison of indicator responses was accomplished primarily through correlation analysis. Indicator comparisons were performed with the U.S. data set. Combination tables were also developed to assess categorically the frequency with which individual sites were classified the same by different indicators. Venn diagrams were developed to assess the degree of overlap in threshold exceedences among indicators.

E. Quality Assurance Evaluation

Participants successfully sampled 99% of the sites targeted for study during the survey period. Three sites were not sampled due to miscommunication.

Participants successfully analyzed all 753 of the samples collected and targeted for analysis, exceeding the data quality objective of 95%.

During the course of data checking, it was discovered that less than 1% of the reported samples had fecal coliform levels that were higher than the total coliform levels. Since fecal coliforms represent a subset of the total coliform group, their number should not exceed the total coliform number in any given sample. On-site audits conducted by the Project QA Officer confirmed that these anomalies resulted from the lack of analytical precision when different methods were used for measuring total coliforms and fecal coliforms and did not reflect errors in analytical methodology. The results of fecal coliforms and total coliforms for the two cases in question were 3,000 and 1,300 for one site, and 200,000 and 33,000 for the other site, respectively. Only one of the discrepancies was from a sample that exceeded bacterial indicator standards for fecal coliforms but not for total coliforms.

TABLE II-1. Allocation of Bight '98 shoreline microbiology samples among sampling strata.

Strata	Base Sample Sites	Mexican Sample Sites	Volunteer Sample Sites
Sandy Beaches	37	19	30
Rocky Shoreline	24		
Freshwater Outlets			
Ephemeral	36		
Ephemeral Point Zero	29		
Perennial	39		
Perennial Point Zero	30	10	
	195	29	30

TABLE II-2. Number of sites sampled and laboratory methods used by each of the survey participants.

	Total Coliforms	Fecal Coliforms	Enterococci
Santa Barbara County			
Santa Barbara Health Care Services	22[c]	22[c]	22[d]
City of Santa Barbara	4[b]	4[b]	4[d]
Goleta Sanitation District	5[b]	5[b]	5[b]
Ventura County			
Ventura WWTP	6[b]	6[b]	6[b]
City of Oxnard	5[b]	5[b]	5[b]
Aquatic Bioassay Labs	4[b]	4[b]	4[b]
Ventura Co. EHD	10[c]	10[c]	10[d]
Los Angeles County			
City of Los Angeles	18[a]	18[a]	18[a]
Los Angeles Co. Sanitation Districts	5[a]	5[a]	5[a]
Los Angeles Co. DHS	12[c]	12[c]	12[d]
City of Long Beach	2[a]	2[a]	2[a]
Southern California Marine Institute	30[c,f]	30[c,f]	30[d,f]
Orange County			
Orange Co. Sanitation District	17[b]	17[b]	17[e]
Orange Co. Environmental Health Division	24[b]	24[b]	24[d]
AWMA/SERRA	14[a]	14[a]	14[a]
San Diego County			
Encina Wastewater Authority	7[a]	7[a]	7[a]
City of Oceanside	1[b]	1[b]	1[a]
City of San Diego	31[a]	31[a]	31[e]
San Diego Co. Department of Env. Health	6[b]	6[b]	6[d]
San Elijo Joint Powers Authority	2[b]	2[b]	2[b]
Mexico			
Instituto de Investigaciones Oceanologicas	29[b]	29[b]	29[d]

[a]MF.
[b]MTF.
[c]Colilert®.
[d]Enterolert®.
[e]EPA 1600.
[f]Analyses performed by City of Long Beach.

TABLE II-3. Number of sites sampled and laboratory methods used by each of the survey participants in the method comparison portion (see Appendix B).

	Total Coliforms	Fecal Coliforms	Enterococci
Santa Barbara County			
City of Santa Barbara	4[b,c]	4[b,c]	
Los Angeles County			
City of Long Beach	2[a,c]	2[a,c]	2[a,d]
Orange County			
Orange Co. Sanitation District	18[b,c]	18[b,c]	18[e,d]
Orange Co. Environmental Health	24[b,c]		
San Diego County			
City of Oceanside			1[b,d]
City of San Diego	31[a,c]	31[a,c]	31[e,d]

[a]MF.
[b]MTF.
[c]Colilert®.
[d]Enterolert®.
[e]EPA 1600.

TABLE II-4. Bacterial Indicator Thresholds Used for the Storm Event Shoreline Microbiology Study.

Indicator	Daily Limits (per 100 mL)
Total Coliforms	10,000
Fecal Coliforms	400
Enterococci	104
Total:fecal ratio	When TC >1,000 and TC/FC $\leq$ 10

III. ASSESSMENT OF THE SOUTHERN CALIFORNIA BIGHT

Results

Rainfall ranged from 1.1 to 3.0 inches throughout the study area, between February 20 and 22, with the highest quantity measured near the Los Angeles-Ventura County border (Table III-1). Duration of the rainfall event averaged 39 h bight-wide. A smaller storm that produced rainfall quantities between 0.1 and 0.5 inch preceded this storm event by 3 d.

More than half (58%) of the shoreline miles along the SCB exceeded water quality thresholds following the storm (Table III-2). The stratum with the highest frequency of shoreline-mile exceedences was the perennial point zero freshwater outlets with 87%, followed by the entire perennial freshwater outlet stratum (includes both point-zero and random sites) with 67%, and the sandy beach stratum with 59%. The ephemeral and rocky shoreline strata had the lowest frequency of shoreline-mile exceedences (38% and 34%, respectively).

The exceedence of the enterococci threshold occurred along nearly 100% of the shoreline miles that demonstrated poor water quality (Table III-2). Enterococci exceeded water quality thresholds at twice the frequency of fecal coliforms, and four times the frequency of the total:fecal coliform ratio. Approximately three-quarters of the water quality threshold exceedences were for more than one bacterial indicator following the storm event (Table III-3). Almost 90% of the multiple threshold exceedences were for at least three indicators. The highest frequency of multiple indicator exceedences occurred at the perennial point zero sites, where all four indicators exceeded the thresholds at more than three times the frequency of the bight as a whole.

The vast majority of water quality exceedences, regardless of indicator type, were significantly above the water quality thresholds following the storm (Figure III-1). One hundred percent of the samples collected during the Storm Event Shoreline Microbiology Study for enterococci exceeded the water quality threshold by more than one standard deviation of measurement error, and 92% exceeded the water quality threshold by more than two standard deviations. Similarly, 85% of the fecal and total coliform samples collected during the Storm Event Shoreline Microbiology Study exceeded the water quality threshold by more than the amount attributable to measurement error.

Similar to the sampling results for the United States, the exceedances of water quality thresholds along the Mexican shoreline were widespread following the storm event (Table III-4). For example, 36% of the shoreline miles in Mexico and the United States exceeded the water quality threshold for fecal coliforms. Similarly, 63% of the beaches in Mexico and 66% of the beaches in the United States exceeded the water quality threshold for enterococci. Total coliform was the only exception, where 11% of the shoreline miles along sandy beaches in Mexico exceeded the total coliform threshold compared to 31% in the United States (Table III-4).

For samples that exceeded any one of the indicator bacteria thresholds, median indicator concentrations were 3 to 6 times higher than the threshold value at freshwater outlets, and 3 to 13 times higher than the threshold value at beaches, with enterococci consistently representing the largest factor of exceedance (Table III-5). In general, total coliforms exceeded the threshold value by 2 to 3 times, and fecal coliforms by 3 to 6 times at beaches and freshwater outlets (Table III-5).

Median indicator concentrations for exceedences also were similar between the United States and Mexico, except in the case of total coliforms. However, the total coliform numbers for the United States include values for numbers that are higher than 24,192 MPN/100 mL, which are the result of using the MPN method (Table III-5). Median indicator concentrations for the entire data set were similar along Mexican and United States shorelines, except for fecal coliforms, where median concentrations were noticeably lower in the United States at both freshwater outlets and beaches (Table III-6).

Discussion

The effect of wet-weather runoff from a storm event on shoreline water quality was dramatic. The 56% of shoreline miles exceeding water quality standards was 10 times higher than in a parallel set of studies conducted in 1998 during summer (Noble *et al.* 1999a, Noble *et al.* 2000) and winter non-storm conditions (Noble *et al.* 1999b, Figure III-2). This increase was observed across all shoreline types and among all bacteria indicator types (Figure III-3). Moreover, the magnitude of the exceedences was much greater during the storm event. In the summer, two-thirds of the water quality exceedences were attributable to exceedences of a single bacterial indicator and most of those exceedences were barely above the indicator threshold. During the storm event study, two-thirds of the exceedences were for multiple indicators in which at least one indicator was twice the allowable standard (Figure III-4).

The present study is not the first to find high bacterial values in stormwater. Stormwater monitoring programs from Ventura to San Diego, California routinely find high densities of enterococci, fecal coliforms, and total coliforms in wet-weather discharges (Schiff 1997). Bacterial concentrations in Mission Bay and Santa Monica Bay, California, were significantly correlated to rainfall quantities and proximity to storm drains (Schiff and Kinney in press, SCAG 1988). Moreover, human enterovirus genomes have also been detected in at multiple locations of wet-weather discharges from southern California (Noble and Fuhrman 2000).

Several oceanographic studies previously have demonstrated the large spatial influence of stormwater plumes such as the one used as the basis for our bacterial findings. Bay *et al.* (1999) found that Ballona Creek stormwater plumes typically extend over 6 km^2 and often travel in proximity to the shoreline for long distances. Plumes from the larger watersheds, which contribute significantly more volume and bacteria loads, follow a similar pattern. Plumes from the Santa Clara, Los Angeles-San Gabriel Rivers, and Santa Ana Rivers have been shown to concentrate in surface waters, extend over large areas, “hug” shoreline features, and persist for days (Hickey in press).

Although a large extent of impaired shoreline was observed during this single storm, the ability to extrapolate our results to other storms is affected by three factors. The first factor is the size of the storm. The storm we studied produced more than 2 inches of rainfall in many parts of the study area, whereas the average storm in southern California produces less than 0.5 inch of rainfall, with an average of only one storm per year generating 2 inches of rainfall (Figure III-5, ES 1989). To assess the effect of storm size on bacterial measurements, we examined Los Angeles County shoreline microbiological data, which are collected on a daily basis from 22 fixed sites (Schiff *et al.* in press). These data indicated a significant relationship between storm size and the percent of sample sites exceeding State of California standards (Figure III-6). Approximately twice as many sites were found to exceed standards following a 2-inch storm as compared to a 1/2-inch storm, suggesting that our results overestimate the spatial extent of storm effect.

The second variable that potentially affects our ability to extrapolate our results to other storms is antecedent rainfall, as previous storm events may flush bacteria from the storm drainage system and decrease the potential impact of subsequent storms (ES 1989). Our storm was preceded by a smaller storm by approximately 3 d. Again, we examined the daily monitoring data from Los Angeles County to assess the importance of antecedent storms. We found that while some flushing occurs, bacterial concentrations return to prior levels within approximately 3 d (Figure III-7), suggesting that antecedent rainfall had little effect on our study results.

The third factor that may affect representation of the storm event is the duration of wet-weather exceedences following storm events. The impact of wet-weather discharges on shoreline water quality will persist for a period of time following rain events until bacteria inputs either mix and disperse or degrade in receiving waters. In this study, sampling was conducted 2 d following the storm event. Analysis of daily shoreline monitoring data from previous studies in Santa Monica Bay showed that wet-weather events most dramatically affect shoreline water quality on the first day; the extent of shoreline water quality excursions diminishes by the second day (Figure III-8). Therefore, our study likely underestimates a representative event because no samples were taken on the first day following the storm event.

While high indicator bacteria concentrations were observed in this study following a storm, the public health risk of wet-weather discharges cannot be determined because the risk associated with bacterial indicators is poorly understood at the present time. Most studies relating bacterial indicators to illnesses have been conducted where the primary source of bacteria is human sewage rather than urban runoff, as was the case here. The only epidemiological study that focused on the human health concerns associated with urban runoff was conducted in Santa Monica Bay and was limited to dry-weather runoff during the summer (Haile *et al.* 1999). Currently, most county public health agencies independently issue countywide warnings every time storms of $\geq$ 0.25 inch occur. Our findings of high, spatially extensive bacterial counts suggest that warnings on large spatial scales are appropriate, but additional epidemiological studies to evaluate the health effects of wet-weather urban runoff are advisable before management decisions are made.

FIGURE III-1. Relative frequency of water quality threshold exceedences by magnitude following a storm event in the Southern California Bight.

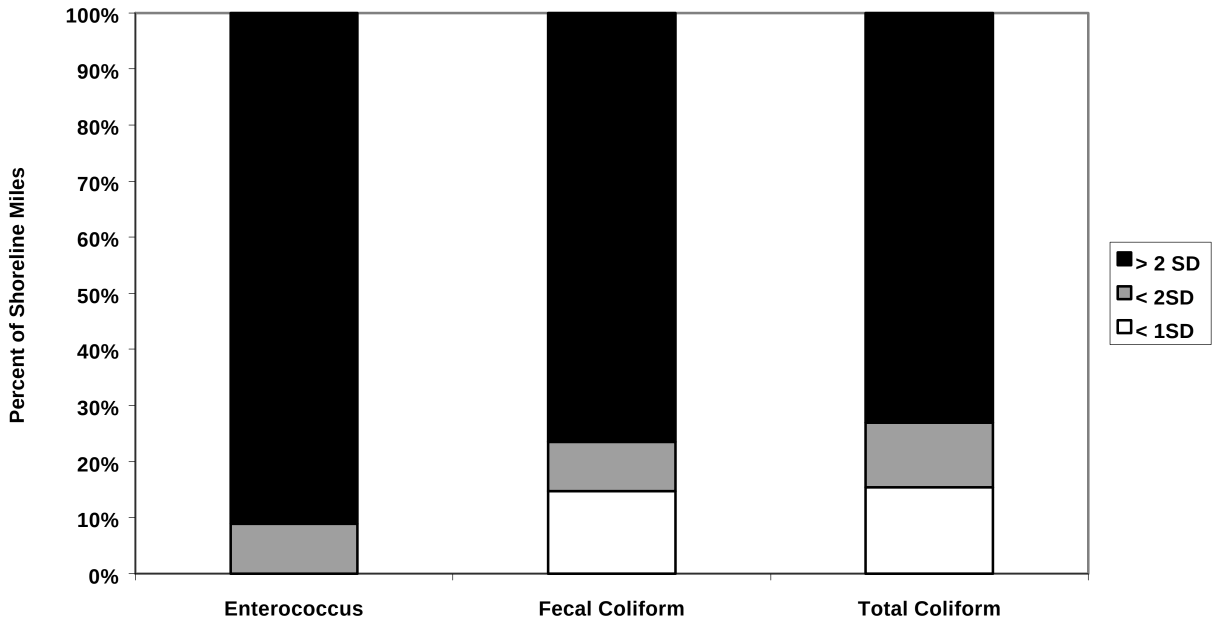


FIGURE III-2. The extent of water quality threshold exceedences during dry conditions in summer and winter compared to a storm event along various shoreline types in the Southern California Bight.

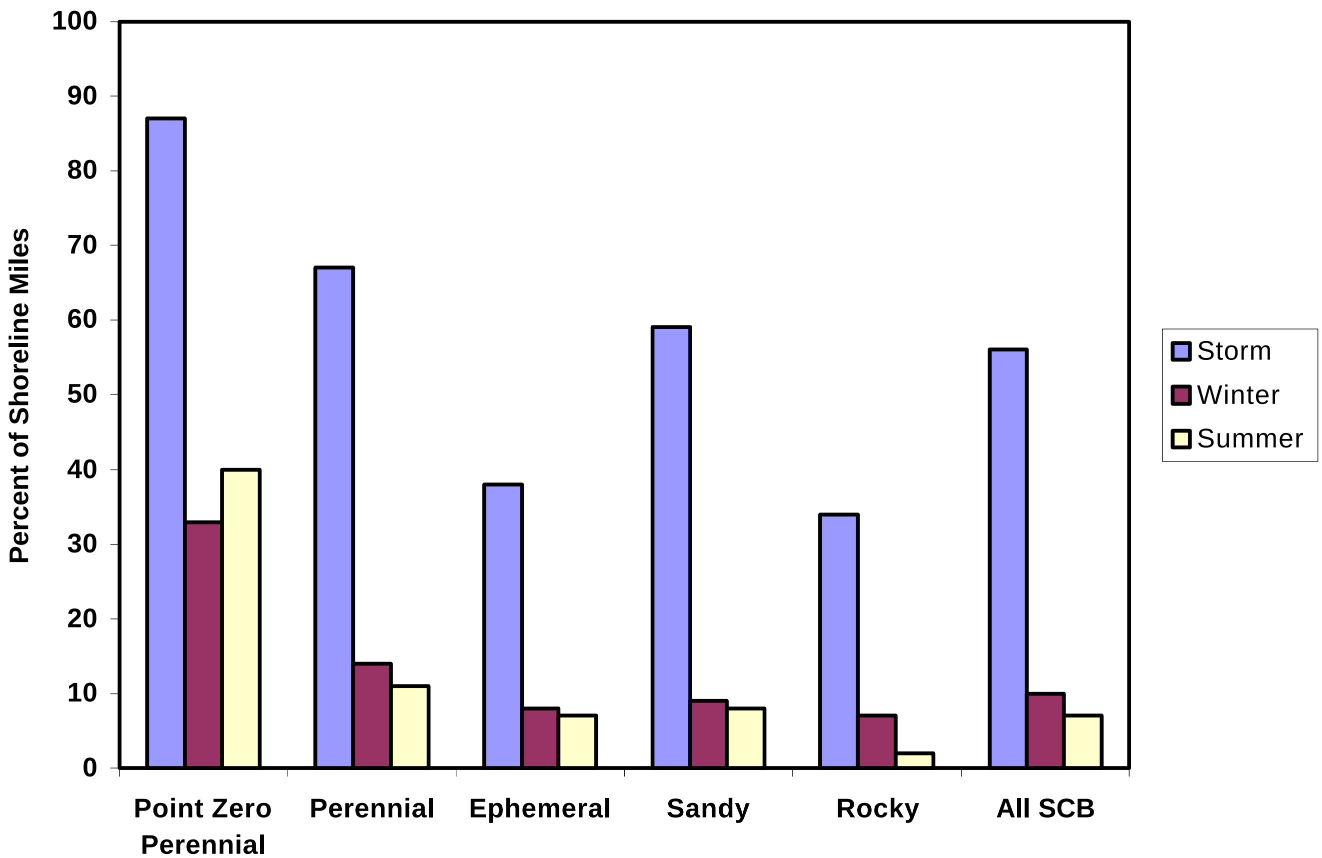


FIGURE III-3. The extent of water quality threshold exceedences during dry-weather conditions in summer and winter compared to storm event conditions among various indicator bacteria in the Southern California Bight.

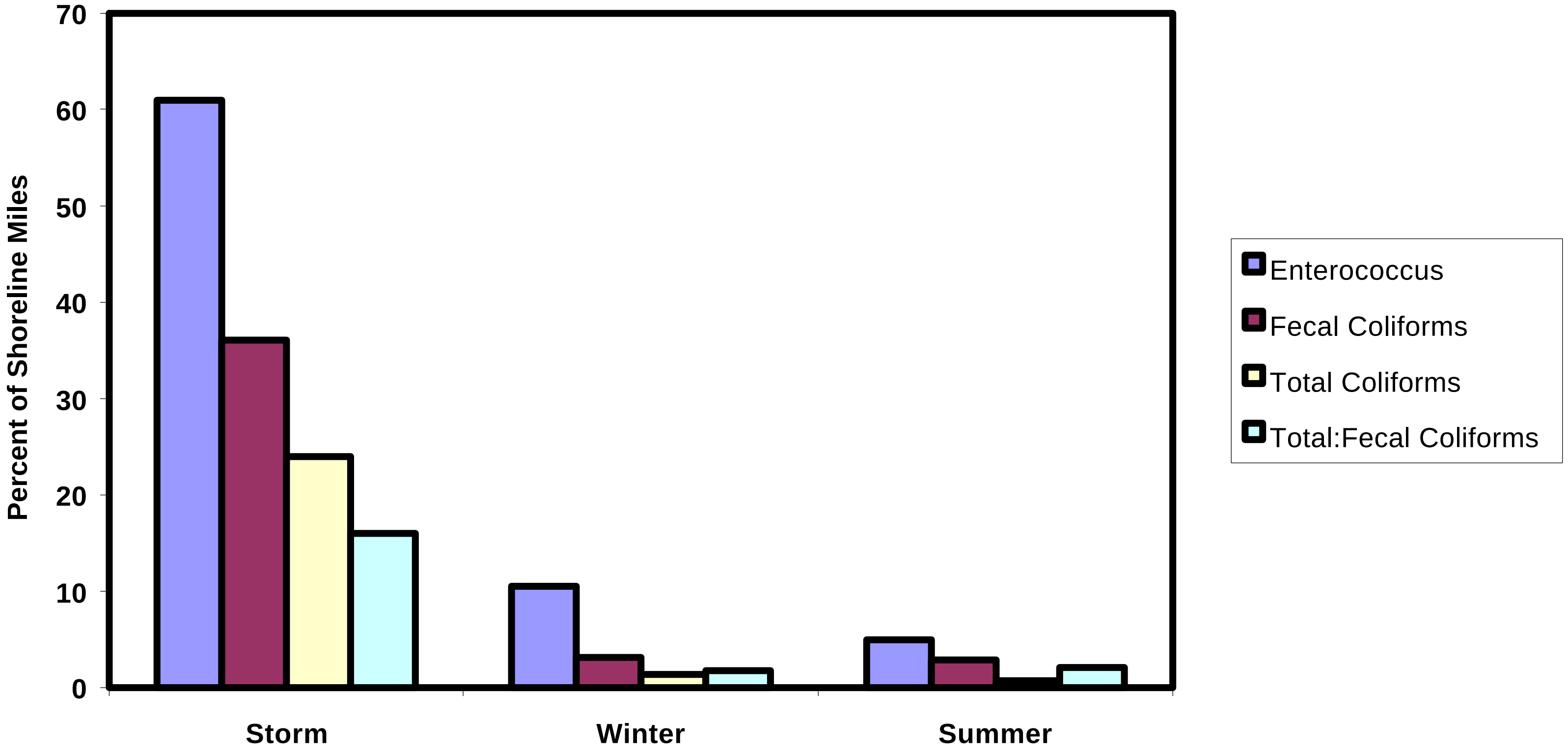


FIGURE III-4. Relative frequency of multiple water quality threshold exceedences during dry-weather conditions in summer and winter compared to storm event conditions in the Southern California Bight.

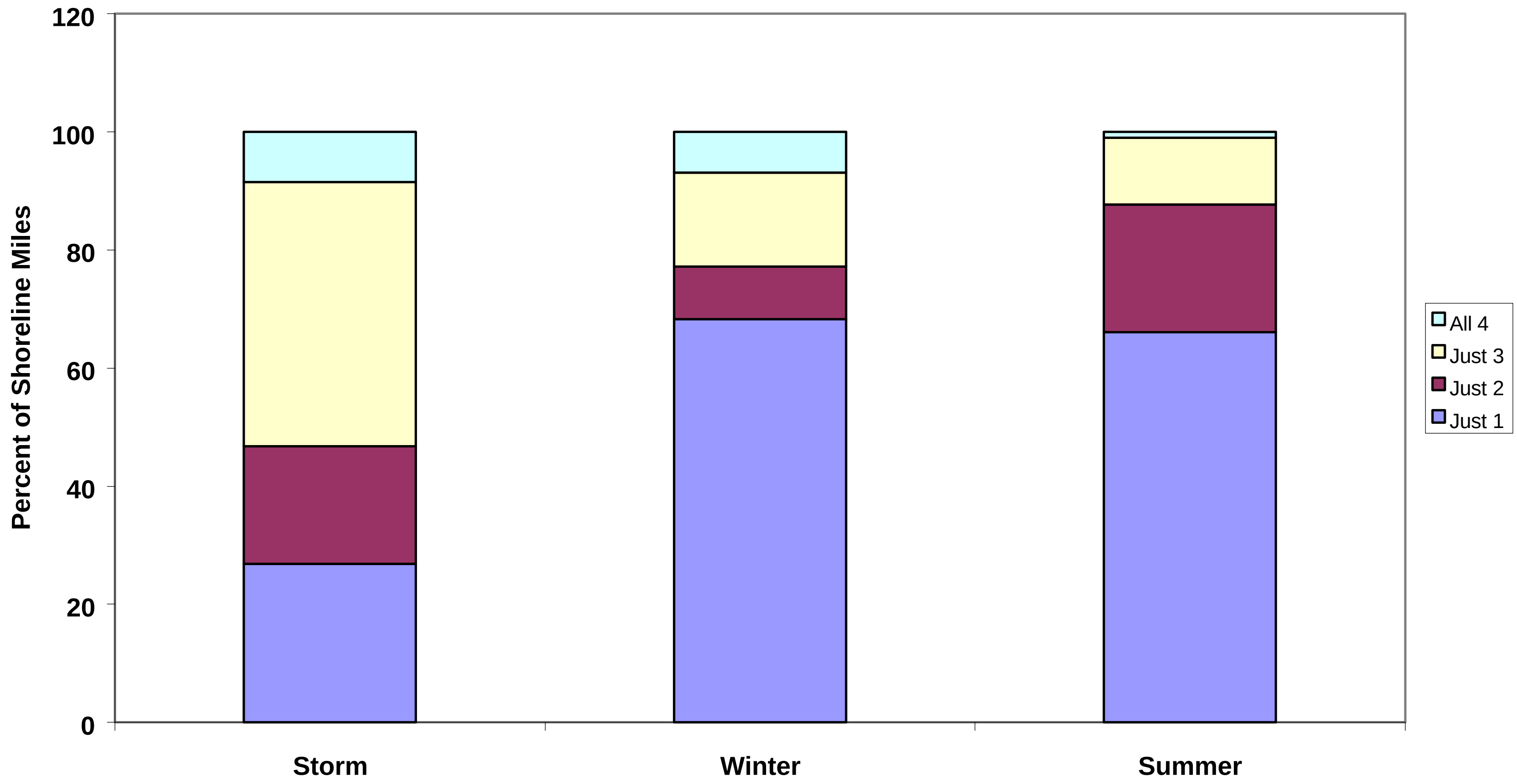


FIGURE III-5: Average annual rainfall frequency at Los Angeles International Airport: 1947-1998.

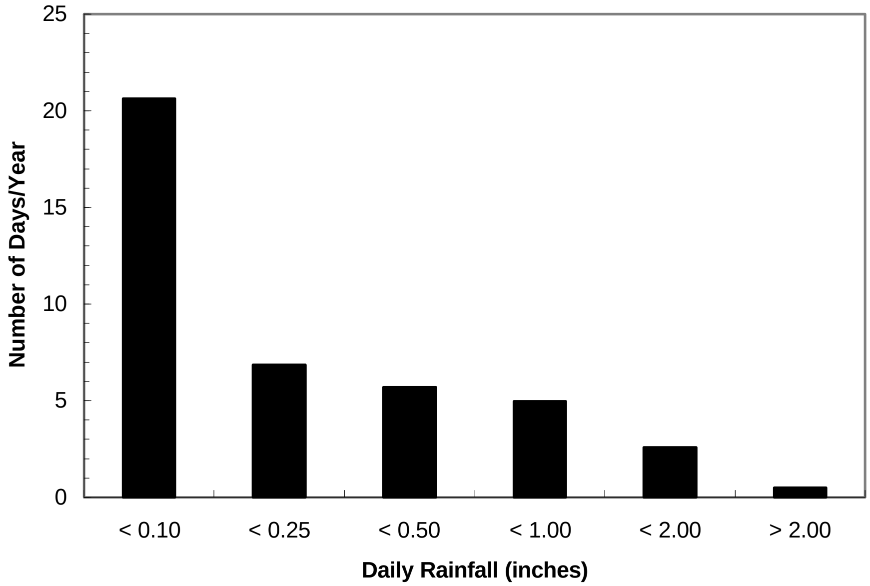


FIGURE III-6. The effect of increasing storm size and extent of water quality threshold exceedences in Santa Monica Bay. Data taken from daily shoreline monitoring in Santa Monica Bay from 1995–2000.

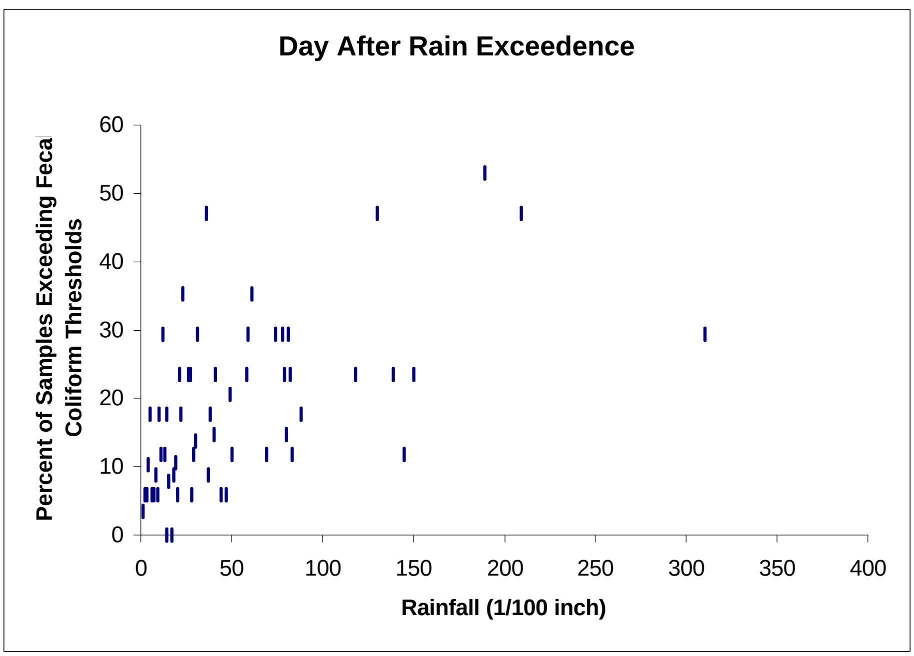


FIGURE III-7. The effect of antecedent rainfall and extent of water quality threshold exceedences in Santa Monica Bay. Data taken from daily shoreline monitoring in Santa Monica Bay from 1995–2000.

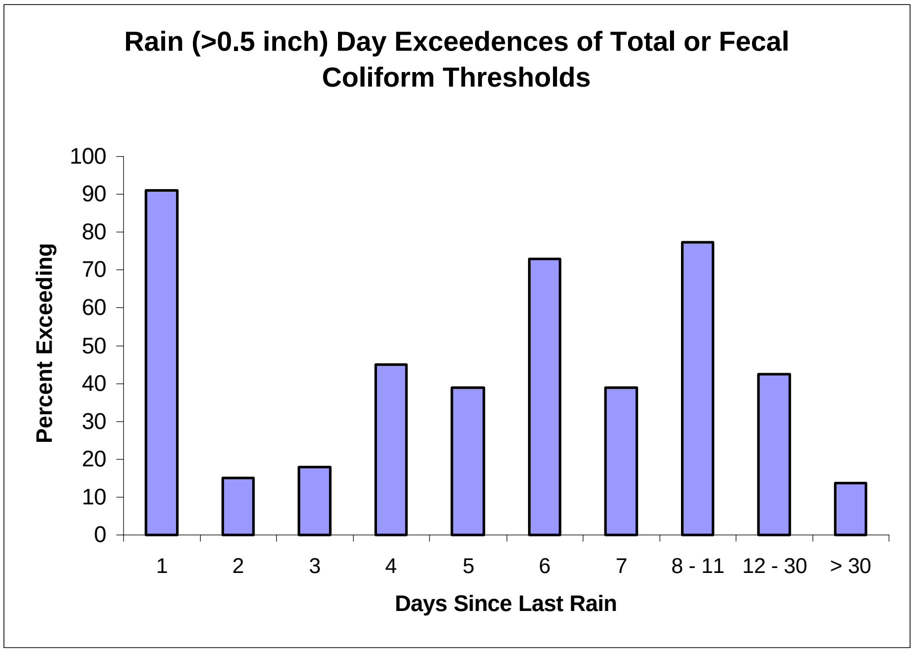


FIGURE III-8. The effect of time since rainfall and extent of water quality threshold exceedences in Santa Monica Bay. Data taken from daily shoreline monitoring in Santa Monica Bay from 1995–2000.

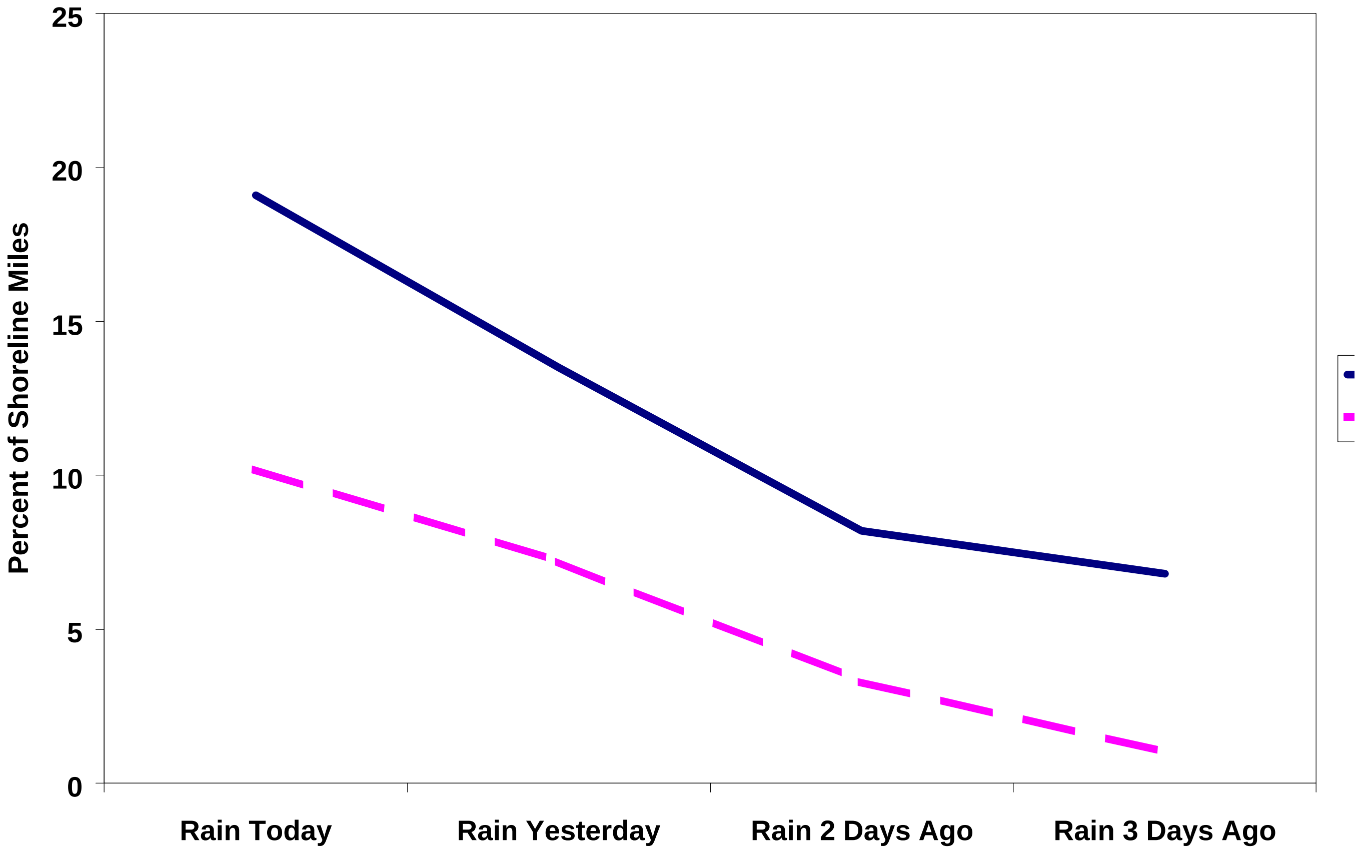


TABLE III-1. Rainfall quantity and duration for the storm sampled during this study (2/20/00–2/21/00). Also included is antecedent rainfall information.

Location	Time Rain Started (2/20)	Time Rain Stopped (2/21)	Duration of Storm (hours)	Rainfall (inches)	Days Since Last Rain	Rainfall from Last Storm (inches)	Duration of Last Storm (hours)
San Ysidro	6:00 am	9:00 pm	40	2.83	3	0.16	9
Plaza Bonita Rd.	6:00 am	4:00 am*	47	1.54	2.5	0.16	23
Fashion Valley	5:00 am	8:00 pm	40	2.01	3	0.28	23
San Onofre	5:00 am	9:00 pm	41	1.10	3	0.12	21
Encinitas	5:00 am	1:00 am*	46	1.06	3	0.16	14
Carlsbad	5:00 am	12:00 am	44	1.61	3	0.20	21
Oceanside	5:00 am	4:00 pm	36	1.43	3	0.12	15
Santa Ana River	6:00 am	4:00 pm	34	1.61	NA		
Coyote Creek	6:00 am	3:00 pm	33	1.27	NA		
Pt. Vicente Lighthouse	6:00 am	8:00 pm	39	1.54	3	0.51	14
Malibu	7:00 am	5:00 pm	35	2.21	3	0.51	18
Oxnard Airport	5:00 am	11:00 am	31	2.78	3	0.17	14
Ventura	4:00 am	12:00 pm	33	2.84	3	0.33	14
Sea Cliff	4:00 am	12:00 am	45	2.94	3	0.43	13
Lechuza Patrol	6:00 am	12:00 pm	31	3.07	3	0.51	17
Pt. Hueneme	4:00 am	11:00 am	32	1.87	3	0.13	17
Santa Barbara	4:00 am	3:00 am*	48	2.71	3	0.37	13
UCSB	5:00 am	10:00 pm	42	2.89	>19		
Overall Range			31–48 hours	1–3 inches	3 days	0.12-0.51 inches	9–23 hours

* Rain stopped on 2/22.

TABLE III-2. Percent of shoreline miles that exceeded water quality thresholds in the Southern California Bight following a significantly sized storm event in February 2000.

	Enterococci	Fecal Coliforms	Total Coliforms	Total:Fecal Ratio < 10	Any Indicator
Ephemeral Point Zero	52	26	11	22	52
Ephemeral	38	13	3	11	38
Rocky	36	19	6	7	34
Sandy	59	42	31	18	62
Perennial	67	28	20	17	67
Perennial Point Zero	87	43	33	30	87
All SCB	56	36	24	16	58

Table III-3. Percent of shoreline miles that exceeded water quality thresholds by number of indicator thresholds in the Southern California Bight following a storm event in February 2000.

	Any Indicator	Only 1 Indicator	Any 2 Indicators	Any 3 Indicators	All 4 Indicators
Ephemeral Point Zero	52	19	11	19	4
Ephemeral	38	13	14	8	3
Rocky	34	8	19	4	5
Sandy	62	14	10	33	5
Perennial	67	29	13	18	8
Perennial Point Zero	87	40	3	27	17
All SCB	58	15	12	26	5

TABLE III-4. Comparison of the percent of shoreline-mile water quality exceedences in Mexico and the United States following a large storm event.

	MEXICO		
	Enterococci	Fecal Coliforms	Total Coliforms
Sandy Beach	63	32	11
Point Zero	80	50	20
Entire Shoreline	66	36	15
	UNITED STATES		
	Enterococci	Fecal Coliforms	Total Coliforms
Sandy Beach	66	42	31
Point Zero	87	43	33
Entire Shoreline	61	36	24

TABLE III-5: Median indicator concentrations for exceedences. Reported as MPN or cfu/100 mL.

	Enterococci	Total Coliforms	Fecal Coliforms
Beach			
Mexico	855	82,000	1,700
United States	1,368	24,192	1,300
Freshwater Outlet			
Mexico	925	147,000	1,300
United States	597	30,500	2,290

TABLE III-6: Median indicator concentrations for all samples. Reported as MPN or cfu/100 mL.

	Enterococci	Total Coliforms	Fecal Coliforms
Beach			
Mexico	330	490	220
United States	130	900	80
Freshwater Outlets			
Mexico	310	1,450	515
United States	228	1,400	80

IV. INDICATOR COMPARISON

Results

Total and fecal coliform concentrations were strongly correlated (r = 0.85), with similarly strong correlations observed between these indicators and enterococci (r = 0.83 and r = 0.86, respectively; Table IV-1, Figures IV-1 through IV-3). The correlation between indicators was largely independent of the laboratory method used to analyze the samples, although slightly stronger correlations were found between indicators measured with MF (Table IV-1). Correlation coefficients using Idexx kits were nearly identical to those using MTF, except for total and fecal coliforms, where the Idexx kits had a slightly lower correlation (Table IV-1). This result may be due to *E. coli* being used as the standard of measurement with the Idexx kits, rather than the more inclusive fecal coliform group.

The correlations between indicators varied slightly among strata, with stronger correlations occurring for samples collected in the rocky shoreline and sandy beach strata than in the freshwater outlet stratum. For example, the correlation between total and fecal coliforms at the freshwater outlet strata (e.g., r = 0.68 for point zero freshwater outlets) was noticeably weaker than that of the rocky shoreline and sandy beach strata (r= 0.90 and r = 0.92, respectively; Table IV-2).

Although three bacterial indicators were measured, four bacterial thresholds were examined because the State of California uses the total:fecal ratio as one of its standards. Only 13% of the 131 samples that exceeded at least one bacterial indicator threshold exceeded all four bacteria thresholds (although 51% exceeded at least three thresholds and 69% exceeded at least two thresholds) (Table IV-3). Enterococci was found to be the most conservative indicator, detecting 99% of all threshold exceedences and accounting for all but one of the samples in which a single indicator exceeded the threshold (Figure IV-4, Table IV-3). In contrast, only 57, 40, and 38% of exceedences were detected for fecal coliforms, total coliforms, and total/fecal ratio, respectively (Table IV-3). The extent to which the three indicators agreed with respect to four thresholds differed by sampling stratum. The majority (52%) of freshwater outlet sample exceedences were for enterococci only (Figure IV-5), while the majority (58%) of beach sample exceedences were for all three indicators (Figure IV-6).

Discussion

Concordance among indicators was considerably higher in this Storm Event Shoreline Microbiology Study than in either of the two dry-weather studies (Summer 1998 Study and Winter 1999 Study). In the Storm Event Shoreline Microbiology Study, 38% of the exceedence events were for all three indicator bacteria compared to only 13% of the exceedence events in the Summer 1998 Study. This difference was even more pronounced for the shoreline stratum, in which 58% of the threshold exceedences were for three indicators during the Storm Event Shoreline Microbiology Study compared to no three-indicator exceedences during the Summer 1998 Study.

Enterococci was the most responsive indicator during the Storm Event Shoreline Microbiology Study, as 99% of the bacterial exceedences would have been identified with the use of enterococci as the sole indicator and 42% percent of threshold exceedences were for enterococci alone. Enterococci was also the most responsive indicator in the Summer 1998 and Winter 1999 studies, although 25% of the total threshold exceedences in the Winter 1999 Study were not detected by enterococci. Our finding that enterococci is the most responsive of the three indicators does not appear limited to southern California (Nuzzi and Burhans 1997). One possible explanation for the higher rate of enterococci threshold exceedences is that the thresholds may be set at different levels of sensitivity, which is consistent with our observation of high correlation among indicator values. This could have resulted from the different approaches used to generate the thresholds (Cabelli 1983a, Cabelli 1983b).

Another possible explanation is that enterococci survive longer in the marine environment and are more resistant to environmental stress than total or fecal coliforms (Hanes and Fragala 1967). This explanation is consistent with the much higher correlation among indicators in the winter season since the summer season is a low-flow period, which allows substantial time for degradation to occur before source materials reach the beach; in contrast, a much higher correlation was observed following the storm event, when high flows allowed little time for degradation during transport. Thus, the difference in concordance may reflect the freshness of the source material.

The United States Environmental Protection Agency (U.S. EPA) has promoted through its national guidance documents the use of a single bacterial indicator, enterococci, for marine waters. This endorsement by the U.S. EPA contrasts with guidance given by the State of California Health Department, which through implementation of Assembly Bill 411 requires that the health departments within the State of California measure three bacterial indicators (total coliforms, fecal coliforms, and enterococci) at high-use beaches during the months of April through October. In practice, most counties have extended this requirement to use three indicators to all beaches and to all seasons.

Our results support enterococci as the choice for a single indicator of bacterial pollution of recreational waters, if a single indicator must be selected. In the present wet-weather study, only one instance was identified in which a coliform measure indicated an exceedence for which enterococci did not. In the two dry-weather studies, three times as many enterococci threshold exceedences were found compared to either of the coliform measures. In the wet-weather study, enterococci threshold exceedences were twice those of coliforms. While more exceedences alone do not make enterococci a better indicator, its greater sensitivity combined with a demonstrated correlation with illnesses at the threshold levels (Haile *et al.* 1999) suggest that enterococci is the most appropriate single indicator.

The more difficult question is whether a single indicator is appropriate. For dry-weather conditions, the results of the Summer 1998 Study appear to be consistent with the State of California's directive to measure three indicators, as poor agreement was found among indicators and insufficient scientific evidence exists at the present time to select one indicator over the others (Noble *et al.* 1999a). The case for measuring three indicators during wet weather is less clear, since 99% of the water quality exceedences identified in the present study through the measurement of total coliforms or fecal coliforms were also identified through the measurement of enterococci. Consistency among indicators in dry-weather conditions found in the Winter 1999 Study was intermediate between the other two studies, making the decision problematic. Naturally, measuring all three indicators would be preferable; but if budgets are limited, the effort expended in monitoring three indicators during the winter months might be more cost-effectively expended by sampling more beach sites or sampling at more frequent intervals.

Addressing which, and how many, indicators should be measured will ultimately require additional research to understand how the bacterial indicators relate to the presence of pathogens that directly affect public health concerns. Investigators have shown that enterococci and coliphage have similar survival characteristics in receiving lake waters (Rajala and Heinonen-Tanski 1998). If the etiology of swimming-associated gastroenteritis is viral, and if coliphage react to physical and environmental stressors in a manner similar to human enteric viruses, then enterococci alone might be a better predictor of adverse health outcomes from exposure to fecal contamination. Cabelli (1982) and Dufour (1984) showed that enterococci correlated better with swimming-associated gastroenteritis at marine and freshwater bathing beaches with wastewater influences. This relationship between enterococci and swimming-associated gastroenteritis has been more recently examined by Kay *et al.* (1994), who demonstrated a significant dose-response relationship between gastroenteritis and fecal streptococci (of which enterococci are a subgroup) concentrations. On the other hand, different indicators may be predictors of specific diseases. Haile *et al.* (1999) found that the relative risk differed by indicator when its particular threshold was exceeded. The most appropriate indicator will be that which is most closely correlated in occurrence and in degradation history to the microbes that are pathogenic. Studies to address this issue will improve the quality of public warning systems, as well as the cost efficiency of monitoring, by more closely relating existing measures to health risk.

TABLE IV-1. Correlation between enterococci, fecal coliforms, and total coliforms in the Bight '98 Shoreline Microbiology survey, for all results, and for all methods.

	Total Coliforms: Fecal Coliforms	Fecal Coliforms: Enterococci	Total Coliforms: Enterococci
Entire Data Set	0.85	0.86	0.83
Membrane Filtration	0.93	0.93	0.90
Multiple Tube Fermentation	0.79	0.77	0.80
Idexx Kits	0.88	0.84	0.82

TABLE IV-2: Correlations between bacterial indicators by strata.

	Enterococci: Fecal Coliforms	Enterococci: Total Coliforms	Fecal Coliforms: Total Coliforms
Overall	0.86	0.83	0.85
Eph Point Zero	0.90	0.70	0.73
Ephemeral	0.81	0.63	0.61
Rocky	0.85	0.81	0.90
Sandy	0.88	0.85	0.92
Perennial	0.87	0.90	0.91

TABLE IV-3: Agreement between all four indicator thresholds, reported in percent.

	Enterococci	Fecal Coliforms	Total Coliforms	Total:Fecal Ratio
Single	31	0	0	0
Enterococci		6	2	11
Fecal Coliforms			0	1
Total Coliforms				0
Fecals and Totals	25			
Fecals and Ratio	12			
Totals and Ratio	0			
All 4 Indicator Types 13				

FIGURE IV-1. Correlation of enterococci and fecal coliforms. Lines represent thresholds of 104 for enterococci and 400 for fecal coliforms.

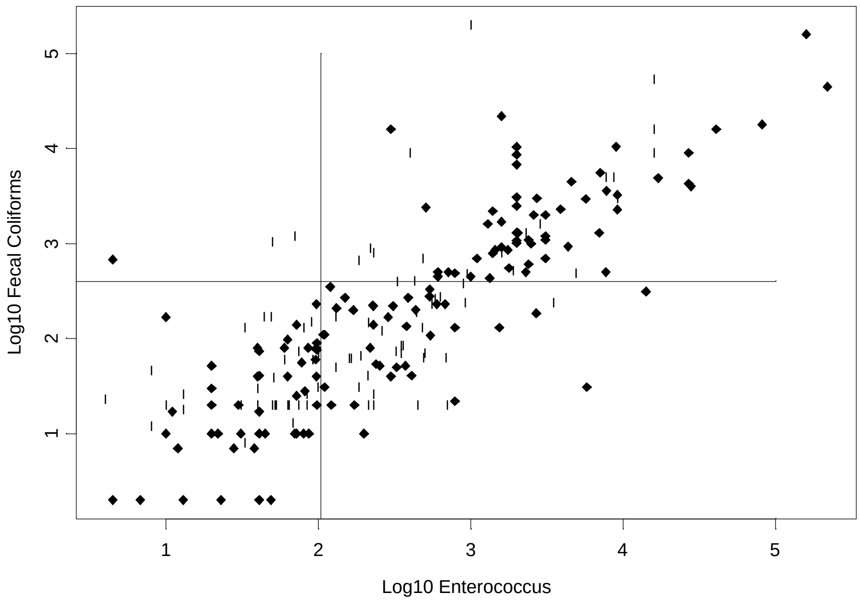


FIGURE IV-2. Correlation of enterococci and total coliforms. Lines represent thresholds of 104 for enterococci and 10,000 for total coliforms.

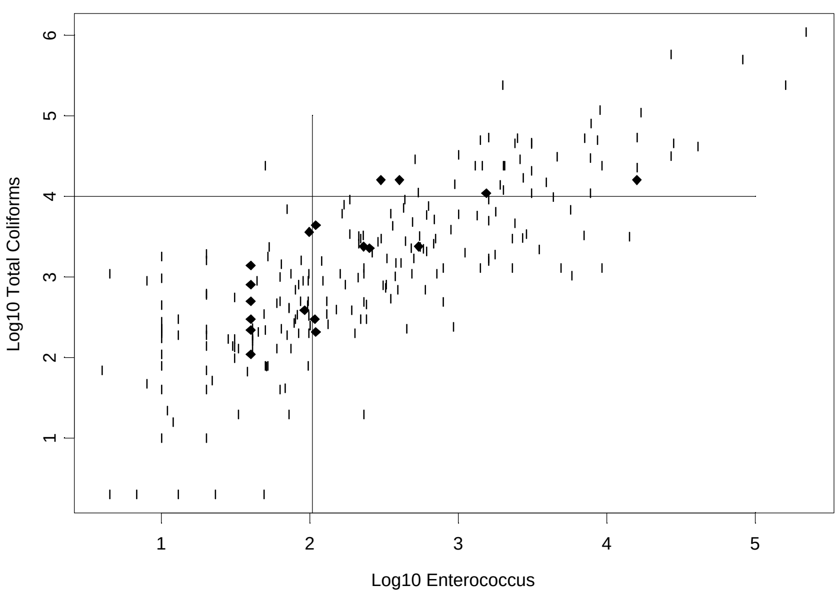


FIGURE IV-3. Correlation of fecal coliforms and total coliforms. Lines represent thresholds of 400 for fecal coliforms and 10,000 for total coliforms.

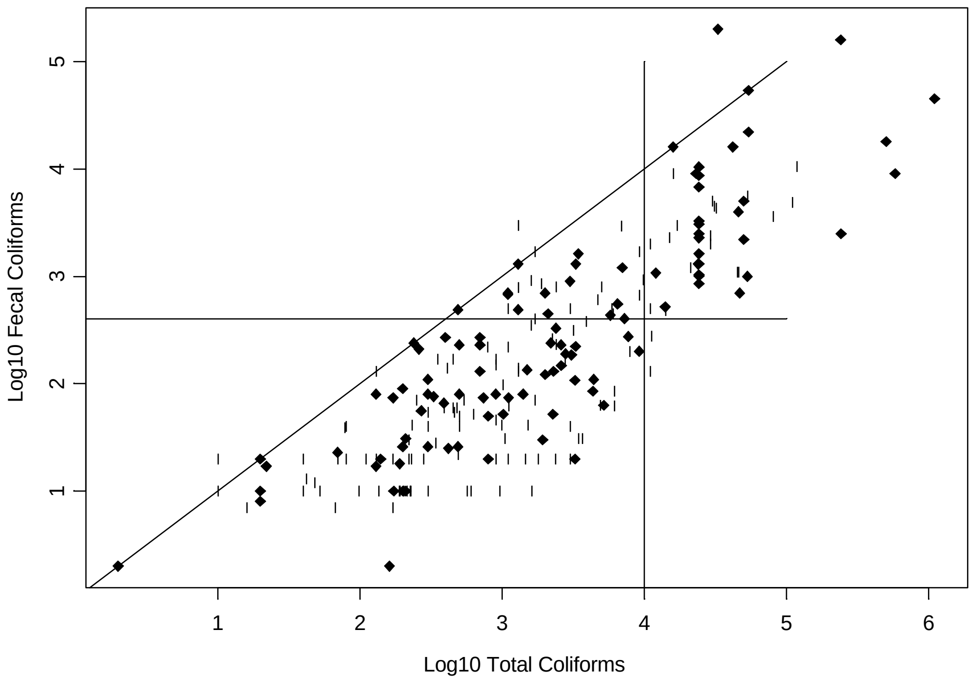


FIGURE IV-4: Percent correspondence of indicator threshold exceedences for all shoreline stations.

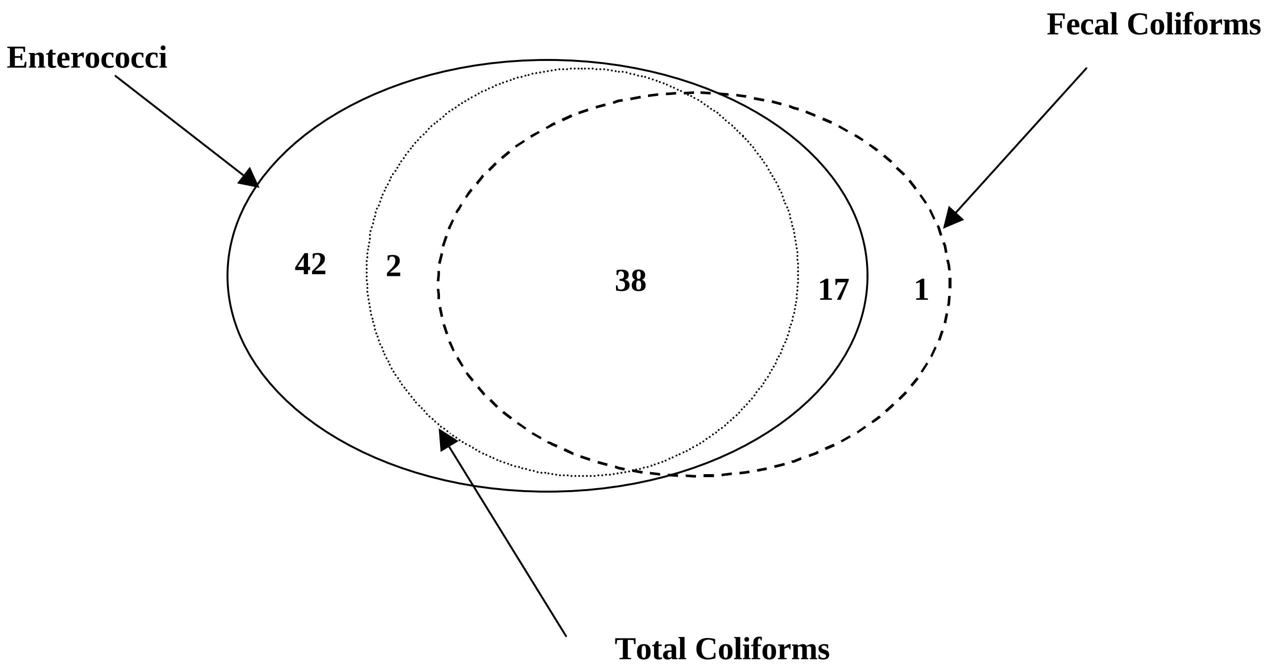


FIGURE IV-5: Percent correspondence of indicator threshold exceedences at beaches.

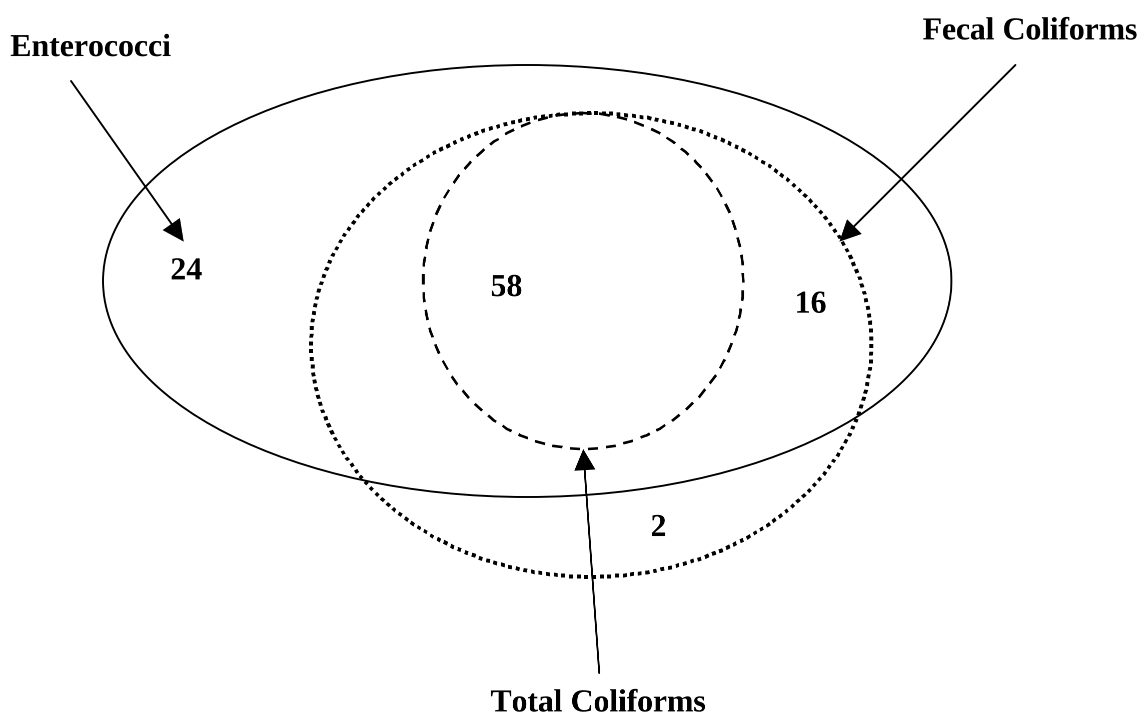
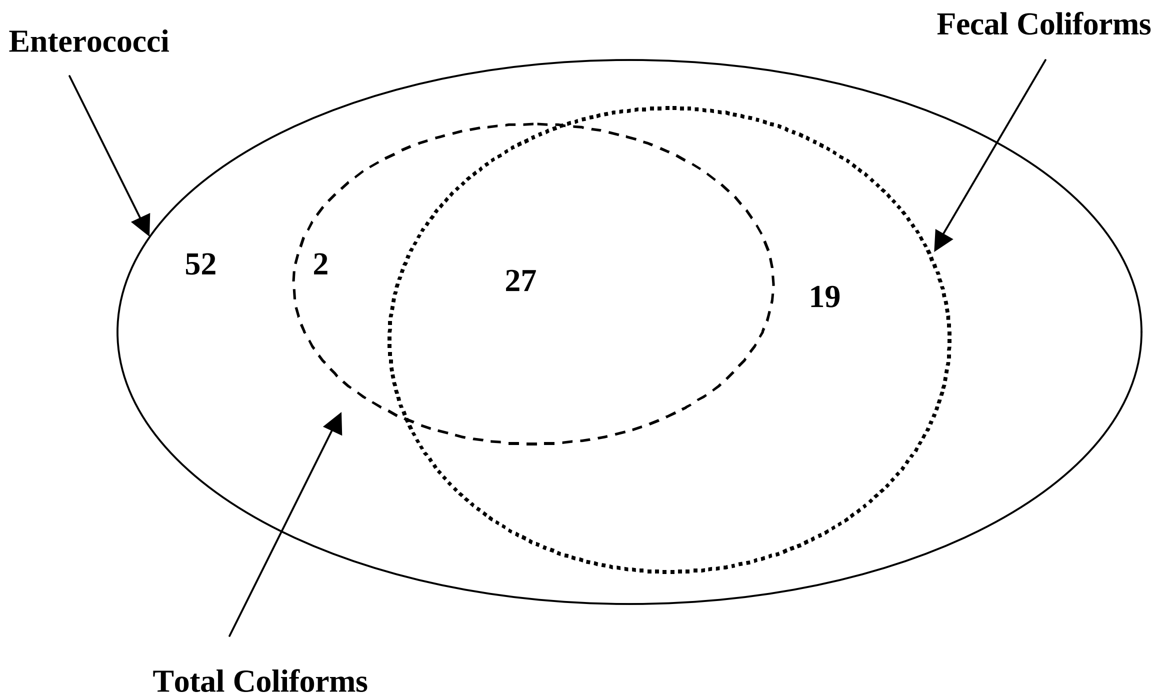


FIGURE IV-6: Percent correspondence of indicator threshold exceedences at freshwater outlets.



V. CONCLUSIONS

- **While the vast majority of beaches met State of California water quality standards during dry–weather conditions, the majority of beaches exceeded State of California water quality standards during wet-weather (storm event) conditions.**

  Fifty-six percent of the shoreline exceeded water quality standards during the Storm Event Shoreline Microbiology Study, compared to less than 6% that exceeded standards in a similar summer study conducted during a dry period (Summer 1998 Study). Moreover, the magnitude of the exceedences was much greater during the Storm Event Shoreline Microbiology Study. During the Summer 1998 Study, two-thirds of the water quality exceedences were attributable to the exceedence of a single bacterial indicator and most of those exceedences were barely above the indicator threshold. During the Storm Event Shoreline Microbiology Study, two-thirds of the exceedences were for multiple indicators in which at least one indicator was twice the allowable standard.

- **The ocean waters adjacent to freshwater outlets (storm drains) demonstrated the worst water quality regardless of the weather.**

  Following a rain event, the ocean waters adjacent to 90% of southern California's freshwater outlets exceeded water quality standards. During dry weather, 60% of freshwater outlets were associated with poor water quality. The magnitude of water quality exceedences near freshwater outlets was always higher than the magnitude of exceedences away from freshwater outlets regardless of wet or dry weather.

- **A much greater degree of consistency was found among bacterial indicators during a storm event than during dry weather.**

  The State of California requires the measurement of three bacterial indicators from April through October. In dry weather, these indicators were poorly correlated and almost 80% of the water quality threshold exceedences were attributable to a single indicator only. Following a storm event, less than half of the exceedences were for just a single indicator and the correlation of indicator values was considerably higher than in dry-weather conditions. In both wet-weather and dry-weather conditions, the number of exceedences attributable to enterococci was much higher than for either of the other two indicators.

- **The newly developed chromogenic substrate test method yielded results comparable to standard methods.**

  Laboratories in southern California have historically used two state-certified methods, membrane filtration and multiple tube fermentation, to measure bacterial concentrations in ocean water. Recent advances have produced a new method, the chromogenic substrate technique, which is less expensive and faster than conventional tests. This method, produced commercially by Idexx Laboratories, Inc., is now being used by some monitoring organizations in southern California. Previous tests conducted in southern California have shown this method to yield results comparable to standard methods in laboratory comparisons. This study was the first to compare these methods in side-by-side field tests conducted during wet–weather conditions. The results produced by the use of chromogenic substrate methods were comparable to those from conventional methods.

VI. RECOMMENDATIONS

- **Additional studies be conducted to explore the relationship between flow from freshwater outlets and the extent of runoff impacts.**

  Health care agencies in southern California routinely issue 72-h advisories regarding poor beach water quality following a rain event that produces at least 0.1 inch of precipitation. This study demonstrates that microbiological water quality is dramatically degraded following a rainstorm. It was also found that following a rainstorm of more than 2 inches, some storm drains were still demonstrating very limited flow. A retrospective analysis of monitoring data from the Santa Monica Bay suggests that a storm's degrading effects on water quality begin to diminish within 24 h. Also, anecdotal evidence suggests that most storms of 0.25 inch or less do not cause the widespread water quality exceedences observed in this study. As a consequence and because of the economic importance of the region's beaches, the public will benefit from additional research to define the relationship between the size of a storm, the discharges from storm drains both during storms and during dry weather, and the duration of water quality impairment at specific beaches.

- **A greater emphasis be placed on upstream investigations to identify the sources of runoff contamination, which will require better integration between stormwater management agencies and the county health departments.**

  This study, when combined with the previously reported regional dry-weather studies, suggests that most of the water quality exceedences on southern California beaches originate with land-based runoff. Solving this problem requires a better understanding of upstream contamination sources. Some work has begun in this area, and the State of California has required the development of investigation protocols (as the result of Assembly Bill 538), but very little work has been accomplished and the relationship between upstream sources and beach impacts is poorly understood. More research is necessary to develop practical tools that can develop this source-impact linkage.

  One important aspect in solving the upstream contamination problem will be the integration of stormwater management agency efforts with those of organizations presently monitoring water quality on the beaches. Virtually all of the routine monitoring in ocean waters near freshwater outlets is conducted by county health departments or by sewage treatment agencies with ocean discharges. These organizations have jurisdictions too limited to address the problems observed near freshwater outlets. This dissociation between the organizations that conduct coastal monitoring and the organizations that bear the responsibility for managing runoff problems is inefficient and usually ineffective in protecting the public's health. Several of the stormwater management agencies in southern California maintain bacterial monitoring programs for inland waters, but these programs are not integrated with the ocean monitoring programs.

The role of stormwater agencies in the shoreline monitoring network should be an important one. Their participation will allow them to react promptly to the monitoring results and will establish a framework for the integration of their inland efforts with the shoreline monitoring programs. An active partnership with the stormwater agencies is beginning to occur. The City of Los Angeles Stormwater Division recently began sharing the costs of routine shoreline bacterial monitoring in the Santa Monica Bay, and the stormwater agencies for Orange, Riverside, and San Bernardino counties were co-sponsors of this regional monitoring program. It is recommended that this cooperative interaction be continued and expanded.

- **The use of three bacterial indicators during rain events months be reevaluated.**

  The implementation of Assembly Bill 411 requires that the health departments within the State of California measure three bacterial indicators (total coliforms, fecal coliforms, and enterococci) at high-use beaches from April through October. In practice, most counties have extended this requirement to include all beaches and all seasons. In contrast, the U.S. EPA's national guidance advocates the use of a single indicator, enterococci, for marine waters. Our studies suggest that enterococci is the best indicator if a single indicator is to be selected. However, the measurement of three indicators appears to be warranted in summer, as poor agreement has been observed among these indicators in summer and insufficient scientific evidence exists to select one indicator over the others. However, the case for three indicators is less clear in the winter, when much greater agreement occurs among the indicators. This was most pronounced during the Storm Event Shoreline Microbiology Study, during which 99% of the water quality exceedences identified through the measurement of total coliforms or fecal coliforms were also identified through the measurement of enterococci. The effort expended in monitoring three indicators during the winter months might be more cost-effectively expended by sampling more beach sites or sampling at more frequent intervals, especially for groups with limited resources.

- **The State of California and the U.S. EPA should approve the Idexx chromogenic substrate method for use in the bacterial testing of marine waters.**

  The State of California Department of Health Services and the U.S. EPA certify laboratory procedures for the measurement of bacterial indicators and have presently certified two methods, membrane filtration and multiple tube fermentation, for use in marine waters. This study, combined with our previous studies, demonstrates that Idexx's commercial chromogenic substrate method produces results comparable to these conventional test methods. The new method is also faster than conventional tests, allowing county health departments to issue more timely warnings to the public. The State Department of Health Services has approved the chromogenic substrate method on an interim basis for use in marine waters and the U.S. EPA has certified the method for use in freshwater. This more cost-effective method will not be fully implemented until these organizations certify it for bacterial testing in marine waters on a permanent basis. A firm scientific foundation appears to support such certification.

VII. LITERATURE CITED

American Public Health Association (APHA). 1995. Standard Methods for the Examination of Water and Wastewater, 18th Edition. Edited by A.D. Eaton, L.S. Clesceri, and A.E. Greenberg. Washington, DC.

Bay, S., Jones, B.H., and Schiff, K. 1999. Study of the Impact of Stormwater Discharge on Santa Monica Bay. Executive Summary Report prepared for the Los Angeles County Department of Public Works, Alhambra, CA. USC Sea Grant Program, Los Angeles, CA. (USCSG-TR-02-99)

Cabelli, V.J. 1983a. Public health and water quality significance of viral diseases transmitted by drinking water and recreational water. Water Science Technology 15:1-15.

Cabelli, V.J. 1983b. A marine recreational water quality criterion consistent with indicator concepts and risk analysis. Journal of the Water Pollution Control Federation 55:1306-1314.

Cabelli, V.J., A.P. Dufour, L.J. McCabe, and M.A. Levin. 1982. Swimming-associated gastroenteritis and water quality. American Journal of Epidemiology 115:606-616.

Cross, J., K. Schiff, and H. Schaefer. 1992. Surface runoff to the Southern California Bight. pp. 19-28 *in*: J. Cross (ed.), Southern California Coastal Water Research Project Annual Report 1989-90. Long Beach, CA.

Dufour, A.P. 1984. Bacterial indicators of recreational water quality. Canadian Journal of Public Health 78:49.

Engineering Science, Inc. (ES). 1989. Santa Monica Bay Stormwater Pollutant Reduction Study. Prepared for the City of Los Angeles Wastewater Program Management Division. Engineering-Science, Inc. Pasadena, CA.

Gold, M., M. Bartlett, J. Dorsey, and C.D. McGee. 1990. An Assessment of Inputs of Fecal Indicator Organisms and Human Enteric Viruses from Two Santa Monica Storm Drains. Santa Monica Bay Restoration Program. Monterey Park, CA.

Gold, M., M. Bartlett, J.H. Dorsey, and C. McGee. 1991. Storm Drains as a Source of Indicators to Nearshore Waters of Santa Monica Bay. Santa Monica Bay Restoration Project. Monterey Park, CA.

Gold, M., M. Bartlett, C. McGee, and G. Deets. 1992. Pathogens and Indicators in Storm Drains within the Santa Monica Bay Watershed. Santa Monica Bay Restoration Project. Monterey Park, CA.

Haile, R.W., J.S. Witte, M. Gold, R. Cressey, C.D. McGee, R.C. Millikan, A. Glasser, N. Harawa, C. Ervin, P. Harmon, J. Harper, J. Dermand, J. Alamillo, K. Barrett, M. Nides, and G. Wang. 1999. The health effects of swimming in ocean water contaminated by storm drain runoff. J. Epidemiology 104:355-363.

Hanes, N.B., and C. Fragala. 1967. Effect of seawater concentration on the survival of indicator bacteria. Journal of the Water Pollution Control Federation 39:97.

Hickey, B. and N. Kachel. In press. The influence of river plumes in the Southern California Bight. *Continental Shelf Research.*

Kay, D., J.M. Fleisher, R.L. Salmon, F. Jones, M.D. Wyer, A.F. Godfree, Z. Zelenauch-Jacquotte, R. Shore. 1994. Predicting likelihood of gastroenteritis from sea bathing: Results from randomized exposure. Lancet 344:905-909.

Messer, J.J., C.W. Ariss, J.R. Baker, S.K. Drousé, K.N. Eshleman, P.N. Kaufmann, R.A. Lithurst, J.M. Omernik, W.S. Overton, M.J. Sale, R.D. Shonbrod, S.M. Stanbaugh, and J.R. Tutshall, Jr. 1986. National Surface Water Survey: National Stream Survey, Phase I - Pilot Survey. EPA-600/4-86-026. U.S. Environmental Protection Agency. Washington, DC.

Noble, R.T., J.H. Dorsey, M.K. Leecaster, M. Mazur, C.D. McGee, D. Moore, V. Orozco-Borbon, D. Reid, K. Schiff, P.M. Vainik, and S.B. Weisberg. 1999a. Southern California Bight 1998 Regional Monitoring Program: Volume I. Summer Shoreline Microbiology. Southern California Coastal Water Research Project. Westminster, CA.

Noble, R.T., J.H. Dorsey, M.K. Leecaster, C.D. McGee, D. Moore, V. Orozco-Borbon, P.M. Vainik, and S.B. Weisberg. 1999b. Southern California Bight 1998 Regional Monitoring Program: Volume II. Winter Shoreline Microbiology. Southern California Coastal Water Research Project. Westminster, CA.

Noble, R.T., J. Dorsey, M. Leecaster, D. Reid, K. Schiff, S.W. Weisberg. 2000. A regional survey of the microbiological water quality along Southern California Bight shoreline. Environmental Monitoring and Assessment. In press.

Noble, R.T., and J.A. Fuhrman. 2000. Enteroviruses detected in the coastal waters of Santa Monica Bay, California: Low relation to bacteriological indicators. In press. Hydrobiologia.

Nuzzi, R., and R. Burhans. 1997. The use of enterococcus and coliforms in characterizing bathing-beach waters. Journal of Environmental Health 60:16-22.

Overton, S.W. 1987. A sampling and analysis plan for streams, in the national surface water survey conducted by EPA. Technical Report No. 117. Department of Statistics, Oregon State University. Corvallis, OR.

Rajala, R.L., and H. Heinonen-Tanski. 1998. Survival and transfer of faecal indicator organisms of wastewater effluents in receiving lake waters. Water Science and Technology 38 (2) 191-194.

Schiff, K. 1997. Review of existing stormwater monitoring programs for estimating Bight-wide mass emissions from urban runoff. pp. 44-55 *in:* Weisberg, S.B., and C. Francisco (eds.), Southern California Coastal Water Research Project Annual Report 1996. Southern California Coastal Water Research Project. Westminster, CA.

Schiff, K., J. Dorsey, and S.B. Weisberg. 2000. Microbiological monitoring of marine recreational waters in Southern California. In press. Environmental Management.

Schiff, K., S. Weisberg, and J. Morton. In press. Retrospective evaluation of shoreline water quality *in*: Weisberg, S., and D. Elmore (eds.), Southern California Coastal Water Research Project Annual Report 1999-2000. Westminster, CA.

Schiff, K., and P. Kinney. In press**.** Tracking sources of bacterial contamination in stormwater discharges from Mission Bay, California *in*: Weisberg, S. and D. Elmore (eds.), Southern California Coastal Water Research Project Annual Report 1999-2000. Westminster, CA.

Sieracki, M. 1980. The effects of short exposures of natural sunlight on the decay rates of enteric bacteria and coliphage in a simulated sewage outfall microcosm. Master of Science Thesis, University of Rhode Island.

Southern California Association of Governments (SCAG). State of the Bay Scientific Assessment. 1988. Microbial risk assessment.

Stevens, D.L., Jr., and T.M. Kincaid. 1997. Variance estimation for subpopulation parameters from samples of spatial environmental populations. Proceedings of the American Statistical Association Section on Statistics and the Environment. American Statistical Association. Alexandria, VA.

Thompson, S.K. 1992. Sampling. Wiley and Sons. New York, NY.

United States Lifesaving Association (USLA). 1998. National lifesaving statistics.
.

APPENDIX A: LIST OF PARTICIPANTS IN THE SOUTHERN CALIFORNIA BIGHT REGIONAL MONITORING PROGRAM

[a] Denotes participants in the Storm Event Shoreline Microbiology component.

AES Corporation
Algalita Marine Research Foundation
Aliso Water Management Agency (AWMA)[a]
Aquatic Bioassay and Consulting (ABCL)[a]
California Coastal Conservancy
Central Coast Regional Water Quality Control Board[a]
Channel Islands National Marine Sanctuary (CINMS)
Chevron USA Products Company
City of Long Beach[a]
City of Los Angeles Environmental Monitoring Division (CLAEMD)[a]
City of Los Angeles Stormwater Division[a]
City of Oceanside[a]
City of Oxnard[a]
City of San Diego[a]
City of Santa Barbara[a]
City of Ventura[a]
Columbia Analytical Services
Commission for Environmental Cooperation[a]
Divers Involved Voluntarily in Environmental Rehabilitation & Safety (DIVERS)
Encina Wastewater Authority[a]
Goleta Sanitation District[a]
Granite Canyon Marine Pollution Studies Lab
Houston Industries, Inc.
Instituto de Investigaciones Oceanologicas, Universidad Autonoma de Baja California (UABC)[a]
Los Angeles Department of Water and Power
Los Angeles County Department of Beaches & Harbors[a]
Los Angeles County Department of Health Services[a]
Los Angeles Regional Water Quality Control Board[a]
Los Angeles County Sanitation Districts (LACSD)[a]
National Fisheries Institute of Mexico (SEMARNAP)
NOAA-NOS International Programs Office[a]
NRG Energy, Inc.
Orange County Environmental Health Division[a]
Orange County Public Facilities and Resources Department (OCPFRD)
Orange County Public Health Laboratory[a]
Orange County Sanitation District (OCSD)[a]
San Diego County Department of Environmental Health[a]
San Diego Interagency Water Quality Panel (Bay Panel)
San Diego Regional Water Quality Control Board[a]

San Elijo Joint Powers Authority[a]
Santa Ana Regional Water Quality Control Board[a]
Santa Barbara Public Health Department[a]
Santa Monica Bay Restoration Project
South East Regional Reclamation Authority (SERRA)[a]
Southern California Coastal Water Research Project (SCCWRP)[a]
Southern California Edison (SCE)
Southern California Marine Institute (SCMI)[a]
State Water Resources Control Board (SWRCB)[a]
Surfrider Foundation [a]
USC Wrigley Institute for Environmental Studies (WIES)[a]
University of California, Santa Barbara
United States EPA Region IX
United States EPA Office of Research and Development
United States Geological Survey
United States Navy, Space & Naval Warfare Systems Center, San Diego (USN)
Ventura County Health Department[a]
Ventura County Environmental Health Division[a]

APPENDIX B: METHODS COMPARISON

Introduction

Coastal waters are an important economic and recreational resource that is influenced by human activities. Treated wastewater discharges, and non-point-source industrial inputs and surface runoff all affect coastal water quality and create the impetus for extensive water quality monitoring programs. The main criterion for assessing the potential health risk of recreational waters to swimmers is the density of indicator bacteria. Although indicator bacteria do not necessarily cause illness, they are abundant in human waste where pathogenic organisms, such as pathogenic bacteria, viruses, and parasites, are also likely to exist. The bacteria most commonly used as indicators of fecal contamination are total coliforms, fecal coliforms, *Escherichia coli (E. coli),* and enterococci.

Three methods are commonly used to quantify bacterial densities, membrane filtration (MF), multiple tube fermentation (MTF), and chromogenic substrate (CS) techniques. These methods differ in (1) the speed of results, from 18 to 96 h depending upon the indicator and test method used; and (2) costs for training personnel, analysis time, reagents, and supplies. If these methods were to produce comparable results, then the fastest, least expensive method would be preferred.

Numerous studies have compared results between MF and MTF, but only a few have assessed comparability between these methods and the newer CS method (Abbott *et al.* 1998, Budnick *et al.* 1996, Eckner 1998, Palmer *et al.* 1993, Bej *et al.* 1991, Covert *et al.* 1989, Noble *et al.* 1999).   These studies, conducted mostly under dry-weather conditions, have generally found high comparability between CS and the other methods. In southern California, rainfall affects ocean water quality more dramatically than in other parts of the country because the coast is highly urbanized and the flood control system is independent of the sewage system, bringing urban runoff directly into the ocean after a storm. This urban runoff has the potential to interfere with microbiological tests because it contains high levels of suspended solids. In addition, certain types of native marine bacteria, such as *Vibrio* and *Aeromonas*, can potentially contribute (as false positives) to total coliform numbers when CS methods are used.

In this study, samples were collected throughout southern California immediately after a rain event to compare results from the Idexx kits, which involve commercial application of the CS methods, to the standard methods of MF and MTF. Comparisons were conducted for enterococci, fecal coliforms (*E. coli*), and total coliforms.

Methods

Samples were collected from 79 sites along the Southern California Bight (SCB) coastline on February 22, 2000, which was one day after a storm produced from 1.1 to 3.0 inches of rain over the entire region in sufficient quantities to induce flow of the major freshwater outlets into the ocean. The sample sites were selected using a stratified random sampling design, stratified by open beach (31 sites) and sites located within 100 meters of a freshwater outlet (48 sites). All samples were collected in ankle-deep water on an incoming wave just prior to receding, with the sampler positioned downstream from the bottle and the mouth of the bottle facing into the current.

Samples were split and processed using both the Idexx method and the methods used as the standard operating procedure by the six laboratories that participated in the study. Standard methods used included 9221B, C and E, 9222B and D, 9230B and C in *Standard Methods for the Examination of Water and Wastewater*, APHA, AWWA, WEF, 18^{th} Edition, 1995, and EPA Method 1600 (for enterococci) (APHA 1995). Not all laboratories used both methods on all samples, yielding 75 analyses for total coliforms, 51 analyses for fecal coliforms (the Idexx method is limited to *E. coli*), and 48 analyses for enterococci.

The bacterial densities were compared between methods using both paired t-tests and Pearson correlation coefficients, after log transformation. These analyses were conducted using all data, as well as by method type and sample site type. The comparisons were also conducted categorically by assessing the consistency of sample classification with respect to the State of California's State Beach Water Quality Standards (10,000 MPN or cfu/100 mL for total coliforms, 400 MPN or cfu/100 mL for fecal coliforms, and 104 MPN or cfu/100 mL for enterococci).

Results

The correlation between results obtained using the Idexx kits and standard methods was high (0.91-0.92) for all indicators (Table 1, Figure 1). Except for the comparison of fecal coliforms/*E. coli* by MTF, correlations for individual standard methods also exceeded 0.91. Similarly, correlations between methods were high regardless of whether the samples were collected on open beaches or near freshwater outlets. However, the correlation was somewhat lower (0.84) for fecal coliforms at freshwater outlets. None of the comparisons between indicators were found to be significantly different using the t-test (Table 2).

We found 90 to 95% agreement with respect to the State of California's Beach Water Quality Standards between methods for all three indicators in the categorical analysis (Table 3). The greatest agreement occurred for total coliforms, with 95% agreement; the 5% of samples that disagreed exceeded the standard for the Idexx method while meeting the standard for the laboratory's conventional method. An 8% disagreement rate was found for fecal coliforms, 6% demonstrating higher results for the Standard Method and 2% demonstrating higher results for the Idexx method.

Enterococci results showed the widest variation, with 10% of the samples evenly spread between Idexx being higher and lower than the Standard Methods.

Discussion

Our finding that the Idexx method yielded comparable results to standard methods is consistent with previous cross-laboratory intercalibration studies (Leecaster *et al.* 2000, Noble *et al.* 2000) as well as previous within-laboratory split sample studies. Our study expands upon these studies by sampling a variety of locations during a period of high urban runoff, thereby allowing us to sample a wide range of bacteria concentrations. The bacterial concentration gradients we measured were large, ranging from 10–220,000 for enterococci, 10–200,000 for fecal coliforms, and 10–1,100,000 for total coliforms, with the upper end of the range for each bacterial indicator exceeding State standards by at least 100 fold. The comparability of results over these large ranges, particularly at a time when interferences are likely to be greatest, provides more assurance of comparability in all environmental samples.

Although comparability was high, some systematic differences were noted between the results from the Idexx method and standard methods. Total coliform results using the Idexx method were slightly higher than for standard methods (Figure 1, Table 2), consistent with results reported by Palmer *et al.* (1993). It has been suggested that the Idexx method yields false positives with members of *Vibrio* and *Aeromonas*, which would explain the higher values. However, the difference between the methods was still statistically insignificant, suggesting that this interference is small and/or isolated to a limited number of samples. Enterococci results from Idexx kits are also generally higher (Figure 1), a trend that was observed in one previous study (Eckner 1998). In two of the samples in which the Idexx method was found to be above the threshold while the standard method was not, the Idexx analyses were verified through Vitech analysis. Conversely, in two of the cases where standard methods were found to be above the threshold while Idexx was not, the standard method was verified through Vitech analysis. These confirmations suggest that both methods are providing correct results and that differences between them are attributable to random measurement error.

A more systematic difference found in the comparison of the two types of methods was the lower fecal coliform values recorded by the Idexx method (Figure 1, Table 2). This difference reflects the fact that the Idexx method is limited to measurement of *E. coli*, which is a subset of fecal coliforms. We found a 93% slope to regression between fecal coliforms and *E. coli*. The State of California Department of Health Services has suggested that, because of the difference in target bacteria between these methods, measurements of *E. coli* using the Idexx method should be increased by 20% when comparing results to a fecal coliform standard. Our results suggest that this is too large a conversion factor. Moreover, even though the overall relationship was linear and highly correlated, individual sample values had ratios of *E. coli*/fecal coliforms ranging from 0.05 to 4.1. This finding suggests that the ratio of *E. coli* to fecal coliforms can only be applied to a population as a whole and is not to be used for correcting individual sample values. The additional study of this conversion principle is warranted, but in the absence of such study, the error introduced by the conversion appears to be larger than the difference between methods.

TABLE 1. Correlation between Idexx and other methods. Results are for overall, segregated by method and segregated by sample site type.

	Fecal Coliforms	Total Coliforms	Enterococci
Overall	0.91	0.91	0.92
Membrane Filtration	0.92	0.92	0.93
Multiple Tube	0.79	0.91	NA
EPA 1600	NA	NA	0.94
Beaches	0.95	0.92	0.92
Outlets	0.84	0.92	0.93

TABLE 2. P-value for paired t-test between methods.

Indicator	P – value
Fecal Coliforms	0.27
Total Coliforms	0.35
Enterococci	0.45

TABLE 3. Threshold agreement between methods. Numbers represent the percent of samples within each category.

	FECAL COLIFORMS	
	Standard Method < 400	Standard Method > 400
Idexx < 400	55	6
Idexx > 400	2	37
	TOTAL COLIFORMS	
	Standard Method < 10,000	Standard Method > 10,000
Idexx < 10,000	64	0
Idexx > 10,000	5	31
	ENTEROCOCCI	
	Standard Method < 104	Standard Method > 104
Idexx < 104	38	4
Idexx > 104	6	52

FIGURE 1. Standard method results versus Idexx results for each indicator. Diagonal lines represent one-to-one relationship. Horizontal and vertical lines at threshold values.

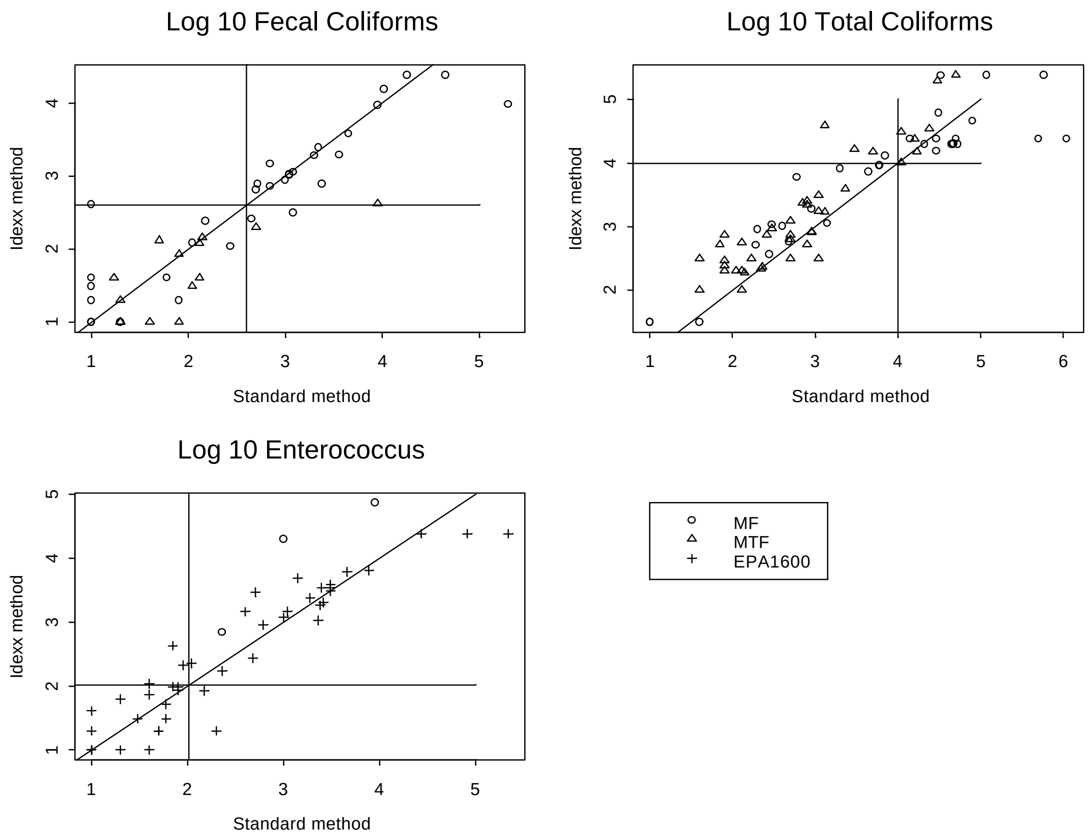
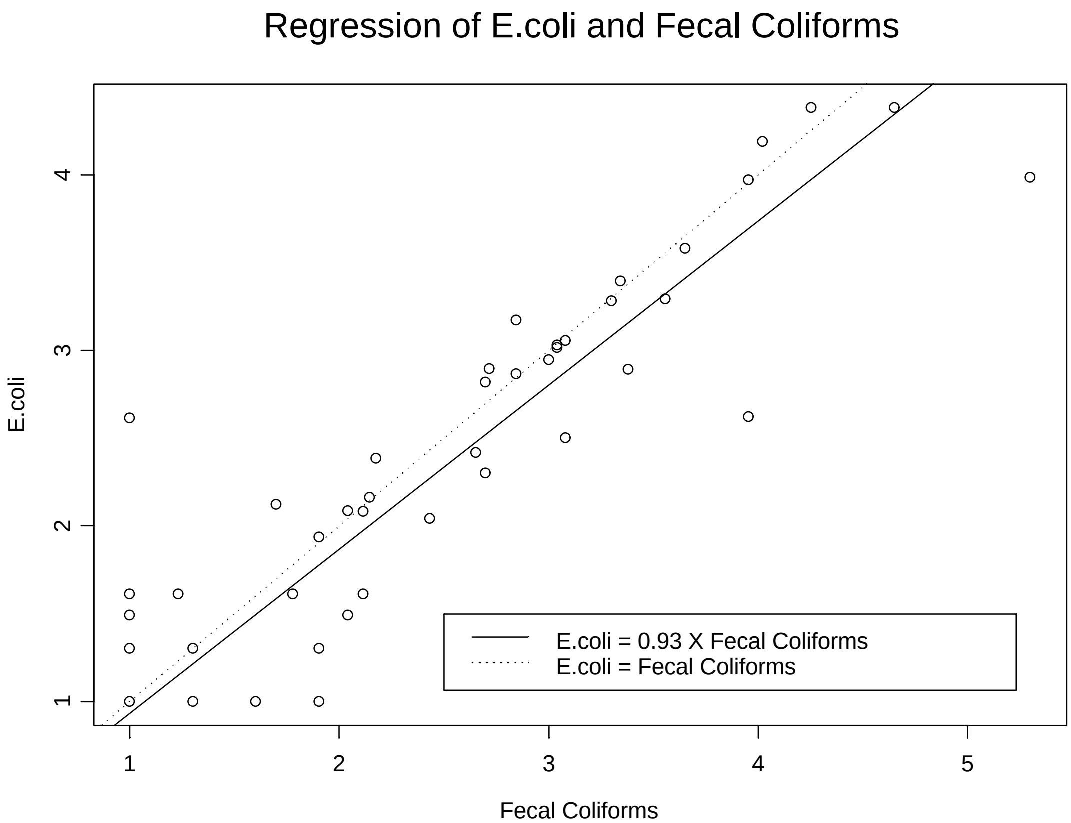
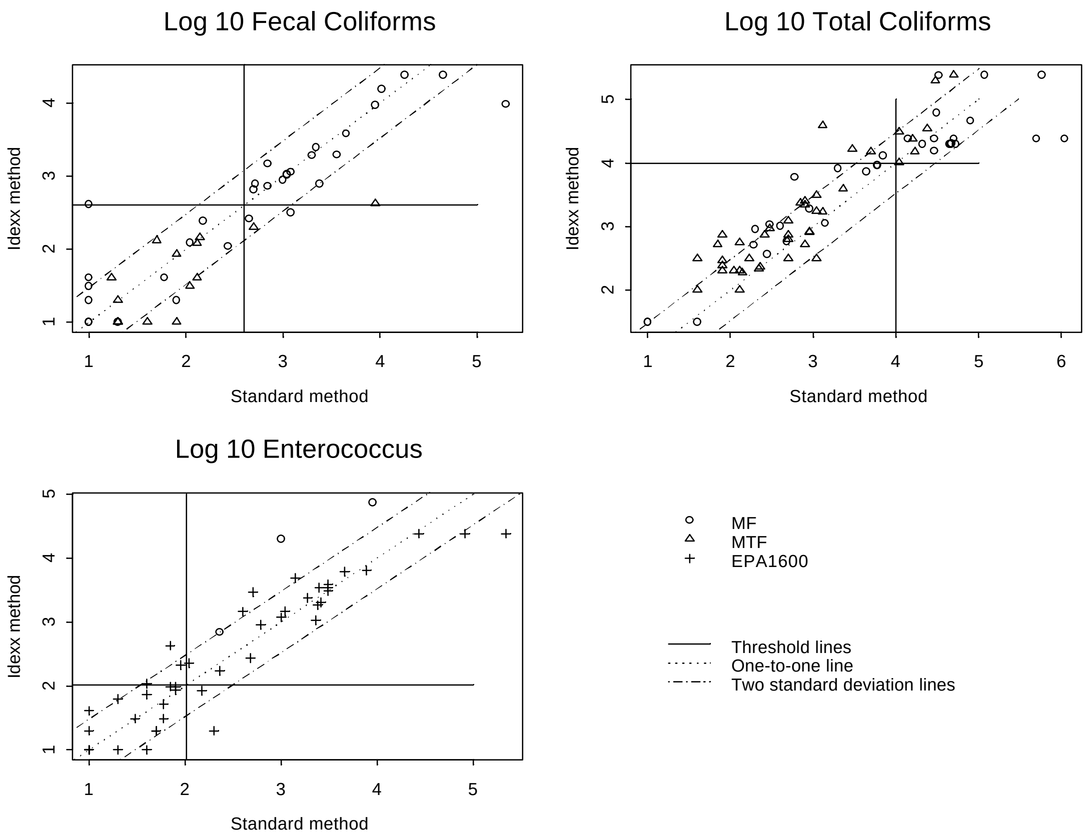
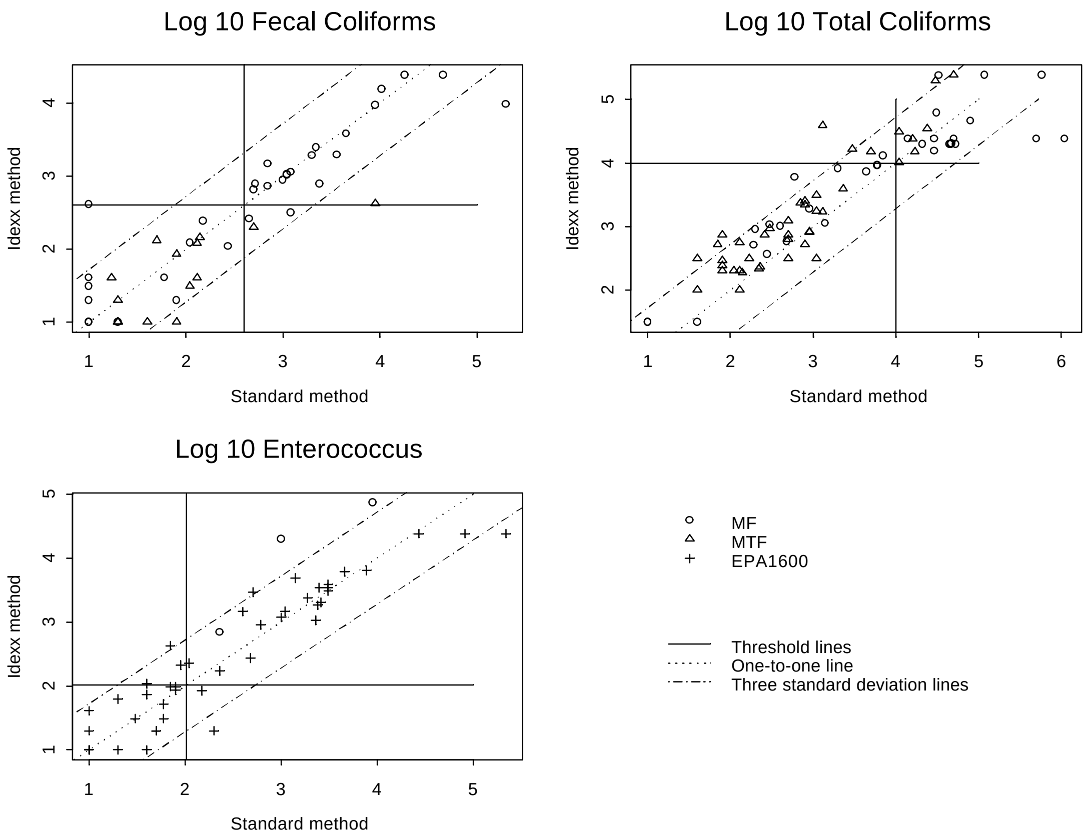


FIGURE 2. Regression comparison of fecal coliforms and *E.coli* counts.




Log 10 Fecal Coliforms
Log 10 Total Coliforms
Log 10 Enterococcus
Idexx method
Standard method
MF
MTF
EPA1600
Threshold lines
One-to-one line
Two standard deviation lines


Log 10 Fecal Coliforms
Log 10 Total Coliforms
Log 10 Enterococcus
Idexx method
Standard method
1
2
3
4
5
6
MF
MTF
EPA1600
Threshold lines
One-to-one line
Three standard deviation lines

Literature Cited

Abbott, S.B. Caughley, and G. Scott. 1998. Evaluation of Enterolert® for enumeration of enterococci in the marine environment. New Zealand Journal of Marine and Freshwater Research 32:505-515.

American Public Health Association (APHA). 1995. Standard Methods for the Examination of Water and Wastewater, 18th Edition. Edited by A.D. Eaton, L.S. Clesceri, and A.E. Greenberg. Washington, DC.

Bej, A.K., S.C. McCarty, and R.M. Atlas. 1991. Detection of coliform bacteria and *Escherichia coli* by multiplex polymerase chain reaction: comparison with defined substrate and plating methods for water quality monitoring. Applied and Environmental Microbiology 57:2429-2432.

Budnick, G.E., R.T. Howard, and D.R. Mayo. 1996. Evaluation of Enterolert® for enumeration of enterococci in recreational waters. Applied Environmental Microbiology 62:3881-3884.

Covert, T.C., L.C. Shadix, E.W. Rice, J.R. Haines, and R.W. Freyberg. 1989. Evaluation of the autoanalysis Colilert test for detection and enumeration of total coliforms. Applied and Environmental Microbiology 55:2443-2447.

Eckner, K.F. 1998. Comparison of membrane filtration and multiple-tube fermentation by Colilert® and Enterolert® methods for detection of waterborne coliform bacteria, *Escherichia coli*, and enterococci used in drinking and bathing water quality monitoring in southern Sweden. Applied and Environmental Microbiology 64:3079-3083.

Leecaster, M. C. McGee, R.T. Noble, P. Vainik, K. Walker. 2000. Comparison of bacterial indicator measurements among Southern California marine monitoring laboratories. Submitted. Water Research.

Noble, R.T., J.H. Dorsey, M.K. Leecaster, M. Mazur, C.D. McGee, D. Moore, V. Orozco-Borbon, D. Reid, K. Schiff, P.M. Vainik, and S.B. Weisberg. 1999. Southern California Bight 1998 Regional Monitoring Program: Volume I. Summer Shoreline Microbiology. Southern California Coastal Water Research Project. Westminster, CA.

Noble, R.T., J. Dorsey, M. Leecaster, D. Reid, K. Schiff, S.W. Weisberg. 2000. A regional survey of the microbiological water quality along Southern California Bight Shoreline. Environmental Monitoring and Assessment. In press.

Palmer, C.J., Y. Tsai, A.L. Lang, and L.R. Sangermano. 1993. Evaluation of Colilert®-marine water for detection of total coliforms and *Escherichia coli* in the marine environment. Applied and Environmental Microbiology 59:786-790.