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Shoreline Microbiology

SOUTHERN CALIFORNIA BIGHT 1998 REGIONAL MONITORING PROGRAM Vol. I

Southern California Bight 1998 Regional Monitoring Program: I. Summer Shoreline Microbiology

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FOREWORD

This study was a cooperative effort in which 60 organizations (Appendix A) joined to assess the overall condition of the southern California near-coastal ecosystem. This study was coordinated by the Southern California Coastal Water Research Project (SCCWRP) as one component of the Southern California Bight 1998 Regional Monitoring Program (Bight'98), and builds upon the success of a similar SCCWRP-coordinated regional monitoring program conducted in 1994 that assessed the condition of offshore ecological habitats (SCBPP 1994). Copies of this and other Bight'98 reports are available for download at www.sccwrp.org.

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This report is the culmination of many hours of hard work by the dedicated individuals at the 36 organizations that participated in the Shoreline Microbiology component of Bight'98. 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; Dr. Jed Fuhrman for his contributions to the viral indicator studies; and Drs. Mark Gold and Robert Haile for their review of and suggestions about the report.

Definition of Terms:

Enterovirus: Genus (subset) of the human enteric virus family, other genera include reovirus and adenovirus.
Ephemeral Freshwater Outlet: Outlet that typically only flows for a portion of the year, not year-round.
Exceedance: Bacterial indicator level that is equal to or above a threshold.
Freshwater Outlet: Natural or constructed freshwater source associated with multiple land use types (for example: urban, rural, agricultural, or industrial).
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-3.
Perennial Freshwater Outlet: Outlet with year-round flow into the surf-zone.
Point Zero Freshwater Outlet Sample: In this study, a sample that was taken at the mouth of a freshwater outlet, at the location of surfzone-freshwater mixing.
Random Freshwater Outlet Sample: In this study, a sample that was taken at a random location within 100 yards of the mouth of a freshwater outlet (for the study, this was done only at perennial freshwater outlets).
Reverse Transcriptase Polymerase Chain Reaction (RT-PCR): Molecular biology primer-based technique for the detection of RNA sequences.
Standard: Level of water quality measurement (characteristic) for ocean waters set by State statute and regulations, e.g., Assembly Bill 411 which refers to bacteriological indicator levels. See Table II-3.
Storm Drain: Subset of the freshwater outlets that do not have main source from freshwater inputs, rather their source is primarily stormwater (from storm events) and their 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-3.
Urban Runoff: Runoff from a freshwater outlet or storm drain whose watershed is primarily urban land use area.
Viral genome: The complete set of genes contained in a virus particle (can be either RNA or DNA, single or double stranded).

EXECUTIVE SUMMARY

More than 80,000 shoreline bacteriological samples are collected annually in southern California, representing roughly one-half of the total bacteriological monitoring conducted in the United States. Despite this impressive amount of bacteriological monitoring, these data are difficult to integrate for a regional assessment of bacteriological water quality because they are collected by 22 different organizations, many of which have different sampling strategies and different data management systems. Additionally, the monitoring programs are focused upon sampling in known "problem areas," which does not allow for an assessment of typical shoreline microbiological water quality. To address these limitations, all of the organizations that conduct routine monitoring in the Southern California Bight (SCB) pooled their efforts to conduct an integrated survey to assess the overall microbiological water quality of the southern California shoreline during the summer of 1998. The three primary goals for the survey were:

- To determine the percent of shoreline mile-days in the SCB that exceeded bacterial indicator thresholds during August of 1998.
- To compare the response among three bacterial indicators commonly used in California.
- To determine how well these bacterial indicator measures correlated with detection of human enteric virus genetic material.

Samples were collected on a weekly basis at 307 sites between Point Conception, California, and Punta Banda, Mexico, beginning August 2, 1998 and continuing for five weeks. Sampling sites were selected using a stratified random design, with six sampling strata: high- and low-use sandy beaches and rocky shoreline, and ephemeral and perennial freshwater outlets. Samples were collected using standardized protocols. Total and fecal coliforms were analyzed for all samples, and enterococci were measured in approximately 70% of the samples. Molecular analyses to measure the presence of human enteric virus genetic material were performed on samples collected from 15 randomly selected perennial freshwater outlet locations. The presence of this genetic material can be used as a tool to detect human fecal contamination in the coastal zone, but these analyses alone can not be used to infer health risk, as virus genetic material may not be associated with an intact, infective virus.

Before the start of the sampling period, the 22 participating laboratories conducted intercalibration studies to assess data comparability. Thirteen common samples were analyzed by each laboratory to define variability among laboratories, within laboratories, and among methods. Three analytical methods, multiple tube fermentation (MTF), membrane filtration (MF), and chromogenic substrate tests were compared for three bacterial indicators: total and fecal coliforms, and enterococci. Bacterial indicator levels were quantified from common samples to identify differences among laboratories and methodologies. The average difference among methods was less than 6%. The average difference among laboratories was less than 2%. The greatest source of variability was among replicates within individual laboratories. The intercalibration exercises demonstrated that a multi-laboratory, performance-based approach was acceptable for implementing this regional study.

Overall microbiological water quality along the southern California shoreline was good during the study period, with more than 95% of the shoreline mile-days meeting all present and proposed

California bacterial indicator standards. In 98% of the cases where a standard was exceeded, it was exceeded for only one bacterial indicator, while all other bacterial indicators at the same site and at the same time were below bacterial indicator thresholds. Less than 0.2% of the shoreline mile-days exceeded thresholds for all indicators measured at the site.

Freshwater outlets failed to meet bacterial indicator standards in almost 60% of the samples, the worst of all of the strata. Most of the standard failures near freshwater outlets were for multiple indicators and occurred repetitively throughout the five-week study period. Molecular tests demonstrated the presence of human enteric virus genetic material in 7 of the 15 freshwater outlets, with 73% of these detections coinciding with levels of fecal coliforms that exceeded bacterial indicator thresholds.

The probability of exceeding a bacterial indicator threshold differed substantially among indicators. Of the samples that exceeded a bacterial standard and for which all three indicators were measured, only 13% failed for all three indicators, 34% failed for two indicators, and 54% failed for one indicator. Thresholds for fecal coliforms were exceeded at twice the rate of total coliforms and enterococci failed at three times the rate of total coliforms. Less than one-half of the enterococci threshold failures paired with threshold failures by another indicator, while nearly 90% of the total and fecal coliforms threshold failures were partnered with failures of another indicator.

This cooperative study is the first to compare the relative quality of Mexican and United States beaches using similar site selection approaches and coordinated quality assurance methods. Although nearly 75% of the beach samples in Mexico met California's bacteriological water quality standards, the standards were exceeded five times more often on Mexican than on United States beaches. Mexican freshwater outlets were just as likely to exceed a bacteriological water quality standard as those in the United States.

I. INTRODUCTION

The Southern California Bight (SCB), an open embayment in the coast between Point Conception, California, and Cabo Colnett (south of Ensenada), Baja California, is an important and unique recreational resource. World renowned for their recreational waters, southern California beaches annually attract more than 175 million people to sunbathe, surf, swim, skin- and SCUBA-dive (USLA 1998). The SCB is also one of the most densely populated coastal regions in the country, which creates stress upon these recreational resources. Nearly 20 million people inhabit coastal southern California, a number that is projected to increase 20% by 2010 (NRC 1990). With this population growth, and the ensuing development of the land, comes the increased potential for pathogenic microorganisms such as viruses, bacteria, and protozoa to enter the coastal environment. These pathogenic microorganisms impact bacteriological water quality and pose potential health risks to beachgoers.

To assess the extent of this contamination, more than 20 agencies in southern California collectively analyze more than 80,000 samples from 510 locations at or near beaches on an annual basis. Although the scope of this bacteriological monitoring is impressive, the data collected cannot be easily integrated to provide a regional assessment of recreational water quality. Most monitoring programs are spatially focused on a small set of high-use beaches or other areas of concern; therefore, the data from these programs cannot be easily integrated. Moreover, many of the organizations involved in beach monitoring analyze different indicators or use different analytical methodologies to measure the same indicators; interlaboratory exercises to assess data comparability are rare. To address this issue, the California State Legislature recently passed Assembly Bill 411 (AB411) requiring the State Department of Health Services (SDHS) to adopt regulations that provide consistency in monitoring indicators and standards.

Recognizing the need for greater consistency and communication, all of the agencies that routinely monitor bacteriological water quality along the shoreline of the SCB coordinated their efforts for the purpose of conducting a regional survey to assess the overall condition of the southern California shoreline in the summer of 1998. Three main goals were established for this survey:

- To determine the percent of shoreline mile-days in the SCB that exceeds bacterial indicator thresholds during the summer of 1998.

  A regionally based study of microbiological water quality was conducted along the shoreline of the SCB. Sites were selected using a probability based sampling design to ensure an unbiased characterization of the coastline. The study incorporated a performance-based approach, where all participating organizations demonstrated data comparability through a series of laboratory intercalibration exercises. The focus of the effort was on the United States side of the border, but the project also included a coordinated effort conducted by Mexican scientists to extend the study area slong the coast from Tijuana to Cabo Colnett. The international participation provides the first opportunity for cross-border comparison of bacteriological water quality using comparable methods.

- To compare responses among the three bacterial indicators commonly measured in California.

  Some of the most common indicators of fecal contamination used today are total coliforms, fecal coliforms (of which *E. coli* is the major component) and enterococci. Once released into the environment, unfavorable physical and chemical conditions affect the relative survival of the fecal and non-fecal bacteriological components. Fecal coliforms may not survive as well as total coliforms in the unfavorable environment outside the gut of warm-blooded animals (Hanes and Fragala 1967, Sieracki 1980). Comparing the responses of these indicators under the differing conditions of the study strata may provide information about the responses of each indicator organism to different environmental circumstances. These results can be used to understand which indicator organisms are most "conservative" at each of several shoreline types, and to assess potential redundancy among indicators.

- To determine how well these bacterial indicator measures correlate with detection of human enteric virus genetic material

  The conventional method for assessing the sanitary quality of recreational waters worldwide is based upon the presence of indicator bacteria. Epidemiological studies of waterborne illnesses, however, show that the most common etiological agents are more likely to be viruses and protozoa (Moore *et al.* 1994, Seyfried *et al.* 1985, Cabelli *et al.* 1982, Cabelli 1983, Kay *et al.* 1994, USEPA 1986). One part of this survey assesses the presence of waterborne human enteric virus genetic material at freshwater outlets along the coast of the SCB to determine whether the presence of the genetic material of these viruses is correlated with levels of indicator bacteria. Detection of human enteric viral genetic material may be used to infer the presence of human fecal contamination, but the method cannot be used to infer health risk as genetic material is not always evidence of an intact, infectious virus particle.

Chapter II describes the methods used to accomplish the above objectives. In Chapter III, a Quality Assurance Evaluation is provided, demonstrating the successful use of a performance-based approach for the study. Chapter IV addresses the first study goal by providing an assessment of bacteriological water quality along the shoreline of the SCB. Chapter V addresses the second goal by comparing responses among the bacterial indicators measured in the study. Chapter VI addresses the third study goal by comparing the responses between viral and bacterial indicators. Conclusions from the study are presented in Chapter VII, which summarizes the study conclusions and integrates the results and ideas presented in Chapters IV, V, and VI. Chapter VIII provides recommendations that follow from the study results. Chapters IV, V, and VI are intended for a scientific audience and contain detailed technical information that provides the foundation for our conclusions and recommendations. Chapters VII and VIII are intended for a wider audience and provide a more general overview of the study findings.

II. METHODS

A. Sampling Design

The Shoreline Microbiology component of Bight'98 involved sampling at 307 sites along the SCB coastline between August 2 and September 5, 1998. Each site was sampled once per week during the 5-week study period. A 5-week study period was selected to meet the required minimum of 5 weekly samples for calculation of 30-day geometric means under the California Ocean Plan and proposed AB411 regulations. The study was conducted during summer to coincide with the period of maximum beach bathing usage.

The study area extended from Point Conception in Santa Barbara County, California, to Punta Banda, Baja California, just south of Ensenada, Mexico (Figure II-1, Appendix B). This area includes approximately 690 miles of coastline, although the sampling frame for the study included only about 270 miles, or 39% of the coastline. The remaining shoreline was classified as unreachable by swimmers due to the presence of ports, private marinas, private land, military property, or steep cliffs.

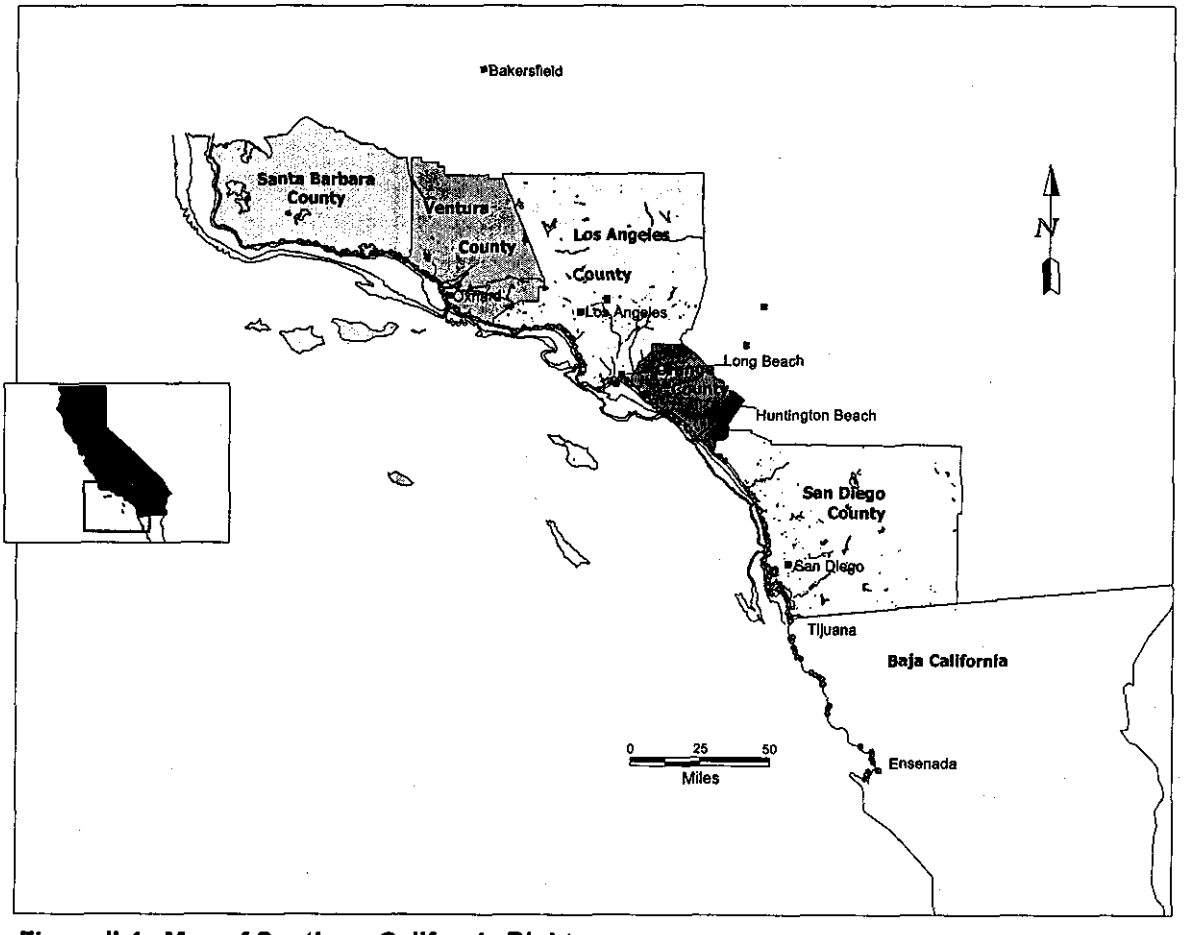


Figure II-1. Map of Southern California Bight

Sampling sites were selected using a stratified random approach, with the strata corresponding to six shoreline types of interest (Table II-1). To implement this design, a GIS layer of shoreline types was created based upon the knowledge of local shoreline conditions by the participating organizations. High- and low-use sandy beaches were differentiated by the presence of lifeguard service. High- and low-use rocky shoreline were differentiated by the presence of known preferred diving or surfing locations. 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 are those outlets that are typically responsible for 99% of the total shoreline runoff inputs to the SCB.

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

Strata	Base Sample Sites	Mexican Sample Sites	Volunteer Sample Sites	Adaptive Sample Sites
Sandy beaches				
High–use	48	19	20	11
Low–use	26			4
Rocky shoreline				
High-use	19			1
Low-use	16			
Freshwater outlets				
Ephemeral	29			5
Perennial	36			10
Perennial point zero	30	10		23
Total	204	29	20	54

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, 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 together into 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 equal lengths. 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 possessed spatial separation of sites as well as a random component to ensure statistical validity.

Sample sites within the perennial water outlet stratum were selected in two ways. 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 32 of the 40 perennial water outlets in southern California. Point zero sites were placed at 30 of the 40 systems, which were selected by availability of effort. Fifteen of these 30 point zero sites were randomly selected to receive analyses for viruses.

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 beach sites were not differentiated between high- and low-use, and point zero sites were associated with the highest flow perennial water outlets.

Volunteer Monitoring

Volunteer organizations enhanced the sampling effort with 14 sampling sites in the Los Angeles and Long Beach Harbor region of San Pedro Bay (between Cabrillo Beach and Seal Beach), and 6 sampling sites in southern Santa Monica Bay (between Ballona Creek and the Palos Verdes peninsula). Volunteer sites were limited to the high-use sandy beach stratum. Volunteer sites were selected as a supplement, rather than as an integrated part of the program, using the same statistical design described. As a supplemental overlay, these samples would not have affected 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 quality assurance requirements, their results were integrated directly into the base program.

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 Public Health Department	24[c]	24[c]	24[d]
City of Santa Barbara	7[b]	7[b]	7[d]
Goleta Sanitation District	6[b]	6[b]	6[b]
Ventura County			
Ventura WWTP	7[b]	7[b]	7[b]
City of Oxnard	7[b]	7[b]	7[b]
Aquatic Bioassay Labs	5[b]	5[b]	5[b]
Los Angeles County			
City of Los Angeles	16[a]	16[a]	16[a]
Los Angeles Co. Sanitation Districts	5[a]	5[a]	5[a]
Los Angeles Co. Dept. of Health Services	14[c]	14[c]	14[d]
City of Long Beach	1[a]	1[a]	0
Southern California Marine Institute	20[c]	20[c]	0
Orange County			
Orange Co. Sanitation District	15[b,c]	15[b,c]	15[a,d]
Orange Co. Environmental Health Division	22[b]	22[b]	0
AWMA/SERRA	16[a]	16[a]	16[a]
San Diego County			
Encina Wastewater Authority	5[a]	5[a]	5[a]
City of Oceanside	3[b]	3[b]	3[a]
City of San Diego	45[a]	45[a]	45[a]
MCB Camp Pendleton	3[b]	3[b]	3[a]
San Diego Co. Department of Env. Health	3[b]	3[b]	0
San Elijo Joint Powers Authority	3[b]	3[b]	3[b]
Mexico			
Instituto de Investigaciones Oceanologicas	29[b]	29[b]	0

[a]MF
[b]MPN
[c]Colilert®
[d]Enterolert®

Adaptive Sampling Sites

In addition to the baseline sampling design, the study also included an adaptive component in which five participating organizations increased sampling activity in areas where elevated indicator bacteria levels were found. Additional sampling took place if a sample exceeded any of the following criteria:

Total coliforms $\geq$10,000 cfu or MPN/100 mL; or
Fecal coliforms $\geq$ 400 cfu or MPN/100 mL; or
Enterococci $\geq$104 cfu or MPN/100 mL; or
Coliforms Index (total:fecal coliforms x 100) $\leq$5, if total coliforms $\geq$ 5,000 cfu or MPN/100 mL.

The adaptive component involved sampling of additional sites on either side of the elevated indicator site within a week following the initial measurement. For sites located on open shoreline, the adaptive sites were located 100 yards on either side of the elevated site; for water outlet sites, the adaptive sites were located 25 yards on either side.

B. Field and Laboratory Methods

Bacteria

Samples were collected in sterile sample bottles or whirl-paks from ankle-deep waters 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 tightly capped and stored on ice in the dark. All samples were returned to the laboratory in time to begin analysis within 6 hours of sample collection.

Total coliforms and fecal coliforms were measured for all sites. Enterococci were measured at roughly 70% of the sites, depending upon the capability and capacity of the participating organization responsible for the site. Enterococci measurements were not performed on samples taken at Mexican or volunteer sites.

Three methods were used to measure bacteria: membrane filtration (MF); multiple tube fermentation (MTF), and substrate technology tests. The first method, MF, is a direct plating method for the detection and enumeration of bacteria in water. 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, Inc. The Idexx kits use either multiple tubes or multiple wells, with an MPN approach, to detect the presence or absence of total coliforms and *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 an enzyme produced only by *E. coli*. This assay is read within 18-22 hours. In this study, *E. coli*, which typically constitute the overwhelming majority of fecal coliforms, were treated as fecal coliforms for data Each participating laboratory used its standard method for sample processing, with a performance-based approach employed to ensure data comparability among labs; intercalibration tests

using common samples were performed before the start of the sampling period. Only laboratories that met the performance criteria were permitted to participate (see Quality Assurance section below). The methods used by each participant are 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.*

Enteric Viruses

The presence of human enteric virus genetic material (such as the genomes of poliovirus, echovirus, and Coxsackie virus) was measured from samples taken at the mouth (point zero site) of 15 randomly selected perennial freshwater outlets using the reverse transcriptase polymerase chain reaction (RT-PCR) technique of Tsai *et al.* (1993). The method used, RT-PCR, is capable of detecting small quantities of virus genetic material in seawater, and is a potentially useful tool for determining the presence of human fecal contamination. However, the method cannot be used to infer health risk as viral genetic material may not be associated with an intact, infectious virus (Sobsey 1998).

Twenty liters of seawater were collected in a plastic carboy from the same site using the same collection procedures used for the bacterial samples. Samples were placed on ice and returned immediately to the lab, where they were pressure filtered (15 psi) through two 142 mm diameter stainless steel filtration units. The first unit housed a glass fiber filter (Whatman, nominal pore size of 1 µm), and the second unit housed a 0.22 µm Durapore filter. While still on ice, the filtrate was ultraconcentrated with a spiral cartridge filtration system (molecular weight cutoff of 30 kDa, SY130, Millipore, Inc.) to a final volume of ca. 150 mL. This sample was further concentrated using Centriprep-30 centrifugal concentration units (Amicon, Inc.). The Centriprep units were centrifuged at 5,000 x g for 30 minutes at 4° C, then the filtrate was poured off and the remaining concentrate was added to the units until the volume was approximately 5 mL. Next, Centricon-30 centrifugation concentration units were spun in a Sorvall SS-34 rotor at 5,000 x g at 10° C to further concentrate the material to approximately 100 mL.

The RT-PCR was performed using a set of pan-enterovirus "universal" primers, EV-L and EV-R, for total enterovirus nucleic acid amplification (Tsai *et al.* 1993). Briefly, a 2 mL subsample of the concentrated seawater sample was heated to 99° C for 5 minutes, and subsequently held at 4° C. This action denatures the protein coat of the virus particles, revealing the RNA genome within. While still at 4° C, reagents for the reverse transcriptase (RT) step were added. The RT step was run with one cycle at 24.0° C for 10 minutes, 42.0° C for 30 minutes, 99.0° C for 5 minutes, and then held at 4.0° C for addition of the PCR reagents, including DNA polymerase. The DNA polymerase catalyzes the extension reaction and a second DNA strand is synthesized. The reaction mixture is then heated again to 99° C to separate the double stranded molecule and expose the primers' target sequences. As the mixture cools, the primers anneal to their targets, and the DNA polymerase continues once again to extend the annealed primers along the target templates to produce amplified DNA fragments of 196 bp. This occurs for 40 cycles, amplifying millions of copies of the original target cDNA. Amplified DNA was visualized by staining a 2% agarose gel with ethidium bromide and illumination with UV light. Lane markers of 100 bp increments were used for size comparison. The expected PCR product for the pan-enteric virus primers is 196 bp.

Negative and positive controls were performed for each RT-PCR run. For the negative controls, 2 µl of deionized water was added to the PCR mixture rather than the seawater sample. A positive control for the RT-PCR kit was performed each time a new kit was used, and involved the amplification of a given

target RNA with random hexamer primers. A positive control for the poliovirus amplification was performed by adding known amounts of high-titer stock poliovirus to the RT-PCR mixture, with amplification using the EV-L and EV-R primer pair. Triplicate analyses were run for each sample by using the RT-PCR protocol for each dilution. Negative and positive signals observed on agarose gels were recorded, and quantitative results were calculated using an MPN approach. The detection limit of our RT-PCR assay ranged from 0.1-1.0 infectious units and was comparable to detection limits reported in similar studies (Tsai *et al* 1993, Rose *et al.* 1997).

Total abundances of viruses and bacteria were determined by small-volume samples preserved with formalin, stained with SYBR Green I, and counted with an epifluorescence microscope (Noble and Fuhrman 1998). When possible, preparation activities were completed under subdued light. Slides were counted immediately, or frozen at -20° C for counting within 1 week. For each filter, 10 to 20 fields were selected randomly and a total of >200 viruses and >200 bacteria were counted on an Olympus BH2 epifluorescence microscope with a 100X D Plan Apochromat UV objective, under blue excitation. Virus particles were distinctly shaped "pinpricks" that fluoresced bright green. Bacterial cells were distinguished from viruses by their relative size and brightness.

C. Quality Assurance

Two distinct but related activities, quality assurance (QA) and quality control (QC), were incorporated into 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.

Methods were standardized across labs by implementing the following actions. Each laboratory was ELAP certified and followed *Standard Methods for the Examination of Water and Wastewater, 18th edition, 1995* (*Standard Methods*). Laboratories also ascribed to common guidelines regarding culture media, water, equipment and instrumentation, and data handling. Commercially available presterilized media were used. Media were sterilized by autoclaving according to the manufacturer's specifications. Water used to prepare culture media and reagents was distilled or demineralized reagent grade, and was stored away from direct sunlight to prevent growth of algae. Ovens, autoclaves, and refrigerators were monitored to ensure proper temperatures. The pH meters were calibrated to maintain an accuracy of 0.1 pH units. Balances were calibrated to provide a sensitivity of at least 0.1 g at a load of 150 g.

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

Intercalibration performance exercises were conducted to assess and control the variability introduced by inclusion of multiple laboratories and measurement methods. These exercises involved preparation of standardized samples, which were distributed to each laboratory for processing. Each laboratory was required to achieve specific accuracy and comparability goals as prerequisites to their participation in the regional survey. Details of the QA intercalibration exercises are presented in Appendix C.

Quality control measures applied during the study were similar to the intercalibration exercises conducted prior to the survey. Each laboratory was required to process two standardized samples, on the second and fourth weeks of the study, that were created by inoculating filtered seawater with raw sewage (from the Orange County Sanitation District).

D. Data Analysis

The assessment of shoreline condition focused upon estimating the percent of shoreline mile-days that exceeded a threshold of concern. Data from adaptive sampling, indicator comparisons (labs where multiple methods were run simultaneously), and Mexican waters were not used for the assessment of shoreline condition. Two sets of thresholds were used, one based upon daily measurements and the other based upon monthly averages (Table II-3). Both sets of thresholds were derived from a combination of State of California draft beach closure thresholds, established in response to the AB411 legislation and primarily applicable to county health departments, and the California Ocean Plan, which proscribe State water quality objectives for NPDES-permitted ocean dischargers.

TABLE II-3. Indicator thresholds used in the Shoreline Microbiology Study

Indicator	Daily Limits (per 100 mL)	Monthly Limits (per 100 mL)
Total coliforms	10,000[a,b,c]	20% of samples >1,000[a,c]
Fecal coliforms	400[b]	200 (GM)[b]
Enterococci	104[b]	35 (GM)[b]
Total:fecal ratio	when TC >1,000 and TC/FC ≤ 10 [b] also, when TC>1,000 and TC/FC≤ 5	
GM = geometric mean		

[a]From California Ocean Plan
[b]From draft regulations developed in response to California Assembly Bill 411
[c]Present California Ocean Water-Contact Sports Standards

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

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

where:

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

Standard error of the response was calculated as:

$$\text{Standard Error} \quad \sqrt{\frac{\sum_{i=1}^{n}((p_i - m)\ w_i)^2}{\left(\sum_{i=1}^{n} w_i\right)^2}}$$

Statistical differences between populations of interest were defined on the basis of non-overlapping confidence intervals. 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 warranted based upon preliminary examination of the data. This assumption is conservative in that its violation would lead to an overestimation 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 entire data set (including adaptive sampling and data from Mexican waters). Contingency tables were also developed to categorically assess the frequency with which individual sites were classified the same by different indicators.

The relationship between bacterial indicators and viral concentrations was assessed in two ways. First, the rank correlation between quantitative results of human enteric virus genome detection by the MPN approach and the levels of each of the bacterial indicators was tested. Second, the correlation between the presence/absence of human enteric virus genomes versus the log transformed bacterial indicator results was tested (logistic regression).

III. QUALITY ASSURANCE EVALUATION

Participants successfully sampled 99% sites targeted for study during the survey period. Of the two stations missed during the study, one was the result of an incorrect sample frame (no public access) and one site was not sampled. Although a five week time frame was defined as the study sampling period, a sixth week was reserved for contingency. Only one agency required the sixth week for sampling; rescheduling allowed them to meet the requirement for a minimum of five sampling events for all of their sites.

Participants successfully analyzed 3,436 of 3,455 (>99%) samples targeted for analysis, exceeding the data quality objective of 95%. All 19 of the missing laboratory analyses were the result of laboratory accidents.

All participants analyzed two external reference samples (seawater samples spiked with sewage effluent) during the survey to quantify measurement error and identify data quality problems. Participating laboratories analyzed these reference samples for total coliforms, fecal coliforms, and enterococci using procedures identical to those used for the pre-survey quality assurance exercises (Appendix C).

The reference sample analysis showed that the cross-laboratory variability established in the pre-survey intercalibration exercises was also achieved during the survey. The only deficiencies identified were laboratory multiplication errors resulting from dilution series. These deficiencies were corrected and all remaining study data were verified for calculation accuracy.

During the course of data checking, it was discovered that 2.5% of reported samples had fecal coliforms levels that were higher than the total coliforms levels. Since fecal coliforms represent a subset of the total coliforms group, their numbers should not exceed the total coliforms numbers. On-site audits conducted by the Project QA Officer confirmed that these anomalies resulted from analytical interferences and not errors in analytical methodology. The median difference between fecal coliforms and total coliforms for these cases was 10. Less than 4% of the discrepancies were from samples that exceeded bacterial indicator standards for fecal coliforms and none exceeded standards for total coliforms.

IV. ASSESSMENT OF THE SOUTHERN CALIFORNIA BIGHT

A. Results

Approximately 95% of the shoreline mile-days in southern California during the five-week study period met bacteriological water quality standards. This high frequency of good region-wide bacteriological water quality was consistent, regardless of whether daily or monthly thresholds were used (Figure IV-1, Table IV-1).

The probability of exceeding a bacterial indicator threshold differed among indicators (Figure IV-2). Enterococci was the indicator for which thresholds were most frequently exceeded, followed in descending order by total:fecal ratios, fecal coliforms, and total coliforms. The shoreline mile-days for which enterococci exceeded thresholds were more than twice those for fecal coliforms, and five times those for total coliforms. Less than one-third of the area that exceeded a threshold for one bacterial indicator exceeded thresholds for multiple indicators measured at the site, whether determined by daily or monthly thresholds (Figure IV-3 and IV-4, respectively, Table IV-2). Only 0.1% of the shoreline, all of which were freshwater outlet sites, failed all indicators on any particular sample.

Few sites exceeded bacterial indicator thresholds for more than one of the five weeks of sampling (Figure IV-5). Less than 2% of the shoreline sample sites exceeded a threshold for a second week for any indicator, and none of the sites away from freshwater outlets exceeded thresholds in multiple weeks for either total or fecal coliforms. Only six of the sites sampled in this study exceeded bacterial indicator thresholds during every week of the study; three were in Mexico and three were in the United States. Five of the six sites were point zero samples taken at freshwater outlet locations.

The frequency with which bacterial indicator thresholds were exceeded varied by shoreline type. The lowest frequency of daily threshold exceedances occurred along high-use rocky shoreline; the lowest frequency of monthly threshold exceedances occurred along low-use sandy beaches; and the highest frequency of exceedances (of both daily and monthly thresholds) occurred at point zero freshwater outlet sites (Figure IV-6, Table IV-1). Nearly 60% of the shoreline mile-days at point zero storm drain sites failed monthly bacterial indicator thresholds for at least one indicator during this study. More than half of the point zero freshwater outlet samples that exceeded a threshold for a single indicator also exceeded the threshold for multiple indicators. Random freshwater outlet samples, taken from sites within 100 yards of perennial freshwater outlets, exceeded indicator thresholds approximately 15% of the time, triple the frequency observed Bight-wide or on high-use sandy beaches (Figure IV-6).

Although nearly 75% of the beach samples in Mexico met bacterial indicator thresholds, beaches and perennial freshwater outlets in Mexico were more likely to exceed a bacterial indicator threshold than those in the United States (Table IV-3). The probability of exceeding the threshold for both total and fecal coliforms on sandy beaches in Mexico was five times that at sandy beaches in the United States. In contrast the probability of exceeding indicator thresholds, including total:fecal ratios, at freshwater outlets was similiar both north and south of the border.

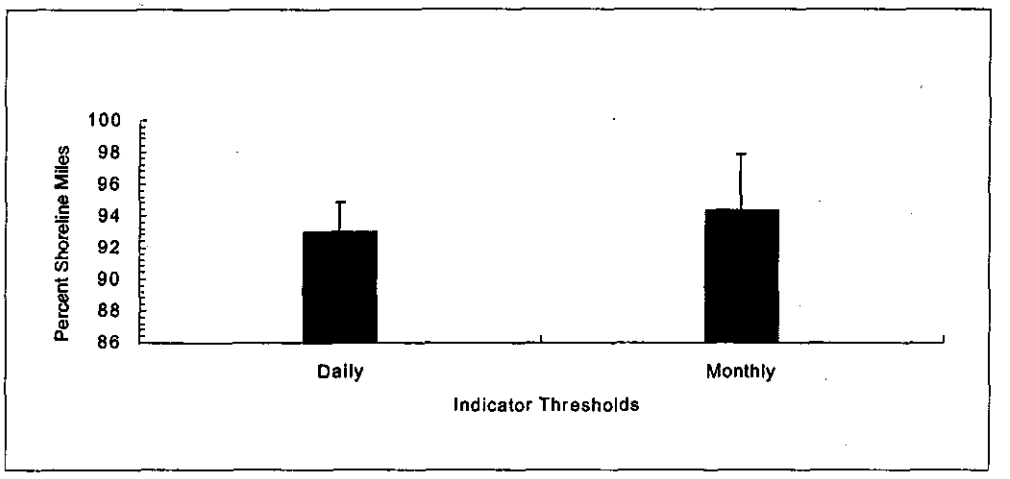


Figure IV-1. Percent of southern California shoreline mile-days that met all bacterial indicator thresholds in August 1998.

TABLE IV-1. Percent of shoreline mile-days exceeding daily bacterial indicator thresholds.

STRATA	Enterococci	Fecal coliforms	Total coliforms	TC:FC <10	TC:FC <5
High-use sandy	6.1	2.5	0.0	1.4	1.3
Low-use sandy	1.2	2.8	2.2	2.1	2.1
High-use rocky	2.4	0.0	0.0	0.0	0.0
Low-use rocky	2.1	0.0	0.0	0	0.0
Perennial outlets	5.7	6.9	1.7	5.4	2.5
Ephemeral outlets	5.0	2.7	0.0	4.0	2.7
Point zero outlets	34.2	24.8	12.0	21.8	17.6
All SCB	4.9	2.9	0.7	2.1	1.8

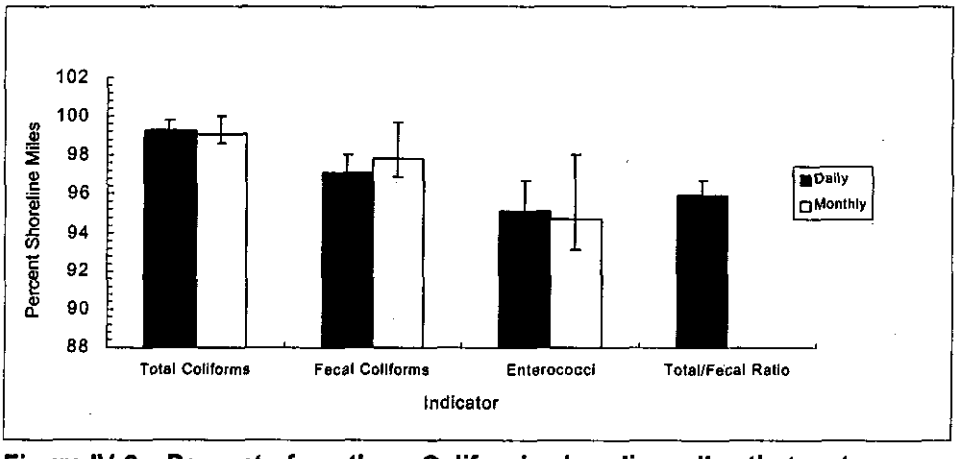


Figure IV-2. Percent of southern California shoreline miles that met indicator thresholds.

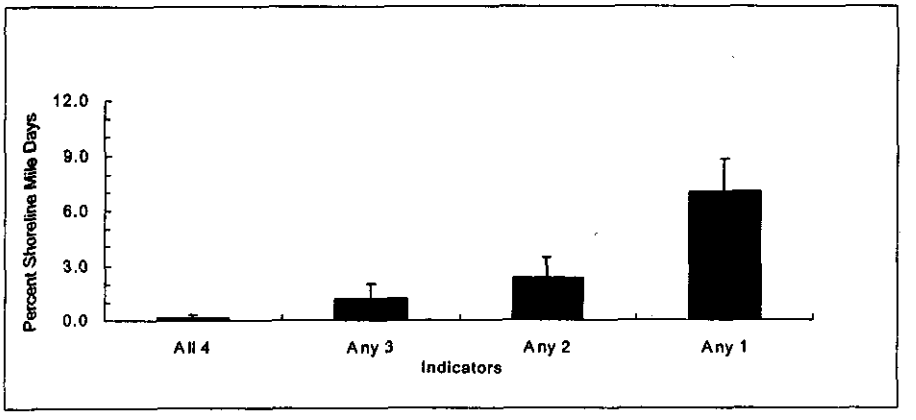


Figure IV-3. Percent of southern California shoreline miles that failed multiple daily bacterial indicator thresholds in August 1998.

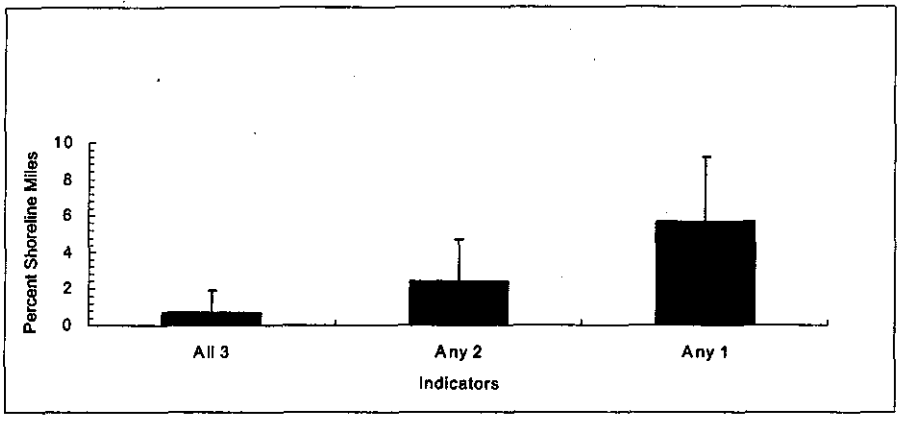


Figure IV-4. Percent of southern California shoreline miles that failed multiple monthly bacterial indicator thresholds in August 1998.

TABLE IV-2. Percent of shoreline mile-days exceeding daily thresholds for all of the indicators, three of the indicators, two of the indicators, and any single indicator. Estimates are based upon the subset of sites at which all indicators were measured.

STRATA	All 4	Any 3	Any 2	Any 1
High-use sandy	0.0	0.4	1.8	7.8
Low-use sandy	0.0	2.2	2.2	4.1
High-use rocky	0.0	0.0	0.0	2.4
Low-use rocky	0.0	0.0	0.0	2.1
Perennial outlets	0.8	3.0	5.2	10.9
Ephemeral outlets	0.0	1.7	4.1	7.3
Point zero outlets	5.8	18.3	26.7	40.0
All SCB	0.1	1.2	2.3	7.0

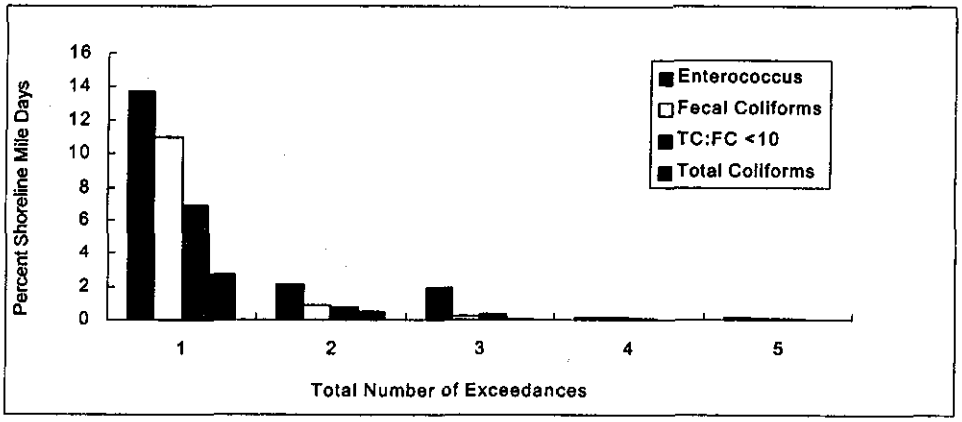


Figure IV-5: Comparison of Repeat Threshold Exceedances by Bacterial Indicator.

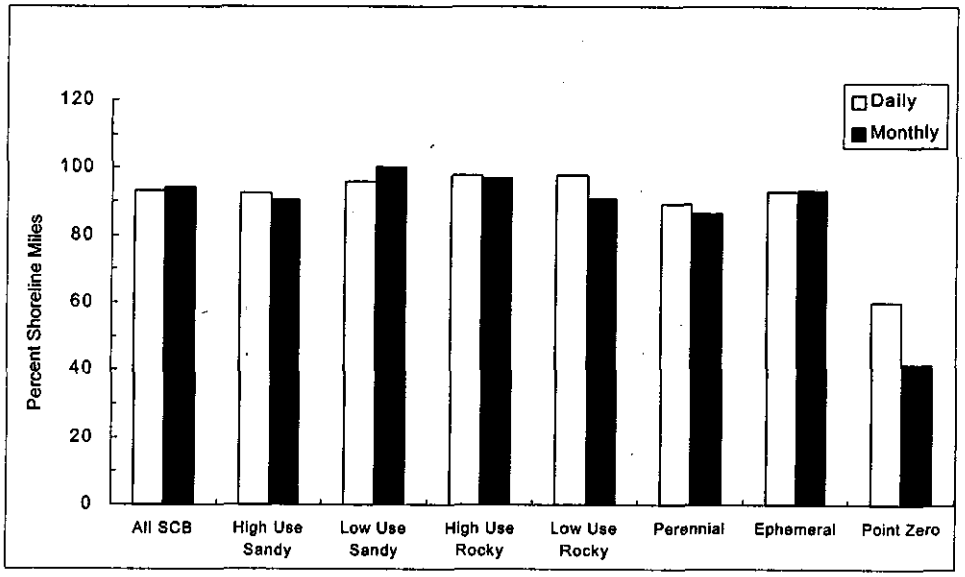


Figure IV-6. Percent of southern California shoreline miles, by shoreline type, that met all bacterial indicator thresholds in August 1998.

TABLE IV-3. Percent of threshold exceedances in Mexico and the United States.

	Total coliforms	Fecal coliforms	TC:FC <10
Sandy beaches			
Mexico	2.6	25.3	16.5
United States	0.5	5.3	2.1
Point zero at perennial freshwater outlets			
Mexico	12.7	32.7	21.8
United States	12.0	24.8	21.8

The magnitude by which thresholds were exceeded differed considerably among shoreline types. Approximately 40% of the measurements along the southern California shoreline away from freshwater outlets were within measurement error standard deviation, as quantified in the this study's intercalibration exercises (Appendix C); an additional 30% of the measurements were within two standard deviations (Figure IV-7). In contrast, two-thirds of the freshwater outlet samples that failed a standard did so by more than two measurement error standard deviations. Nearly 80% of the Mexican samples that failed a standard did so by more than two standard deviations, regardless of whether the sample was collected near a freshwater outlet or on a beach (Figure IV-7).

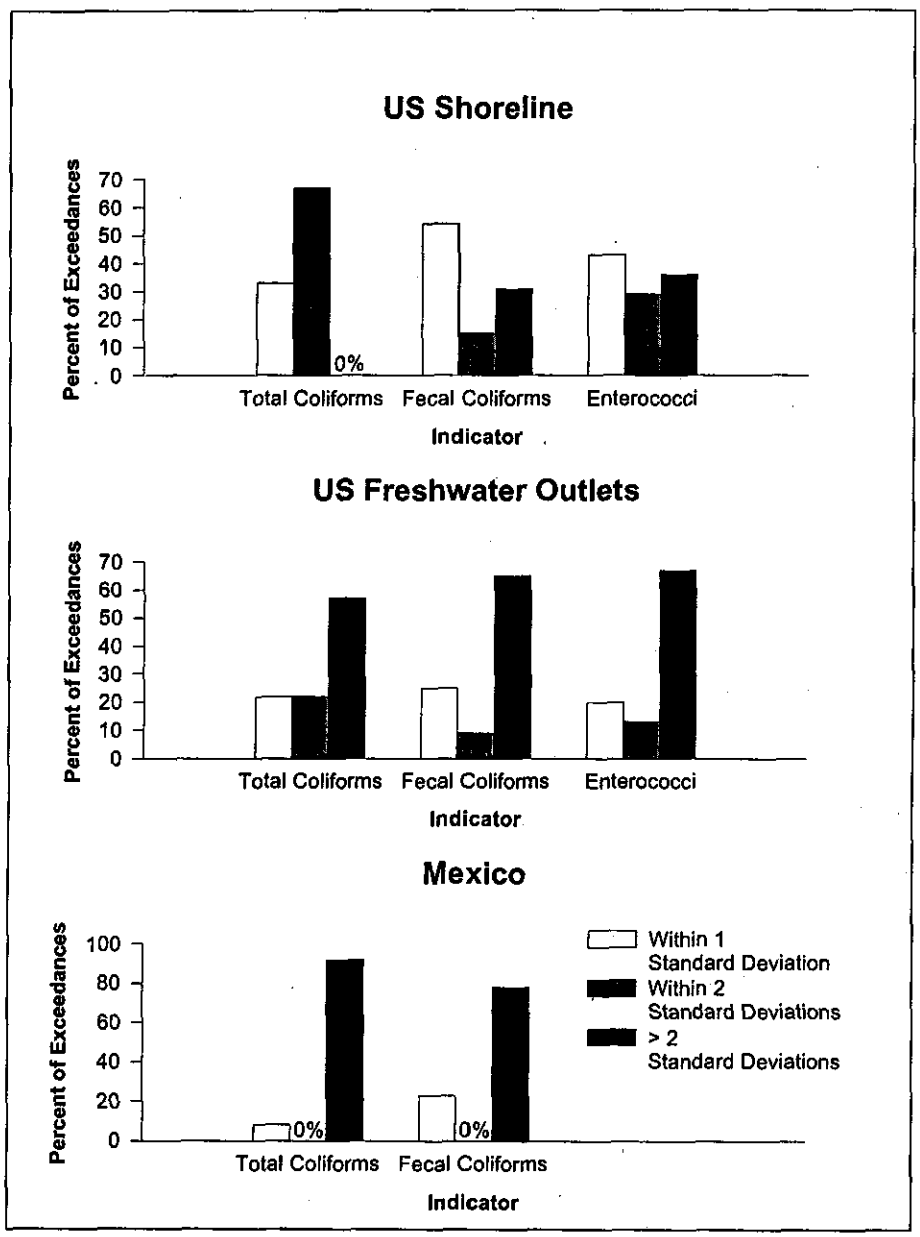


Figure IV-7. Percent of exceedances within 1, 2, or greater than 2 standard deviations for combined US sandy and rocky shoreline sample sites, US freshwater outlet samples sites, and combined Mexican sample sites.

Adaptive sampling was included in the study to quantify the spatial extent of the shoreline affected by individual threshold exceedances. Of the 133 adaptive samples taken upcoast or downcoast from the original site where a threshold was exceeded, less than 25% were found to exceed thresholds at a distance as close as 25 yards and only 5% at a distance between 25 and 100 yards. These findings, in part, reflect the fact that adaptive sampling was conducted up to a week after the original measurement was taken. Less than 35% of the 63 samples that triggered adaptive sampling remained above the threshold a week after the adaptive sample was collected.

B. Discussion

The vast majority of the southern California shoreline had good bacteriological water quality during August 1998. The one exception to this finding was the areas adjacent to freshwater outlets. Most of these outlets are storm drain systems that receive a variety of upstream inputs, including organic debris, non-human fecal matter, accidental sewage spills, illicit sewage connections, sanitary sewer system leaks, leachate from septic systems, runoff from homeless populations, and/or illegal dumping of waste. Storm drains in southern California are independent from sewer systems and their flows receive no treatment or disinfection prior to ocean discharge.

Urban runoff is a large contributor of microorganisms to storm drains, but it is not the sole source of fecal contamination. Waterfowl, dogs, and marine mammals can also contribute bacterial contamination, particularly where lagoonal or embayment systems, which serve as wildlife habitat, immediately precede the confluence of the drainage system with the ocean. Genetic tests of *E. coli* isolates from urban runoff water samples in San Diego and Orange Counties matched DNA sequences observed in wastes sampled from several animal sources (Simmons 1998). These local observations are consistent with the results of studies in other locations. In Massachusetts, for example, an estimated 67% of the coliforms in Buttermilk Bay were derived from waterfowl (Weiskel *et al.* 1996).

While this study is the first to quantify the effect in an unbiased, regional context, it is not the first to conclude that storm drains are areas of concern. High levels of indicator bacteria have been found routinely in storm drain effluents, affecting shoreline bacteriological water quality near these sources throughout southern California. A recent study performed in Santa Monica Bay linked the poor bacteriological water quality of storm drains to the epidemiology of people using the beach for recreation (Haile *et al.* 1996). During dry weather, Gold *et al.* (1992) reported elevated counts of enterococci and total and fecal coliforms in several storm drains in Santa Monica Bay. Indicator bacteria sampled from storm drain effluents during wet weather commonly exceed State water quality objectives (Schiff 1997). Median densities of fecal coliforms ranged between 10^2 and 10^4 cfu or MPN/100 mL in wet weather flows from San Diego to Los Angeles. These high densities of indicator bacteria are reflected in gradients of coliforms and enterococci in the receiving waters of Santa Monica Bay (Gold *et al.* 1990, SCAG 1988). These observations are not unique to southern California; urban runoff yields consistently high densities of fecal coliforms in many metropolitan areas (EPA 1983) and is one of the largest contributors to impaired surface waters in the United States (EPA 1994).

This study is also not the first to detect areas along the Mexican coast with high bacterial counts (Segovia-Zavala and Orozco-Borbón 1986), though it is the first to use consistent sampling approaches to compare the relative quality of United States and Mexican beaches. Water contamination in the northwest-

ern coastal area of Baja California results from rapid urban and industrial growth, and a lack of infrastructure to treat municipal wastewater, mainly near the cities of Tijuana and Ensenada. Previous bacteriological studies in this area (Orozco *et al.* 1994, Segovia *et al.* 1995) have found that the main inputs of total coliforms and fecal coliforms to the area are from storm drains and wastewater discharges along the shoreline. Wastewater discharges increase during summer months with an increase in tourism, while storm water runoff is the principal source in winter (Orozco-Borbón and Sañudo-Wilhelmy 1988). The Mexican government has already taken actions to reduce bacteriological pollution of coastal waters. First, they have adopted the Mexican official standard NOM-001-ECOL-1996 that establishes pollution limits (Secretaría de Medio Ambiente 1997), and have established dates for initiating discharge quality control programs. Additionally, they are improving the existing infrastructure, as well as constructing new facilities to collect, treat and dispose of sewage from the rapidly growing population in the region. The Mexican government has participated in construction of the South Bay International Wastewater Treatment Plant and is planning construction of a series of wastewater treatment facilities along the Tijuana-Ensenada corridor. It is clear, however, that illegal discharges also exist on these waters and that additional measures will have to be taken to correct the problem. The data from this study can be a valuable baseline for assessing the effectiveness of those future actions.

Measurement error is an important factor to consider in interpreting bacterial indicator data. The intercalibration aspect of this study documented that the standard deviation associated with replicate laboratory analysis was nearly 50% of the measured value at concentrations near the State thresholds; this magnitude of measurement error is comparable to that of laboratories outside of southern California and reflects the inherent accuracy of current bacterial measurement technologies. More than two-thirds of standards failures observed in this study, particularly those from samples collected away from storm drains, were within measurement error. County health departments typically collect confirmation samples at sites where a threshold is rarely exceeded, in part to ensure that the failure did not result from measurement error. In areas away from freshwater outlets, we found that less than 0.5% of the shoreline exceeded a threshold in two consecutive samples.

One of the most striking results of this study was the difference in response among indicators. These differences are likely to affect the actions of county health departments in the near future with implementation of AB411 regulations. Present State law requires the use of total coliforms as the indicator to determine recreational water quality and, in the event of exceedances, to post or restrict access to the shoreline. The new proposed standards under AB411 require measurement of three indicators. A failure finding is presently proposed as exceeding a threshold for any one of the three indicators, although early drafts proposed failure as exceeding thresholds for any two of the three indicators. The results of this study indicate that either proposal will lead to a substantial increase in the number of samples failing State standards and may increase the number of beaches posted or closed. Failures of the total coliform standard amounted to 0.7% of shoreline mile-days, while failures of any two indicators amounted to 2.2% of the shoreline, or almost triple those of coliforms alone. If the AB411 regulations are written such that exceeding standards for any one of the three indicators can lead to beach posting or closure, the rate of posting or closure will increase by a factor of 10 (Table IV-1).

One outcome of this study is the recognition of the effectiveness of ongoing beach monitoring programs in southern California. More than 20 programs throughout the SCB cumulatively spend $3 million annually collecting samples from at least 510 sites and conducting more than 80,000 analyses per year, roughly the same amount expended for monitoring activities in the rest of the country combined (Appendix D). The programs in southern California focus the bulk of their resources on monitoring high-use beaches and known problem areas such as storm drains. The present study emphasized the sampling of new, randomly selected locations and did not uncover any previously undisclosed "hot spots" of concern. Only 10 sites that exceeded a bacterial indicator threshold were located more than one-half of a mile from a routine monitoring site; only one of these new sites exceeded a threshold for more than one week. Eight of the ten new sites were located in Ventura and Santa Barbara Counties, where population densities are significantly lower than urbanized areas of the SCB. Recent political and community support has led to the expansion of regularly monitored beach locations in both Ventura and Santa Barbara Counties.

V. INDICATOR COMPARISONS

A. Results

Correlation Analysis

A strong correlation was found between total and fecal coliforms (r = 0.93), while the correlation between enterococci and both total and fecal coliforms was weak (r = 0.29, Table V-1, Figures V-1 through V-3). The correlation between indicators was largely independent of which laboratory method was used to analyze the samples; for example, the correlation between total coliforms and fecal coliforms analyzed by MF was 0.89, whereas the correlation between the two using MTF analysis was 0.93 (Table V-1). Samples analyzed with MTF had marginally improved relationships between indicators compared to MF. Correlation coefficients were nearly identical when comparing the MTF and MF methods to analyses using the Idexx kits (Table V-1).

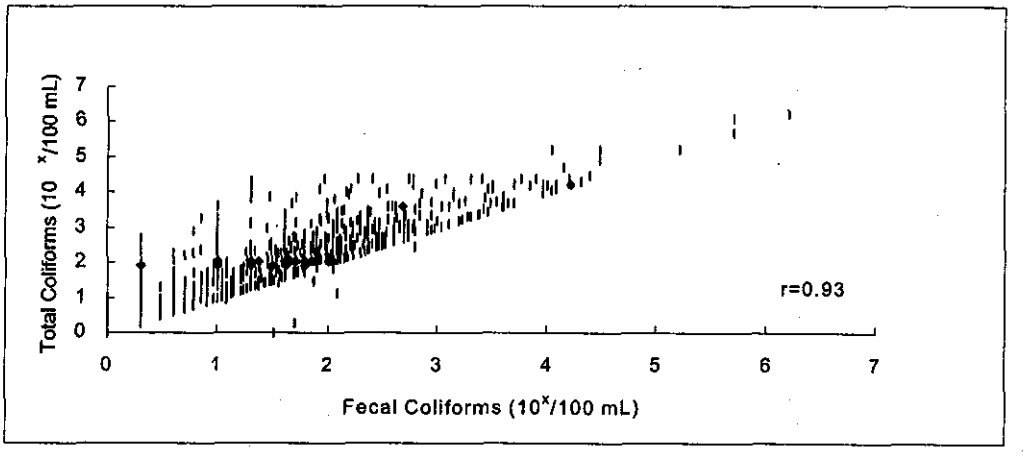


Figure V-1. Correlation of Total Coliforms and Fecal coliforms in August 1998.

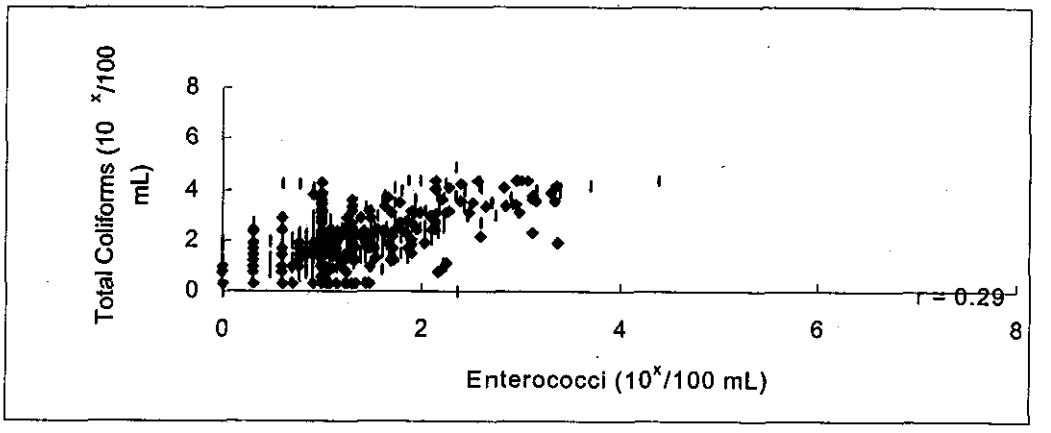


Figure V-2. Correlation of Total Coliforms and Enterococci in August 1998.

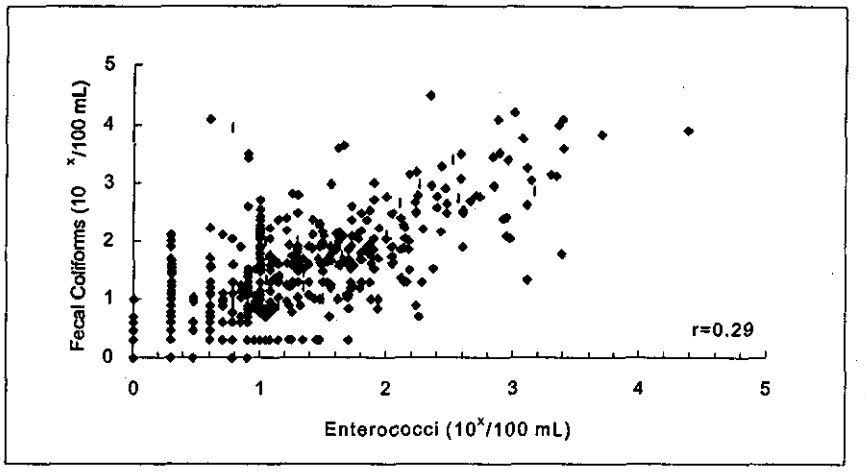


Figure V-3. Correlation of Fecal Coliforms and Enterococci in August 1998.

The correlations between indicators were also similar among the different sampling strata assessed in this survey (Table V-1). The correlation between each of the indicators improved marginally at freshwater outlets compared to high-use sandy beaches. This is noteworthy since freshwater outlets generally demonstrated the highest bacterial densities while high-use sandy beaches had the lowest bacterial densities.

A number of samples from the survey were not quantified because they exceeded the capacity of the dilution series performed; instead they were reported as ">" values. For the analyses above, these values were truncated to the upper end of their quantification range (i.e., converting >16,000 to 16,000). Removing these data points, rather than truncating, had little effect on the correlation between fecal coliforms and enterococci or total coliforms. The correlation between total coliforms and enterococci more than doubled with the reduced data set (Table V-1).

TABLE V-1. Correlation between enterococci, fecal coliforms, and total coliforms density in the Bight'98 Shoreline Microbiology survey.

	Total coliforms: Fecal coliforms	Fecal coliforms: Enterococci	Total coliforms: Enterococci
Entire data set	0.93	0.29	0.29
Membrane filtration alone	0.89	0.38	0.29
Multiple tube fermentation alone	0.93	0.47	0.42
Idexx alone	0.93	0.38	0.30
High-use sandy beaches alone	0.88	0.25	0.25
Water outlets alone	0.93	0.30	0.28
Without truncated values	0.91	0.40	0.77

Threshold Analysis

Of the 880 samples that were tested for all three indicators, 93 exceeded at least one indicator threshold. Of these threshold exceedances, only 13% failed for all three indicators, 34% failed for two indicators, and 54% failed for only a single indicator (Table V-2). Fecal coliforms failed at twice the rate of total coliforms, and enterococci failed at three times the rate of total coliforms. Less than one-half of the enterococci threshold exceedances paired with threshold exceedances by another indicator. Approximately 89% of the total and fecal coliforms threshold failures were partnered with failures of another indicator.

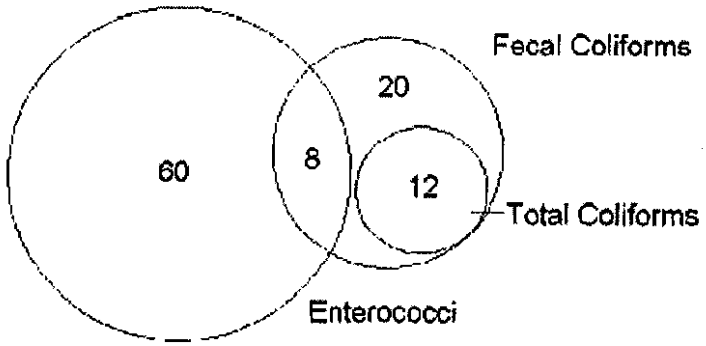
The concordance among indicators was considerably higher at freshwater outlet sites. Near outlets, more than 50% of the samples that failed the threshold for one indicator also failed for another; 18% failed for all indicator thresholds (Figure V-4). In contrast, only 20% of the failures away from outlets were accompanied by the failure of a second threshold. Sixty percent of the failures away from freshwater outlets resulted from enterococci measurements alone. No single sample collected away from freshwater outlets during the entire study failed the standard for both enterococci and total coliforms (Figure V-4).

Table V-2. Allocation of all observed threshold exceedances among indicator combinations (in percent).

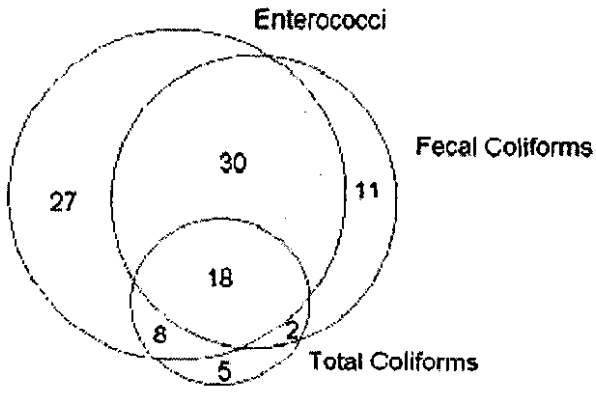
	Total coliforms	Fecal coliforms	Total:fecal ratio	Enterococci
Alone	3.1	6.3	5.3	32.3
Total coliforms				
Fecal coliforms	1.0			
Total:fecal ratio	0.0	6.3		
Enterococci	5.2	3.1	2.1	
Fecal coliforms & total:fecal ratio	3.1			
Fecal coliforms & enterococci	5.2			
Total:fecal ratio & enterococci	0.0	19.8		
All 4 Indicators	7.3			

Figure V-4. Percent correspondance of indicator threshold exceedances at southern California sites near and away from freshwater outlets in August 1998

SHORELINE



FRESHWATER OUTLETS



B. Discussion

Our finding that different indicators do not equally reflect whether a site exceeds thresholds, as well as the higher incidence of enterococci threshold exceedances during summer, is consistent with the observations of the project participants from their routine monitoring programs. This conclusion does not appear to be limited to southern California. Nuzzi and Burhans (1997) compared the responses among total coliforms, fecal coliforms, and enterococci at 143 New York beach sites and found that while indicator values were correlated, the likelihood of exceeding an enterococci threshold was more than twice that for either of the coliform measures.

One possible explanation for the disparity among indicator threshold exceedances is that enterococci survive longer in the marine environment than total or fecal coliforms, resulting in more values that exceed the threshold. Hanes and Fragala (1967) demonstrated that *E. coli* survival in marine water was 0.8 day while enterococci survival was 2.4 days. Sieracki (1980) demonstrated that the rate of enterococci die-off did not increase as the intensity of sunlight increased while *E. coli* demonstrated the converse pattern. Both of these factors could increase the likelihood of enterococci threshold exceedances relative to coliforms.

The applicability of bacterial indicators, and their thresholds, for influencing decisions about beach closures is dependent upon their relationship to the pathogenic organisms that cause illness. Investigators have shown that enterococci and coliphage have similar survival characteristics in receiving lake waters (Rajala 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, resulting in the development of water quality guidelines by the United States Environmental Protection Agency (U.S. EPA) for recreational waters based upon enterococci densities (EPA 1986). This relationship between enterococci and swimming-associated gastroenteritis has been more recently examined by Kay *et al.* (1994), who demonstrated a significant dose response relation 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.* (1996) found that the relative risk differed by indicator when its particular threshold was exceeded. For example, positive associations were observed with skin rashes when total or fecal coliforms thresholds were exceeded. Meanwhile, positive associations of highly credible gastroenteritis (HCGI) and diarrhea were observed when enterococci thresholds were exceeded. These results are also supported by Fleisher *et al.* (1996), who showed that fecal streptococci were predictive of upper respiratory tract illness, while fecal coliform exposure was predictive of ear ailments.

Another possible explanation for the higher rate of enterococci threshold exceedances is that the thresholds for the indicators were generated using different approaches and thus may be measuring different outcomes. Enterococci and total:fecal ratio thresholds were developed to estimate human health risk, based upon correlation of indicator bacteria densities and rates of human illness. Studies conducted by Cabelli (1983) established that enterococci densities correlated with numbers of HCGI in swimmers at beaches influenced by wastewater in New York, New Orleans, and Boston. Similarly, Haile *et al.* (1996) established significant associations between several microbial indicators and rates of human illness at beaches in

Santa Monica Bay influenced by storm drains. Most notable among these were the total/fecal ratios and several different symptoms including HCGI, nausea, diarrhea, and skin rashes. In contrast, the fecal coliform and total coliform thresholds were derived from historical technology-based limits, not upon probability or rates of illness (Cabelli 1983).

The results of this study indicate that measuring multiple indicators may be inefficient. Testing enterococci alone detected 79% of all indicator threshold failures. The cost of measuring multiple indicators at a site is nearly comparable to the cost of measuring an equal number of new sites with a single indicator, and the public's interest might be better served by measuring more sites or measuring selected sites more often using a single indicator. This can only occur if the scientific community agrees upon an epidemiological basis for selecting the most appropriate indicator and threshold. Of particular concern is the need to distinguish indicators and thresholds that most frequently result from the presence of human wastes from indicators of animal wastes, which are unlikely to contain the viral agents of greatest human health concern. The tools necessary to understand relationships between the pathogenic organisms that cause illness (e.g., viruses) and the bacterial indicators routinely monitored are only beginning to be developed. The California State Department of Health Services and the U.S. EPA have independently embarked upon efforts to standardize beach monitoring regionally and nationally. The public's interest, as well as the cost efficiency of monitoring, will be greatly improved by these programs if they focus on the research necessary to better relate existing measures to health risk.

VI. ENTERIC VIRUSES

A. Results

Seven of the 15 samples examined for human enteric virus genetic material (virus genomes) by reverse transcriptase polymerase chain reaction (RT-PCR) were positive (Table VI-1). The number of human enteric virus genomes detected ranged from 4 to 75 per 100 µL of concentrate, from an original volume of 20 L. Inhibitory substances, as evidenced by higher concentrations in more dilute samples in serial dilutions, were present in only a single sample from the Los Angeles River.

Correlations between human enteric virus genomes and each of the bacterial indicators (total coliforms, fecal coliforms, and enterococci) were statistically insignificant using rank correlation analysis. A significant logistical correlation was found between fecal coliforms concentration and the presence or absence of human enteric virus genomes. In 73% of the samples, the presence of human enteric virus genomes coincided with the exceedance of the fecal coliforms threshold of 400 cfu/100 mL.

TABLE VI-1. Human enteric virus genome and bacterial indicator concentrations at virus sampling sites. Bold face type indicates a threshold exceedance for that bacterial indicator (nm = not measured).

Freshwater Outlet Site	Sampling Date	Virus Genomes per 100 mµ	Total Coliforms	Fecal Coliforms	Enterococci
Tijuana River	8/3/98	75	30	8	10
Los Penosquitos Lagoon	8/3/98	75	8	4	2
San Luis Rey River	8/10/98	14	800	80	24
Los Angeles River	8/18/98	4	9,000	**1,700**	2
Aliso Creek	8/17/98	66	140	20	64
Ballona Creek	8/31/98	75	5,000	**1,600**	**170**
Malibu Creek	8/31/98	75	1,353	**616**	**175**
San Diego River	8/3/98	0	36	38	54
Moonlight Beach	8/10/98	0	3,000	230	nm
San Juan Creek	8/17/98	0	160	70	20
Goleta Creek	8/24/98	0	314	314	20
Mission Creek	8/24/98	0	240	85	10
Arroyo Burro	8/24/98	0	**24,192**	**589**	99
Carpinteria Creek	8/24/98	0	41	20	10
Calleguas Creek	8/31/98	0	1,100	170	**140**

B. Discussion

Human enteric viruses, unlike most bacterial indicators, are direct indicators of the presence of human fecal contamination. In this study, we specifically focused upon the detection of the genetic material (genome) of enteroviruses, a subgroup of the entire human enteric virus family. Enteroviruses are members of the picornaviridae, a family of single stranded RNA viruses. The family includes 67 human serotypes, including poliovirus, Coxsackie virus, echovirus, and other enteroviruses. Vaccine-strain poliovirus, although not a public health risk because it is an attenuated version of the virus, is also detected using our RT-PCR technique, and is a direct indicator of human fecal contamination. Vaccine-strain poliovirus may be found in elevated quantities in fecal material from children, as it is actively shed by those that have been recently vaccinated. Other viruses that can be found in human fecal material, but were not pursued as part of this study, include astrovirus, adenovirus, Norwalk virus, coronavirus, and Hepatitis A virus.

This study is not the first to examine the presence of human enteric viruses in the coastal waters of the Southern California Bight. A pilot study performed in Santa Monica Bay in 1989 used cell culture techniques and revealed the presence of infective human enteric viruses at 11 of 15 samples taken at a single storm drain in Santa Monica Bay, and repeat testing in 1990 revealed positive results in 3 of 4 samples (Gold *et al.* 1990). In another study in 1991, human enteric viruses were detected at all five of the storm drains tested in Santa Monica Bay (Gold *et al.* 1992), and one of the enterovirus isolates was identified as Coxsackie B virus, a known etiological agent. More recently, in an epidemiological study in Santa Monica Bay in 1995, infectious human enteric viruses were detected at all 3 of the storm drain systems tested (Haile *et al.* 1996). The virus research performed using RT-PCR in this study supports the previous studies in Santa Monica Bay, and demonstrates the positive detection of human enteric virus genomes at both of the Santa Monica Bay storm drains tested (Table 1), with quantitative results suggesting that the levels of human enteric virus genomes at these sites were among the highest of the freshwater outlet sites studied.

While enteroviruses are responsible for a variety of illnesses or symptoms, including upper respiratory tract infections, meningitis, myocarditis, and hemorrhagic conjunctivitis, the measurement techniques used in this study do not provide direct information about infectivity of the observed virus particles. The RT-PCR works by identifying the presence of viral RNA based upon conserved sequences of RNA found within the viral genome of specific virus families, in this case enteroviruses, without distinction as to whether the viral RNA is free or contained within an intact, infective virus particle. It is a valuable technique for detecting virus material found in human fecal contamination, and therefore has the potential to be used as a tool to distinguish between human and animal waste. The technique must be combined with other measures, such as direct plating of coliphages or cell culture techniques to assess infectivity.

Although we found a correlation between the presence of human enteric virus genomes and fecal coliforms, the correlation was weak and did not extend to all of the other bacterial indicators. This mirrors the findings of Noble and Fuhrman (1997), who conducted similar studies in Santa Monica Bay and found no apparent correlation between any of the bacteriological indicators and the presence of enteroviruses. The poor relationship between bacterial and viral indicators may indicate the substantial presence of non-human sources of bacterial contamination. All of the samples from this study were taken in the surf zone immediately adjacent to the storm drain outlets. Many of these outlets drain lagoonal systems that are inhabited by waterfowl, which can contribute large amounts of animal wastes. The two sites where we observed high bacterial counts in absence of human enteric virus genomes, Calleguas Creek and Arroyo

Burro, had hundreds of birds near the storm drain at the time of sampling. If animal wastes are a significant source, then bacterial indicators may provide an overly conservative estimate of microbiological water quality conditions, since animal waste does not typically contain pathogens of concern to humans.

An alternative explanation for the poor correlation between bacterial and viral indicators is the differential survival of pathogens in seawater (McNeill 1992). There are many complex factors that influence the persistence of pathogenic microorganisms, among them sedimentation, turbulence, sunlight intensity, temperature, and predation. Under some circumstances, viral pathogens can survive longer in the marine environment than indicator bacteria as they adsorb to solids that can protect them from inactivation by biological, chemical, and physical factors (EPA 1985). Conversely, McNeill (1992) has shown that coliforms and enterococci not only persist, but can grow in the marine environment at warmer water temperatures found in tropical areas. Understanding the relative degradation rates between bacterial indicators and the viral pathogens of human health concern, and how various environmental factors such as temperature affect their relative rates of attenuation, is essential to knowing how well bacterial indicators predict human health threats in marine waters.

The RT-PCR technique presented here provides a potential mechanism for distinguishing between human and animal fecal contamination and more closely identifying sources of possible human health risk. Although RT-PCR detection of human enteric virus material cannot be used to infer infectivity, RT-PCR radically improves upon the time required to detect the presence of human pathogens in seawater, taking a day rather than the weeks required for conventional cell culture techniques. Additionally, RT-PCR can be used to detect a variety of human pathogenic viruses not detectable by cell-culture techniques. The cost of RT-PCR, however, remains 50 times higher than that for bacterial indicator measurements. Further refinements to reduce cost will be required before the technique is feasible on a routine basis for addressing management decisions about local coastal health hazards.

VII. CONCLUSIONS

The Bight'98 Shoreline Microbiology Study represents the most comprehensive regional assessment of microbiological water quality along the Southern California Bight shoreline conducted to date. The regional and unbiased nature of the sites sampled provides the opportunity to make assessments that cannot be accomplished by examining data from individual sites or from samples collected by an individual monitoring agency. The study also is the first to compare the relative bacteriological water quality along Mexican and United States shoreline using similar site selection approaches and coordinated quality assurance methods. The survey participants, representing every agency that conducts routine microbiological monitoring in southern California plus a group of Mexican scientists, have reached the following conclusions based upon the findings of this study:

- Bacteriological water quality was consistently good along the southern California shoreline during the summer of 1998.

Nearly 95% of the shoreline mile-days from Santa Barbara through San Diego during August met all of the State of California's present and proposed bacterial water quality standards. Ninety-eight percent of the samples that exceeded a State standard did so for only one bacterial indicator, whereas other indicators measured at the site were within State standards. Less than 0.2% of the shoreline mile-days exceeded thresholds for all indicators measured at a single site. Except for those locations immediately adjacent to freshwater outlets, most of the threshold exceedances were temporally sporadic. Only three sites along the United States shoreline, other than those near a freshwater outlet, exceeded an indicator threshold for more than one of the five weeks sampled.

- Areas adjacent to freshwater outlets exhibited the worst microbiological water quality, both in the United States and in Mexico.

Areas adjacent to freshwater outlets, which constitute only a small of fraction of the southern California coastline, had poor microbiological water quality. Almost 60% of the shoreline mile-days in these areas failed State standards based upon monthly thresholds. Most of these exceedances were for multiple indicators and occurred repetitively throughout the five-week study period. Human enteric virus genetic material was detected in samples taken from 7 of 15 freshwater outlet locations; 73% of these detections coincided with an exceedance of a bacterial indicator threshold for fecal coliforms. Mexican freshwater outlets were about just as likely to exceed a bacteriological water quality standard as those in the United States.

- Mexican beaches exceeded indicator bacteria thresholds more frequently than beaches in the United States.

This cooperative study is the first to compare the relative water quality along Mexican and United States shoreline using similar site selection approaches and coordinated quality assurance methods. Although nearly 75% of the beach samples in Mexico met California's bacteriological water quality standards, the standards were exceeded five times more often along Mexican than United States beaches. The magnitude by which standards were exceeded was also higher in samples taken from Mexican beaches. This

information provides valuable base-line information that can be used to assess progress in efforts by Mexican authorities to improve their shoreline bacteriological water quality.

- Dry-weather beach closure decisions in southern California are sensitive to which indicators are measured at the site; closure rates are likely to increase with proposed new regulations.

In this survey, the enterococci standard proposed under AB411 was exceeded approximately twice as often as the proposed fecal coliform standard, and three times as often as the present total coliform standard. In areas away from freshwater outlets, 60% of the standards failures were for enterococci alone. Only 13% of the samples that failed one of the standards failed all standards. Beach closure decisions are made by local (county or city) health departments utilizing standards set by the State. For the last several decades, the standard has been based upon total coliforms. Proposed regulations drafted under AB411 require measuring all three indicators. Various drafts of the regulations have defined failure as (1) exceeding the threshold for any one indicator or (2) exceeding the thresholds for any two indicators. Either proposal will lead to a substantial increase in the number of sites failing State standards. If regulations are written such that exceeding thresholds for any two of the three indicators constitutes failure, the rate of posting or closure will increase by a factor of three. If the failure standard is written as exceeding a threshold for any of the three indicators, the rate of posting or closure will increase by a factor of ten.

- Data quality was high and comparable among all of the participating laboratories.

Three laboratory techniques, membrane filtration, multiple tube fermentation, and defined substrate technology, are variously used by different laboratories in southern California for routine monitoring. The quality assurance exercises conducted as a part of this study, which were the first nationally to compare all of these methods on marine samples, demonstrated that all three techniques provided comparable results. We also found a high degree of comparability among laboratories participating in the project, including volunteer monitoring organizations, indicating that the degree of protection the public receives in southern California does not differ as a function of which laboratory processes their local beach samples. We caution, however, that the conclusions about methods comparability are based only upon processing summer samples. These results may not extrapolate to winter samples, which can contain a higher number of interferences introduced by stormwater runoff.

- Southern California beach monitoring programs are highly effective.

More than 20 southern California organizations maintain shoreline bacteriological monitoring programs. Cumulatively, these organizations spend $3 million annually collecting samples from more than 500 sites and conduct more than 80,000 analyses per year in southern California. Most of this effort is focused on high-use beaches and known problem areas. The present study directed considerable efforts into new locations and did not uncover previously unmonitored "hot spots" of concern. Only 10 sites that exceeded a State threshold were located more than one-half of a mile from a routine monitoring site, and only one of these new sites exceeded a threshold for more than one week. Eight of the ten sites were located in Ventura and Santa Barbara Counties, where recent political and community support have led to the expansion of regularly monitored beach locations.

- Volunteer monitoring efforts can contribute valuable data to southern California monitoring programs.

An increasingly large component of beach monitoring in some areas is performed by volunteer organizations. One consideration in evaluating the effectiveness of a monitoring program is whether data produced by volunteer organizations is of sufficient quality to include in integrated beach assessments. The volunteer organizations participating in this study demonstrated through quality assurance exercises that they can produce data comparable to those of the certified professional laboratories. The volunteers involved in the study were more experienced than most, having conducted their own monitoring activities for many years. They also benefited from U.S. EPA-sponsored training and working closely with a local university. Regardless, they demonstrated that with a similar level of training, volunteer organizations can become full partners in developing regional beach quality assessments.

VIII. RECOMMENDATIONS

- Integrate stormwater management agencies into routine shoreline microbiology monitoring networks.

Ocean waters immediately adjacent to 60% of the freshwater outlets in southern California were found to exceed State standards for indicator bacteria, which accounted for more than 90% of the standards failures observed in this study. At present, virtually all of the routine monitoring in ocean waters near freshwater outlets is conducted by county health departments or by ocean-discharging sewage treatment facilities, both of which have limited jurisdiction to address problems observed near freshwater outlets. This dissociation between the organizations that design and implement ocean monitoring programs and the organizations that bear most of the management responsibility for correcting observed problems is inefficient for protecting the public's interest. 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 ensure continuing and expanded monitoring efforts near freshwater outlets; will allow them to react immediately to the results produced by these monitoring programs; and will establish the framework for their inland efforts to be integrated with the ocean area 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 Santa Monica Bay, and the stormwater programs for Orange, Riverside and San Bernardino Counties were co-sponsors of this regional monitoring program. This cooperative interaction should be expanded.

- Reassess the relationship between bacterial indicator thresholds and health risk.

This study found a high degree of inconsistency among the three bacterial indicators proposed as the basis for beach posting/closure decisions. The epidemiological evidence upon which indicator thresholds are based is scant and derived largely from studies conducted on the east coast under conditions that are vastly different from southern California. Moreover, the tools necessary to understand relationships between the pathogenic organisms that cause illness and the bacterial indicators monitored routinely by many southern California monitoring agencies are in the early stages of development. As a result, most agencies measure multiple indicators, which will soon be required under AB411 derived regulations. This method is inefficient, resulting in higher costs as each agency triples its effort to capture largely redundant information, since these bacterial indicators correlate. Agency expenditures might be better spent using one indicator to monitor more locations, or to monitor existing locations more frequently, but this can only occur if the scientific community agrees upon an epidemiological basis for selecting the most appropriate indicator and threshold. The California State Department of Health Services and the U.S. Environmental Protection Agency have independently embarked upon efforts to standardize beach monitoring data collection regionally and nationally. The public's interest, as well as the cost efficiency of monitoring, will not be greatly improved by these programs unless they focus on the research necessary to more closely relate existing measures to health risk.

- Quantify magnitude of bacterial densities.

Many of the measurements taken in this study, as well as in routine monitoring programs, yielded truncated values (for example, >16,000 for total coliforms) because standard methods do not mandate that these large indicator density values be "bracketed" by the analytical dilution series employed. The doctrine not to extend the dilution series to quantify all values has its roots in the health advisory framework where exceeding a threshold yields an advisory, regardless of whether the exceedance is small or large. More detailed quantification is important for several reasons:

1. The extent of public health risk is dependent on the concentration of bacteria, not simply on a categorical exceedance;

2. Draft beach closure standards associated with AB411 include indicator ratios, which cannot be calculated if one of the indicator values is not quantified;

3. Risk managers, particularly stormwater agencies, need to focus their mitigation efforts in places and times (seasons) of greatest health risk. Without quantification, it is difficult to assess relative risk among systems or time periods; and

4. Risk managers also need a means for assessing progress, which is most appropriately accomplished by trends in indicator densities. This is most efficiently done when indicator values are quantified.

Increasing the level of quantification, however, may not be logical at all sites and all times. The additional endpoints must be selected judiciously since the cost of an extra dilution series for a site nearly equals the cost of monitoring additional sites. Risk managers need to weigh the relative value of quantification in selecting the site locations and sampling intervals that are optimum to fully quantify their results.

- Conduct a similar cooperative regional survey during the wet season.

This study found the shoreline to be in good condition, but areas near freshwater outlets were consistently of concern. The study was conducted in the summer, under low flow conditions, when the influence of freshwater and stormwater inputs is lowest. It is unclear how much larger an area would be affected during higher flow conditions. The study also established a series of indicator relationships that begin to form the basis for refining monitoring strategies. A wet season study is needed to examine the consistency of those relationships between wet and dry conditions.

IX. LITERATURE CITED

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APPENDIX A. PARTICIPANTS IN THE SOUTHERN CALIFORNIA BIGHT 1998 REGIONAL MONITORING PROGRAM (BIGHT'98). [a] Denotes participants in the Shoreline Microbiology component.

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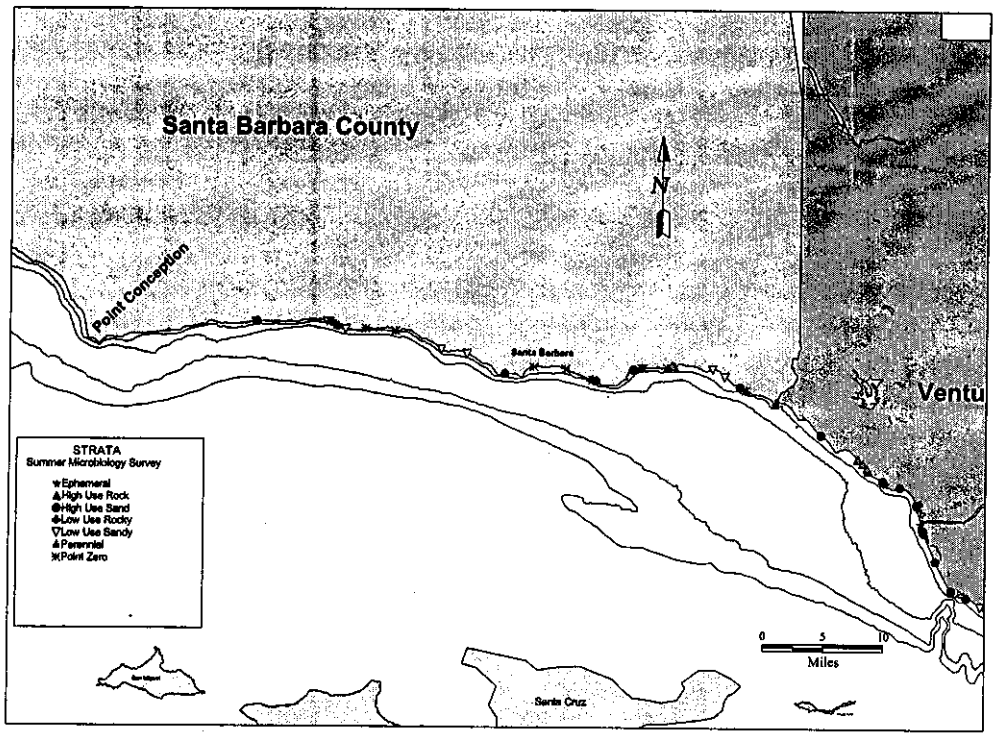
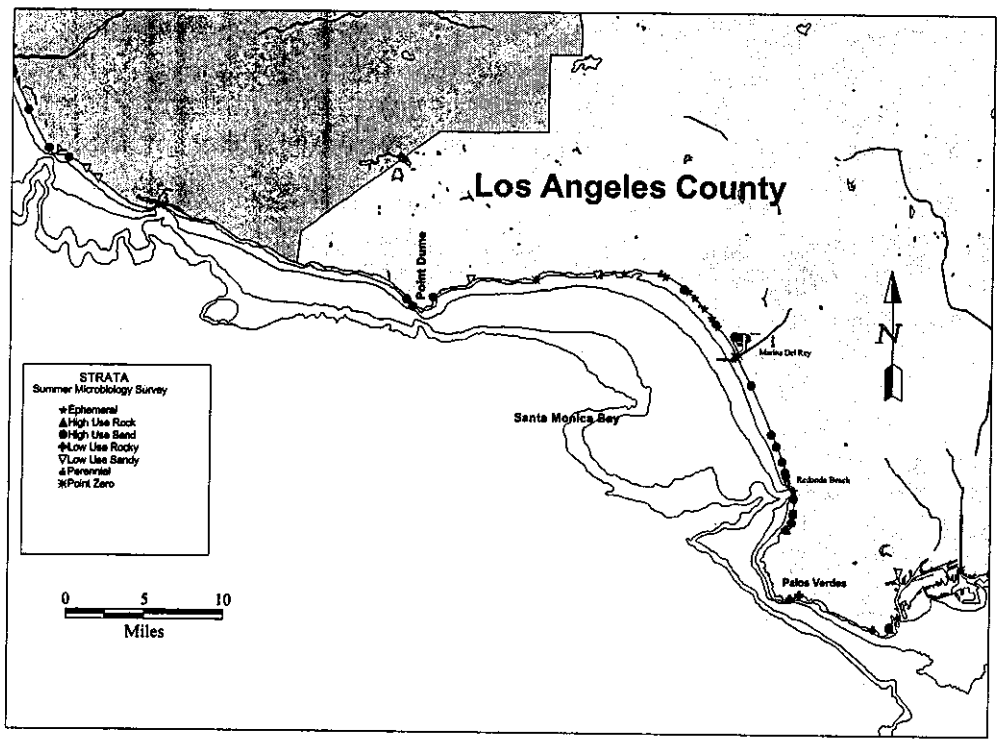
Algalita Marine Research Foundation
Aliso Water Management Agency(AWMA)[a]
Aquatic Bioassay and Consulting (ABCL)[a]
California Coastal Conservancy
Central Coast Regional Water Quality Control Board[a]
Channel Islands National Marine Sanctuary (CINMS)
Chevron USA Products Company
Cities and County of Riverside Stormwater Program
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 Laboratory
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]
Marine Corps Base Camp Pendleton[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 Bernardino County Stormwater Program
San Diego County Department of Environmental Health[a]

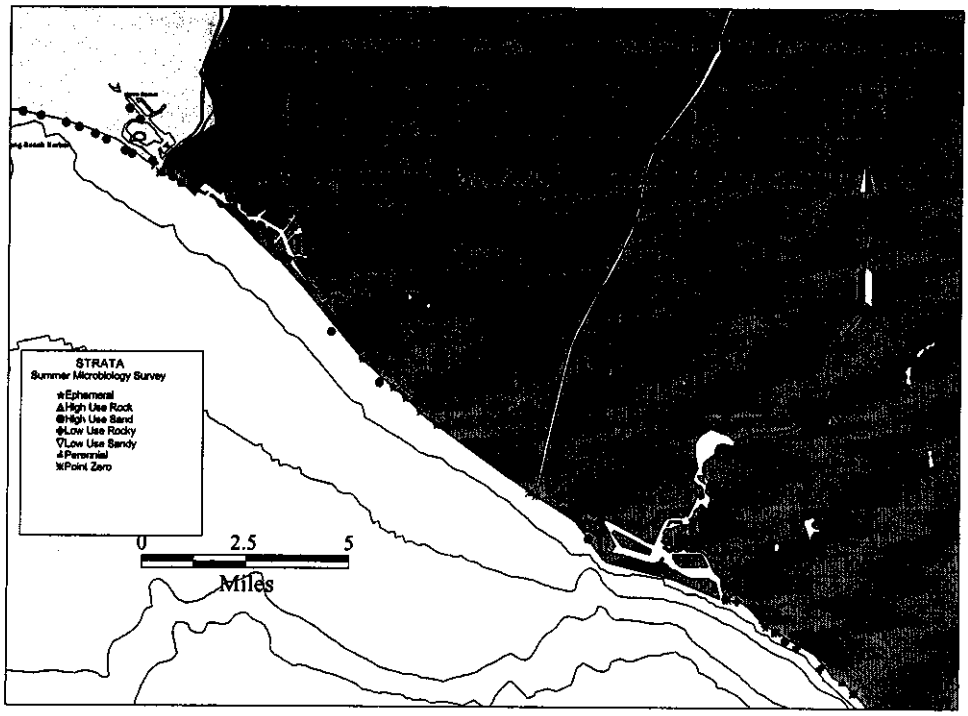
Appendix A (continued). Participants in the Southern California Bight 1998 Regional Monitoring Program (Bight'98). [a] Denotes participants in the shoreline microbiology component.

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
Southeast 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
US EPA Region IX
US EPA Office of Research and Development
US Geological Survey
US Navy, Space & Naval Warfare Systems Center, San Diego (USN)
Ventura County Health Department[a]

APPENDIX B. MAP OF STUDY AREA



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●High Use Sand
◆Low Use Rocky
▽Low Use Sandy
⊿Perennial
✱Point Zero
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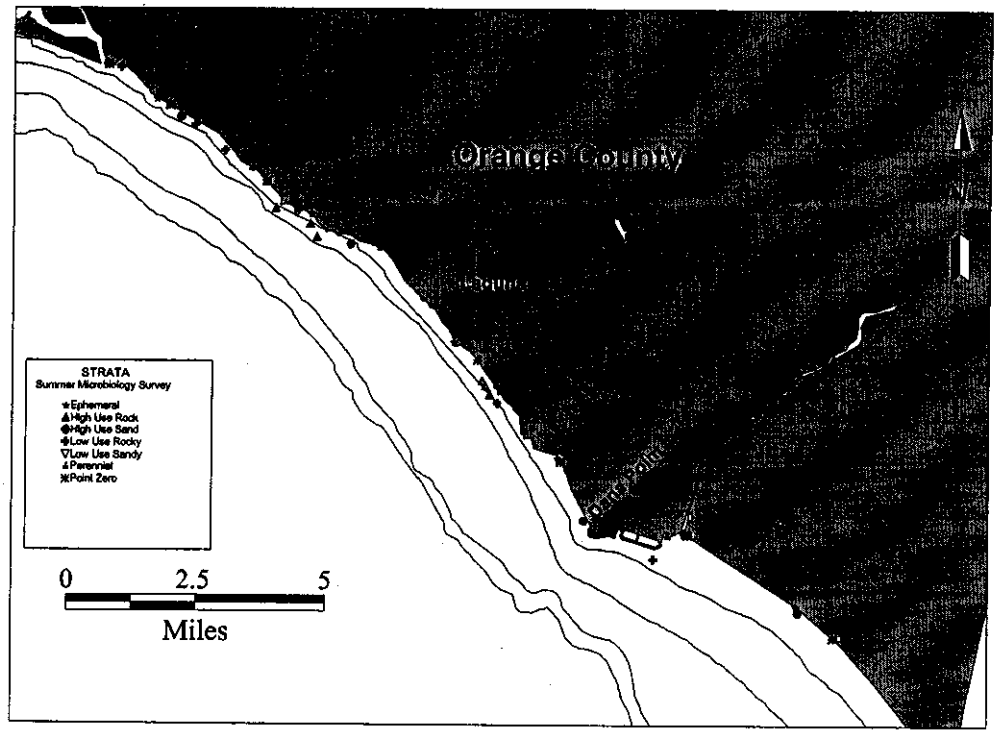
5
Miles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STRATA
Summer Microbiology Survey
★Ephemeral
▲High Use Rock
●High Use Sand
◆Low Use Rocky
▽Low Use Sandy
⊿Perennial
✱Point Zero
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2.5
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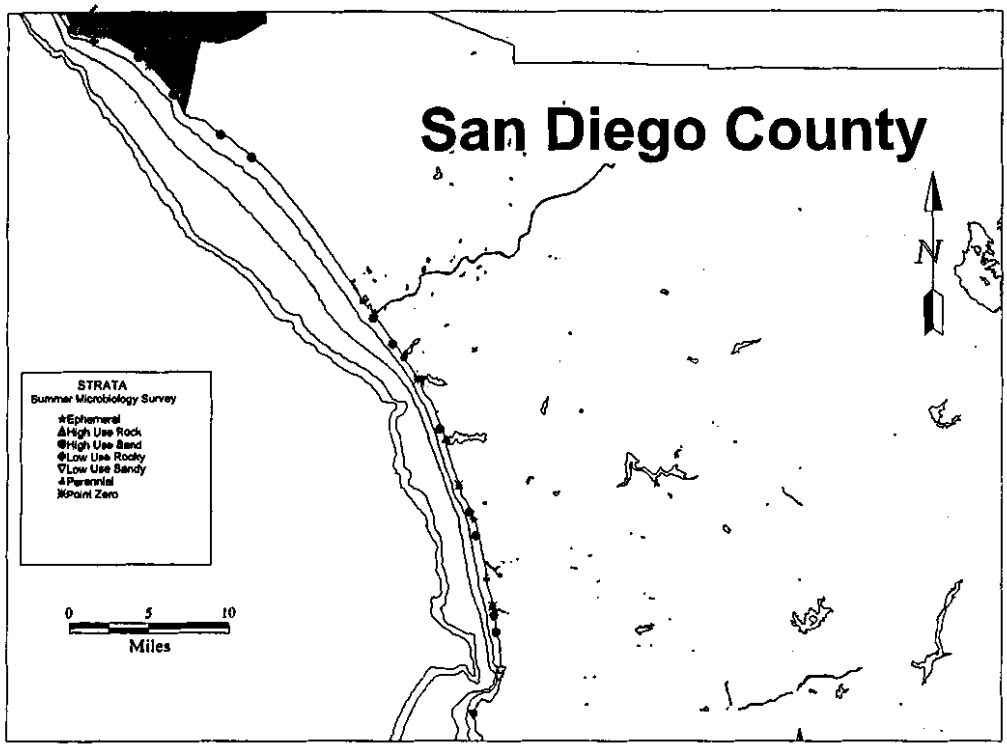
Miles


San Diego County
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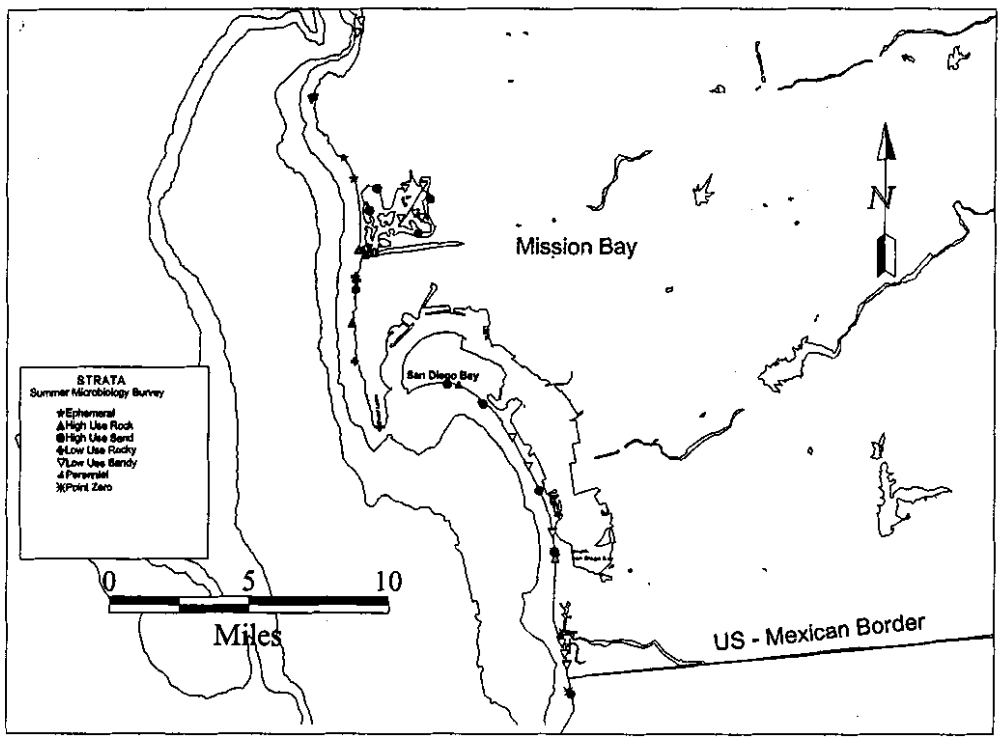
STRATA
Summer Microbiology Survey
★Ephemeral
▲High Use Rock
●High Use Sand
◆Low Use Rocky
▽Low Use Sandy
4 Perennial
✳Point Zero
0 5 10
Miles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Mission Bay
San Diego Bay
STRATA
Summer Microbiology Survey
★Ephemeral
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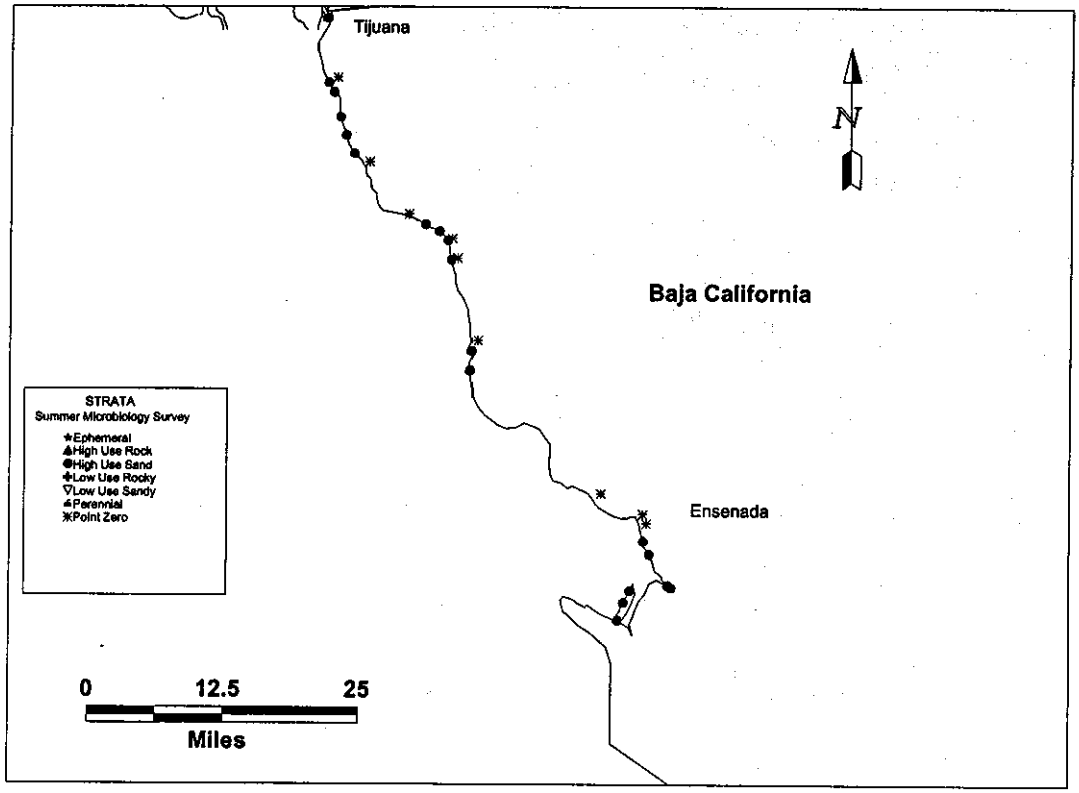
✳Point Zero
0 5 10
Miles
US - Mexican Border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Summer Microbiology Survey
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Low Use Rocky
Low Use Sandy
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Miles

APPENDIX C. COMPARISON OF BACTERIAL INDICATOR MEASUREMENTS AMONG SOUTHERN CALIFORNIA MARINE MONITORING LABORATORIES

ABSTRACT

Recent initiatives to develop regional/national assessments of beach quality require consolidation of bacteriological data across multiple laboratories. In southern California, 22 laboratories routinely measure bacterial indicators of fecal contamination using several methods. To assess data comparability, each of these labs quantified total coliforms, fecal coliforms or *E. coli*, and enterococci density from thirteen common samples. Three sources of variability (among laboratories, among analytical methods and within laboratory) were also quantified and compared. The average difference among methods was less than 6%. The average difference among laboratories was less than 2%. The greatest source of variability was among replicates within individual laboratories. Combining data from all laboratories using different methods increased variability by only about 30% over that which would be expected if a single laboratory using a single method generated all of the data.

INTRODUCTION

Coastal waters are an important economic and recreational resource that is influenced by human activities. Treated wastewater discharges, industrial inputs, and surface runoff all affect coastal water quality and create the impetus for extensive water quality monitoring programs. An important criterion for assessing the potential health risk of recreational waters to swimmers is the density of bacteria associated with fecal contamination. The bacteria most commonly used as indicators of fecal contamination are total coliforms, fecal coliforms, *Escherichia coli (E. coli)*, and enterococci. Although indicator bacteria do not necessarily cause illness, they are abundant in human waste where pathogenic organisms, such as viruses and parasites, are also likely to exist. Bacterial indicators are measured instead of pathogenic organisms because the indicators occur in much larger numbers and can be measured with faster, less expensive methods than the pathogens of concern.

Nationwide, tens of thousands of marine water samples are analyzed annually for indicator bacteria (Natural Resources Defense Council 1998). Most of the analyses are part of sampling programs that are independently planned and implemented by local or county public health departments, or by Publicly Owned Treatment Works (POTWs) fulfilling federal, state and regional monitoring requirements specified in their permit to discharge wastewater into waters of the United States. In southern California alone, over 20 agencies regularly monitor near-shore water quality (Appendix D), but the data are rarely combined to provide estimates or comparisons of conditions on a regional scale.

Several recent initiatives require the merger of data at regional and national levels. These initiatives, which reflect public desire for a more comprehensive assessment of beach water quality, include California Assembly Bill 411; USEPA's Beaches Environmental Assessment, Closure, and Health (BEACH) program; and the World Health/USEPA Expert Consultation of Safety of Recreational Waters. One concern that arises when consolidating data from independent programs is that the numerous laboratories that perform the analyses use different analytical methods. Standard enumeration methods for the isolation of viable bacteria from environmental samples include membrane filtration (MF) and multiple tube fermentation (MTF). Each of these enumeration formats can also be used with more than one type of media. For example, the MTF method of enumerating fecal coliforms can be performed using EC or A-1 media.

Enumeration using chromogenic substrate media, media that can detect enzymes produced by specific bacteria or groups of bacteria, are also available and currently being used by several monitoring agencies.

The consistency in response among methods has rarely been quantified. A few studies have compared response between pairs of methods (Eckner 1998, Stasiak and Cheng 1991, Edberg *et al.* 1990, Green *et al.* 1997) and one study examined among-laboratory variability in marine applications (Messer and Dufour 1998). No study has quantified among-method variability for the three methods (MTF, MF and chromogenic substrate kits), nor has any study placed among-method variability within the context of variability among laboratories that use the same methods. California's Environmental Laboratory Accreditation Program (ELAP) attempts to address comparability among laboratories by establishing acceptance criteria for specific test methods, but the program does not rigorously quantify inter-method or inter-laboratory variability. Within-laboratory variability between methods has been assessed on a limited basis when a laboratory demonstrates method comparability in preparation for switching from one analytical method to another.

This study examined comparability of data generated by 22 southern California laboratories when quantifying total coliforms, fecal coliforms (or *E. coli*), and enterococci densities in common samples. Participants included 12 wastewater discharger agencies, five public health departments, three volunteer organizations, one private consulting laboratory and one university laboratory (Table C-1). The study assessed among laboratory, among analytical method and within laboratory variability. The additional variability introduced by pooling data from different monitoring programs using different methodologies was also quantified and placed within the context of natural variability occurring within a single laboratory program.

TABLE C-1. Laboratories participating in the interlaboratory comparison study.

Laboratory	Methods Used
Algalita Marine Research Foundation	Colilert®
Aliso Water Management Authority and Southeast Regional Reclamation Authority	MF, MTF
Aquatic Bioassay and Consulting Laboratories	MTF
City of Long Beach Department of Health & Human Services	MF, MTF
City of Oceanside	MTF
City of Oxnard	MTF
City of Los Angeles Environmental Monitoring Division	MF, Colilert®
City of San Diego	MF, MTF
City of Santa Barbara	MTF, Enterolert®
City of Ventura	MTF
Encina Wastewater Authority	MF
Goleta Sanitation District	MTF
Instituto de Investigaciones Oceanalogicas (UABC)	MTF
Los Angeles County Department of Health Services	MF, MTF, Colilert®, Enterolert®
County Sanitation Districts of Los Angeles County	MF, MTF
Orange County Public Health Laboratories	MTF, Colilert®
Orange County Sanitation District	MF, MTF, Colilert®, Enterolert®
San Diego County Department of Environmental Health	MTF
San Elijo Joint Powers Authority	MTF
Santa Barbara Public Health Department	Colilert®, Enterolert®
Southern California Marine Institute	Colilert®
Surfrider Foundation	Colilert®, Enterolert®

METHODS

Five intercalibration exercises were conducted. The first three exercises involved quantification of total coliforms, fecal coliforms (or *E. coli*) and enterococci in the transport medium. Each of the exercises used three concentrations of the bacterial indicator. The fourth exercise involved quantification of total coliforms and fecal coliforms (or *E. coli*) at a single concentration in seawater and fecal coliforms (or *E. coli*) in transport medium. The final exercise involved quantification of a single concentration of fecal coliforms (or *E. coli*) in seawater.

In the first three exercises, samples were prepared by seeding 24 hour-old stock cultures of *E. coli* (ATCC 75922) or, *Streptococcus faecalis* (ATCC 29212) into 10-liter carboys of NYSDH-1 transport medium (Toombs and Conner 1980). Transport media was prepared prior to the day of the experiment in two-liter volumes and sterilized. Carboys were sterilized separately. Bacteria was added to the transport media and mixed for twenty minutes on a magnetic mixer prior to dispensing the first sample. Targeted seeding densities were 100, 1,000 and 10,000-bacteria/100 mL. Amount of stock culture necessary to achieve the target densities was based on MF analyses begun the preceding day.

In the fourth exercise, *E. coli* was added to both seawater and transport medium. In the final exercise, filtered primary wastewater from the Orange County Sanitation District Plant #1 was added to seawater. Primary wastewater was filtered through Whatman Grade 415 filter paper. To increase homogeneity among aliquots, the seawater was filtered through a sand filter to remove large particulates.

Samples were readied by 8:00 AM, packed in ice, and distributed in time for all laboratories to begin their analyses by 1:00 PM the same day. The originating laboratory analyzed the first and last sample dispensed from each carboy by MF and MTF procedures in order to validate the homogeneity of bacteria in the carboy. Analyses were begun soon after the last sample was collected from the carboy and again four hours later.

Each laboratory was allowed to use its own standard operating procedures. Methods used by participants included 9221B, C and E, 9222B and D, 9230B and C in *Standard Methods for the Examination of Water and Wastewater*, APHA, AWWA, WEF, 18th edition, 1995 and EPA method 1600. Colilert® and Enterolert® (Idexx Laboratories, Inc, Westbrook, ME) kits were used in both 15-tube MTF format and 51 well Quantitray® format. Three to five replicates for each indicator at each density were required. Several laboratories used more than one analytical method, which resulted in more than 22 analytical results reported in some data sets.

Log transformed bacterial density measurements were compared among laboratories and among methods using a nested ANOVA model. Multiple comparisons were performed using Tukey's method, with alpha set to an overall experimental error rate of 0.05. Three components of variance (among-replicate variance within individual laboratories, among-laboratory variance, and among-method variance) were estimated using the sum of squares from the nested ANOVA mode.

RESULTS

Data were highly consistent among laboratories and methods. For only 11 of the 213 analyses performed did a sample result differ by more than 0.5 log unit from the median for the test batch (Figures C-1 - 5). Six of these cases were for fecal coliforms recovery by MF. The remaining five cases were due to procedural errors, which were later identified and corrected. The five outlying values were removed from the data sets prior to performing statistical analysis, although they appear in the figures.

FIGURE 1. Log total coliform density from first exercise. Dashed lines are overall mean +/- 0.5 log.

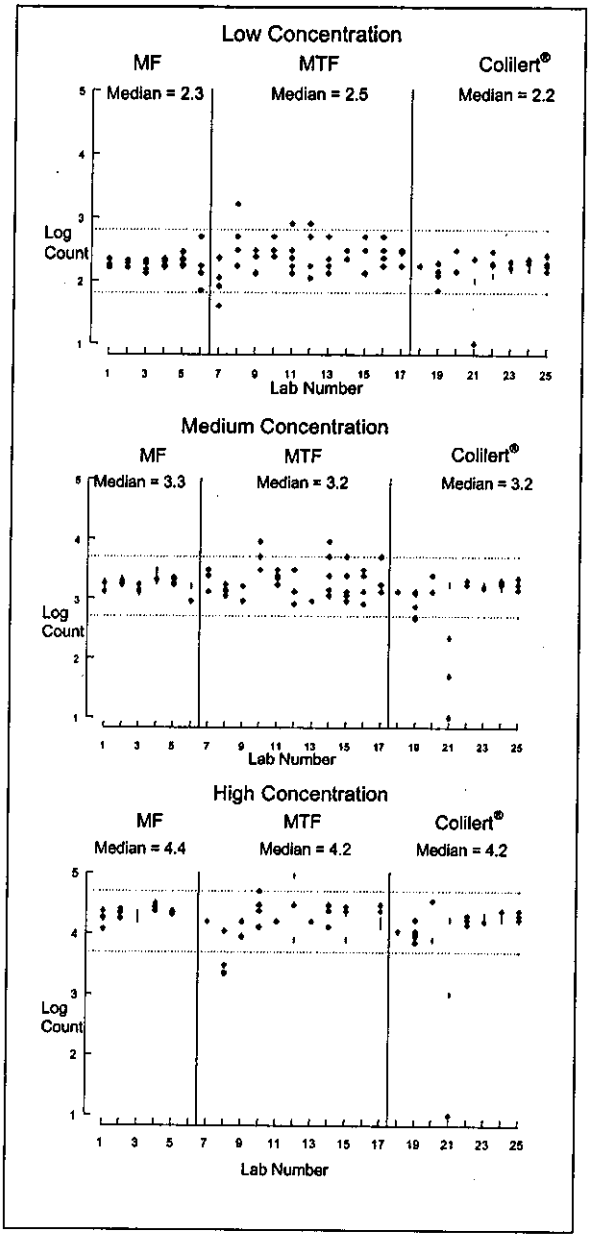


FIGURE 2. Log fecal coliform or *E. coli* density from second exercise. Dashed lines are overall mean +/-0.5 log.

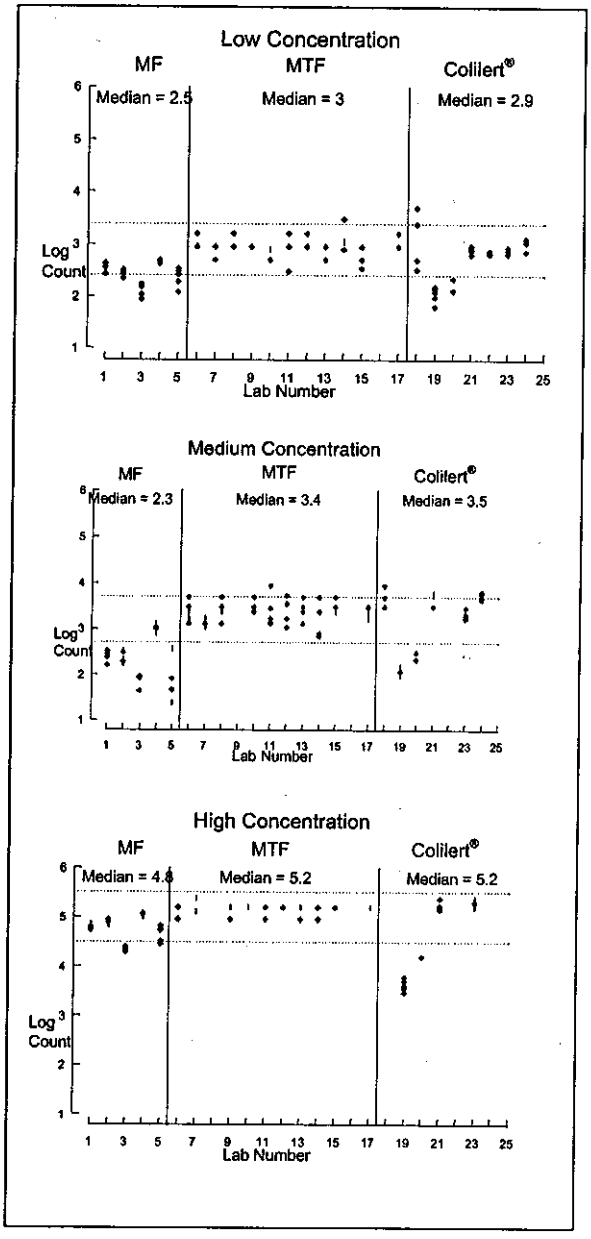


FIGURE 3. Log enterococcus density from third exercise. Dashed lines are overall mean +/- 0.5 log.

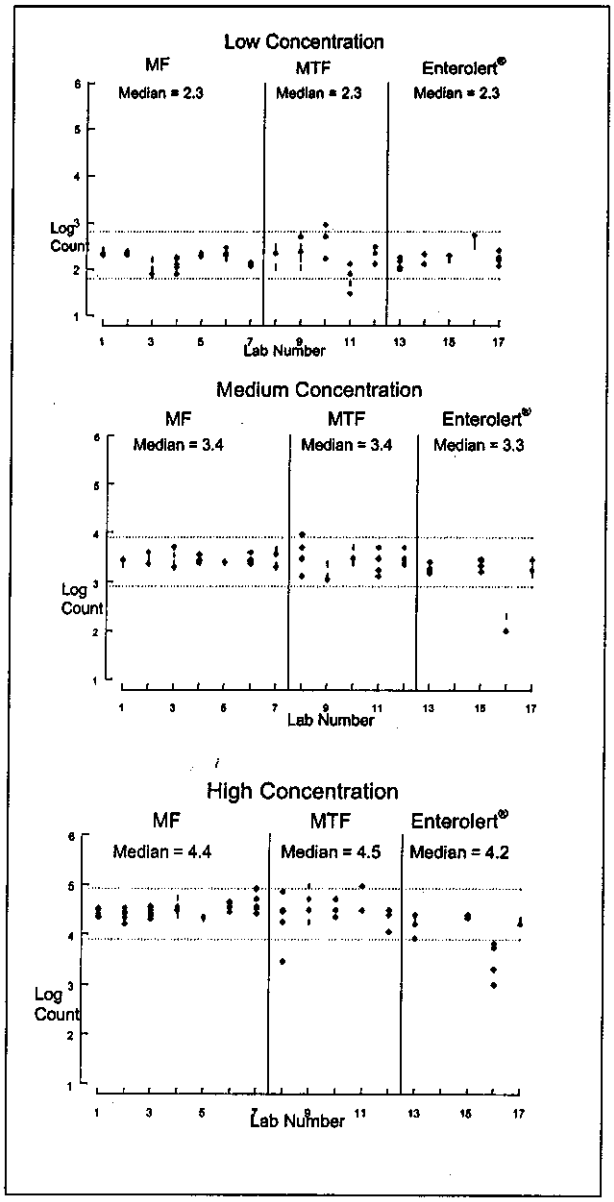


FIGURE 4. Log total coliform and fecal coliform or E. coli density from fourth exercise. Dashed lines are overall mean +/- 0.5 log.

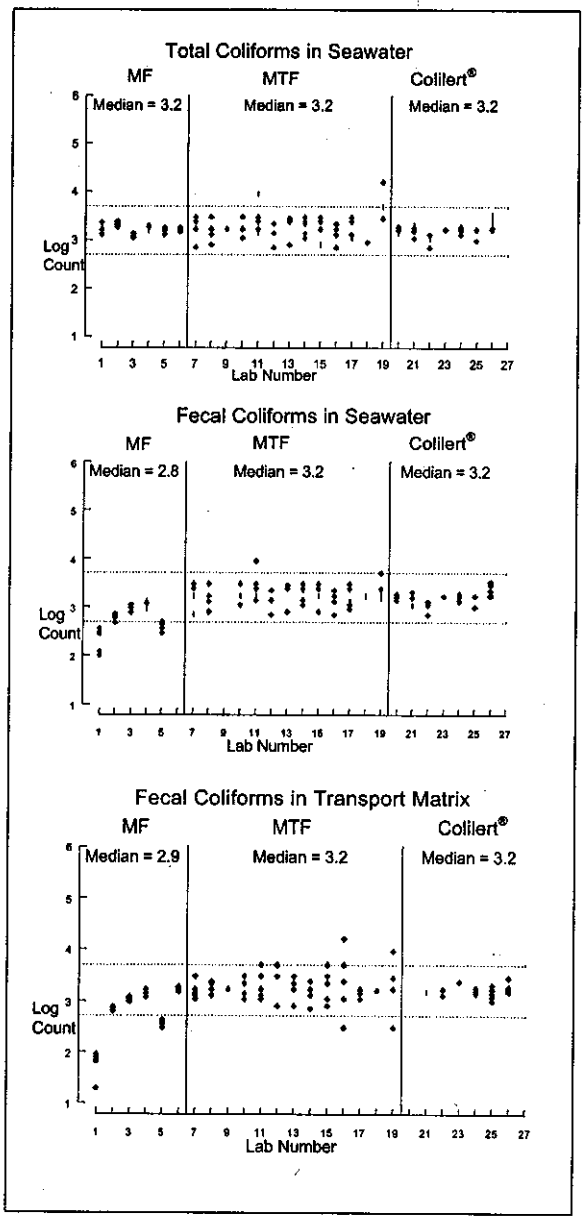
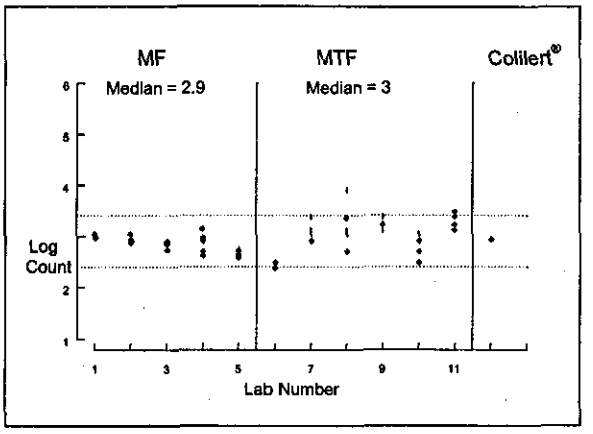


FIGURE 5. Log fecal coliform density from fifth excercise. Dashed lines are overall mean +/- 0.5 log.



Bacterial densities differed among laboratories for seven of the 13 samples analyzed, but most of these differences were small and limited to a few laboratories. Only 7% of all of the pairwise comparisons among laboratories differed significantly, and most of these differences occurred in the early exercises. (Tables 2-4). The largest difference among laboratories was 29%, with an average difference of less than 2%. Among-laboratory differences occurred most frequently for total coliforms (10%) and least frequently for fecal coliforms (3%).

Bacterial density measurements differed significantly among analytical methods for 16 of 37 possible comparisons (43%), but the average between-method difference was less than 6% (Table 5). The largest among-method difference in any of the tests was 41%. Most of the differences among methods were due to low fecal coliforms values measured by MF (Figures 2 and 4). This result remained consistent even after the six values differing by more than 0.5 log units were removed. The *E. coli* stock culture used in these experiments was suspected to be thermophilic with a tendency to clump, which would account for the low densities reported using MF enumeration. To eliminate this potential confounding, filtered wastewater was used in place of a pure culture of *E. coli* in the final exercise. After switching to the wastewater inoculant, MF results did not differ significantly from the other two MTF enumeration formats. The only consistent difference among methods occurred for the Enterolert® method. At low densities, Enterolert® results were statistically indistinguishable from those of the other two methods, but at intermediate and higher densities, Enterolert® underestimated concentrations relative to the other two methods by 5% (Figure 3).

The largest source of variability identified in this investigation was among replicates within individual laboratories (Table 6). The MTF method yielded the greatest within-laboratory variability (Table 6), with recovery values typically ranging between one-third and three times the median value. The MF method had the smallest within-laboratory variance (Table 6), with a typical recovery range of two-thirds to 1.5 times the median value.

Among-laboratory variance was about two-thirds of the within-laboratory variance (Table 6). Similar to the pattern for the within-laboratory variability, among-laboratory variability was greatest for MTF and least for MF. Among-method variability was only about one-third of the within laboratory variance.

DISCUSSION

This investigation demonstrated that data from multiple laboratories using various analytical methods could be pooled without adding an unacceptable level of additional variability. Between-laboratory pairwise differences were generally small and improved in later interlaboratory testing efforts. The difference among methods was small, and the variability added by using multiple methods was less than the normal variability encountered using a single method in a single laboratory. Overall, the increase in variability among measurements from pooled data was approximately 30% higher than data obtained using a single analytical method performed at a single laboratory. Although none of the samples analyzed by participants in this study were environmental samples, the data suggest that a performance-based approach at multiple laboratories is acceptable for measurement of indicators of seawater contamination.

Chromogenic substrate detection methods, such as Colilert®, have not yet been approved as standard methods for marine waters by the USEPA or by the Standard Methods Committee. No significant difference was found in this study between results obtained by Colilert® and those obtained using approved standard methods for coliforms; differences in results between Enterolert® and approved methods for enterococci were small and the differences only occurred at concentrations well above California Ocean Plan standards. Data from this study also demonstrated that variability within laboratories using Colilert® was less than that for the standard MTF methods, which probably results because Colilert® is based on a 51-well format while MTF is typically performed in 15 tubes.

While these findings support the use of chromogenic substrate tests, they are not comprehensive. The bacteria measured in the first four tests were laboratory strains, with no background bacteria to compete or interfere with analyses. In informal field tests, some of the participating laboratories have noted that *Vibrio* sp. can interfere with, and lead to overestimates of, total coliforms. Also, none of the samples contained high levels of suspended solids. Low turbidity is typical in southern California in the summer-dry season, but not always during the winter-wet season. Side-by-side testing of samples from the natural environment, particularly during high turbidity conditions, is a logical next step in evaluating these candidate methodologies.

An increasingly large component of beach monitoring in some areas is performed by volunteer organizations. One consideration in creating integrated beach assessments is whether data produced by volunteer organizations is of sufficient quality for inclusion. The volunteer organizations involved in this study produced data comparable to that of the certified professional laboratories. The volunteers involved in our study were more experienced than most, having conducted their own monitoring activities for many years. They also benefited from EPA-sponsored training and working closely with a local university. Regardless, our data show that with proper training, volunteer organizations can become full partners in developing regional beach quality assessments.

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Edberg, S.C., M.J. Allen, D.B. Smith, and N.J. Kriz. 1990. Enumeration of total coliforms and *Escherichia coli* from source water by the defined substrate technology. *Applied and Environmental Microbiology* 56:366-369.

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Stasiak, M.C. and S.H., Cheng. 1991. Coliforms detection using membrane filtration and Colilert, and *E. coli* detection using nutrient AGAR Plus MUG and EC Plus MUG, pp. 741-748 *in:* Proceedings of the Water Quality Technology Conference, Orlando. American Water Works Association, Denver, CO.

Toombs, R.W. and D.A. Conner. 1980. Proficiency test sample media for single and mixed pure cultures of water pollution indicator bacteria. *Applied and Environmental Microbiology* 40:883-887.

TABLE C-2. Percent significant difference in fecal coliforms or *E. coli* density between pairs of laboratories. Randomly assigned laboratory numbers are in the first row and column. NS indicates no significant difference between laboratory pairs.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
2	NS															
3	NS	NS														
4	NS	NS	NS													
5	NS	NS	NS	NS												
6	NS	NS	NS	NS	NS											
7	NS	NS	NS	NS	**4**	NS										
8	**19**	**20**	**19**	**22**	NS	NS	**21**									
9	NS	NS	NS	NS	NS	NS	NS	**13**								
10	NS	NS	**14**	NS	NS	**14**	NS	**19**	**16**							
11	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS						
12	NS	NS	NS	NS	NS	NS	NS	**23**	NS	NS	NS					
13	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS				
14	NS	NS	NS	NS	NS	NS	NS	**20**	NS	NS	NS	NS	NS			
15	NS	NS	NS	NS	NS	NS	NS	**19**	NS	NS	NS	NS	NS	NS		
16	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	
17	NS	NS	NS	NS	NS	NS	NS	**19**	NS	NS	NS	NS	NS	NS	NS	NS
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29	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
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31	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
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17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
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18	**25**	**19**	NS	**23**	NS	**20**	**15**	NS	NS	NS	NS	NS			
NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**19**		
NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	
NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**19**	NS	NS

TABLE C-3. Percent significant difference in total coliforms density between pairs of laboratories. Randomly assigned laboratory numbers are in the first row and column. NS indicates no significant difference between laboratory pairs.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
2	NS															
3	NS	NS														
4	NS	NS	NS													
5	NS	NS	NS	NS												
6	NS	NS	NS	NS	NS											
7	NS	NS	NS	NS	NS	NS										
8	NS	NS	NS	NS	NS	NS	NS									
9	NS	NS	NS	NS	NS	NS	NS	NS								
10	NS	NS	NS	NS	NS	NS	NS	NS	NS							
11	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS						
12	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS					
13	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS				
14	**22**	NS	NS	**14**	NS	NS	NS	NS	NS	NS	NS	NS	NS			
15	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**17**		
16	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	
17	**21**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**16**	NS
18	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
19	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
20	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
21	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
22	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
23	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
24	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
27	**18**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
28	**20**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**15**	NS
29	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
30	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
31	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
32	NS	**21**	**18**	NS	**16**	NS	NS	NS	NS	NS	NS	NS	NS	**29**	NS	NS
33	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

17	18	19	20	21	22	23	24	27	28	29	30	31	32
NS													
NS	NS												
NS	NS	NS											
NS	NS	NS	NS										
NS	NS	NS	NS	NS									
NS	NS	NS	NS	NS	NS								
NS	NS	NS	NS	NS	NS	NS							
NS	NS	NS	NS	NS	NS	NS	NS						
NS	NS	NS	NS	NS	NS	NS	NS	NS					
NS	NS	NS	NS	NS	NS	NS	NS	NS	NS				
NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS			
NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS		
28	NS	NS	NS	NS	NS	NS	NS	**26**	**28**	NS	NS	NS	
NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

TABLE C-4. Percent significant difference in enterococci density between pairs of laboratories. Randomly assigned laboratory numbers are in the first row and column. NS indicates no significant difference between laboratory pairs.

3	5	6	8	9	10	11	12	13	14	15
NS										
NS	**26**									
19	NS	NS								
NS	NS	**26**	NS							
NS	NS	**25**	NS	NS						
NS	NS	NS	NS	NS	NS					
NS	NS	NS	NS	NS	NS	NS				
NS	NS	**22**	NS	NS	NS	NS	NS			
NS	NS	NS	NS	NS	NS	NS	NS	NS		
NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	
NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
NS	NS	**16**	NS	NS	NS	NS	NS	NS	NS	NS
NS	NS	**21**	NS	NS	NS	NS	NS	NS	NS	NS
NS	NS	**12**	**18**	NS	NS	NS	NS	NS	NS	NS
NS	NS	NS	**19**	NS	NS	NS	NS	NS	NS	NS
NS	NS	**16**	NS	NS	NS	NS	NS	NS	NS	NS

TABLE C-5. Average percent difference in median log bacteria density between pairs of methods.

Total Coliforms

	MF	MTF
MTF	4%	
Colilert®	3%	2%

Fecal Coliforms

	MF	MTF
MTF	16%	
Colilert®	15%	1%

Enterococcus

	MF	MTF
MTF	1%	
Enterolert®	3%	3%

18	21	22	24	26

NS				
NS	NS			
NS	NS	NS		
NS	NS	NS	NS	
NS	NS	NS	NS	NS

TABLE C-6. Comparison of variance components.

	MF	MTF	Colilert®Enterolert®	Pooled Over Method
Within lab variance	0.007	0.047	0.021	0.03
Among lab variance	0.01	0.04	0.01	0.02
Merged lab variance	0.01	0.077	0.027	0.05

APPENDIX D. INVENTORY OF MICROBIOLOGICAL MONITORING PROGRAMS FOR MARINE RECREATIONAL WATERS IN SOUTHERN CALIFORNIA

ABSTRACT

An inventory was conducted to assess the amount, type, spatial distribution, and costs of microbiological monitoring programs in southern California marine waters from Point Conception to the United States/Mexico International Border. The location of each sampling site was determined using global positioning system (GPS) and estimates of geographical coverage were determined using geographic information system (GIS) techniques. Twenty-one programs conducted 87,007 tests annually at 576 sites. Sampling effort varied by more than an order of magnitude among counties. The greatest number of sites were sampled in Orange County, whereas the greatest number of tests were performed in Los Angeles County because Los Angeles County monitoring programs focused on daily monitoring. Fifteen of the 21 programs were National Pollutant Discharge Elimination System (NPDES) permitted sewage effluent dischargers who sampled both offshore and shoreline waters and typically tested for three indicator bacteria (total coliforms, fecal coliforms, and enterococcus). Their combined effort comprised 82% of all the microbiological indicator analyses on an annual basis. Five of the remaining monitoring organizations were public health agencies (four county, one city) which typically focused their efforts on testing only for total coliforms. Laboratory methodology also varied considerably, with NPDES permittees predominantly utilizing membrane filtration while public health agencies generally used multiple tube fermentation or premanufactured test kits. Nearly three-quarters of all the effort expended in southern California occurred along the shoreline as opposed to offshore locations. Two-thirds of this shoreline effort was focused on high use sandy beaches and around perennial freshwater outlets (storm drains and creeks), which are frequent sources of shoreline bacterial contamination. Most sampling occurred at a set of fixed sites that were revisited frequently, but represented only about 7% of the total shoreline. Approximately $3M is spent annually on monitoring bathing water quality in southern California, exceeding that spent in any other part of the country.

INTRODUCTION

Southern California coastal waters are an important and unique recreational resource. More than 100 million people visit southern California beaches annually to sunbathe, surf, swim, skin- and SCUBA-dive. On a summer weekend, the average number of visitors to Santa Monica Bay beaches alone is more than 600,000 (Economic Resources Data 1993). These ocean recreation activities contribute approximately $9B to the local economy.

Southern California coastal waters are extensively tested for recreational water quality using indicator bacteria, which include total coliforms, fecal coliforms, and enterococcus. Indicator bacteria are not necessarily pathogenic, but are found abundantly in wastes with human contributions where pathogenic organisms, such as viruses, are likely to exist. The levels of indicator bacteria in bathing waters have been shown to correlate with the incidence of illness in swimmers from New Jersey and Santa Monica Bay (Cabelli 1983, Haile *et al.* 1996) and, unlike the virus tests which are time consuming and expensive, measurements of indicator bacteria are relatively fast and inexpensive.

Many organizations conduct microbiological monitoring of beaches in southern California, but these programs are largely independent with no formal mechanism for integrating their data. These programs are

valuable for assessing the condition of selected individual beaches, but are not currently being used to assess the overall condition of southern California beaches. In this paper we present an inventory of these programs to determine the level of effort being expended by monitoring programs in terms of the amount, type, spatial distribution, and cost. Our goal is to identify similarities and differences among these programs, and to determine the extent to which they could be integrated to provide the public with a comprehensive assessment of southern California's coastal waters.

METHODS

A list of organizations that conduct microbiological monitoring in marine waters was compiled by contacting all of the city and county public health agencies and Regional Water Quality Control Boards in southern California. Monitoring organizations were then surveyed for the following information about each of their sampling sites: station name, location (latitude/longitude, general description, water body type), depth of sampling, analytes measured, analysis methods, and sampling frequency by season. Sites for which latitude and longitude data were unavailable were visited with the sampling organization and recorded using differential GPS.

The relative distribution of sampling effort among habitat types was assessed by differentiating sampling sites into offshore and shoreline; shoreline sites were further differentiated into eight categories: 1) high use sandy beaches; 2) low use sandy beaches; 3) high use rocky shoreline; 4) low use rocky shoreline; 5) perennial freshwater input areas; 6) ephemeral freshwater input areas; 7) embayments, and 8) restricted access areas. Offshore samples were defined as those collected by boat from the open ocean. High use sandy beaches were defined as beaches where lifeguard services are present (an estimated > 50,000 beachgoers per year). High use rocky shoreline was defined as rocky areas popular for diving or surfing activities. Freshwater input areas were defined as within 100 yards of rivers and creeks which drain into the ocean, and were separated into perennial (year-round) and ephemeral (only during storm event) depending on their flow characteristics. Samples from freshwater input areas were only included in the inventory if they were from waters with measurable salt concentration (i.e. monitoring of freshwater creek systems was not included). Embayment samples were defined as those collected by boats or from docks in enclosed water bodies, such as Anaheim, Newport, or Mission Bays; boat-collected samples in embayments were differentiated from offshore samples because of the higher level of recreational activity and likelihood of human water contact in bays. Restricted access areas included military bases, commercial ports, and private shoreline distant from any public access point. These eight shoreline categories were mapped for the entire southern California coast using GIS techniques. Each shoreline type was designated and inserted into the GIS overlay based on the expertise of local monitoring agencies, cross-referencing designations from the most recent NOAA navigation charts, and using maps from the California State Lands Commission, California Coastal Commission, and city/county governments.

Estimating spatial coverage

The spatial coverage of shoreline monitoring (i.e., percent of shoreline miles) was estimated by plotting each station in our microbiological monitoring inventory onto the digitized map of the southern California shoreline, assigning a representative distance of shoreline to each sample and then counting the relative number of monitored and unmonitored shoreline miles for each shoreline category. At freshwater

outlets, it was assumed that a sampling site represented a minimum area of 25 yard upcoast and downcoast (i.e., 50 yard total), based on the findings of Gold *et al.* (1992). All other types of shoreline samples were assumed to represent a shoreline distance of 200 yards (100 yards up and downcoast) based on Haile *et al.* (1996).

Estimating Monitoring Costs

The annual expenditure on microbiological monitoring in southern California was estimated by assessing both analytical laboratory and sampling costs. Analytical laboratory expenses were calculated based upon the current market rate for microbiological testing, which averages $30 per analysis per sample (i.e. $90 per sample if three indicator bacteria are measured). Sample collection costs were calculated by assuming that a single technician making $30/hour (including benefits and overhead), could sample three sites per hour along the shore and two sites per hour for offshore samples (based on conversations with people presently conducting the efforts). Transportation costs were assumed to be $2 per sample for shoreline monitoring ($0.33 per mile) and $50 per sample for offshore monitoring, where vessel and boat crew are required.

The cost of the shoreline monitoring was also expressed per capita, per shoreline mile and per tourist dollar expended within each county. Population statistics for each county were obtained from the State of California Department of Finance (1998). Shoreline miles were gathered from the GIS effort above. Tourism estimates were gathered from California Trade and Commerce Agency, Division of Tourism (1998).

RESULTS

Twenty one programs were found to conduct 87,007 indicator bacteria analyses per year at 576 different sites throughout southern California (Table D-1). Seventy-two percent of these analyses were collected along the shoreline, either along the open coast, in bays and harbors, or near the mouths of creeks and storm drains (Table D-1). The remaining 28% were samples taken from offshore areas (up to 100 meters depth) to supplement water quality measurements for deep ocean outfalls in compliance with NPDES permit requirements. Fifteen of the 21 monitoring programs were NPDES sewage discharge permittees whose outfalls were sighted well offshore. In addition to offshore monitoring, NPDES permittees performed 75% of the shoreline bacterial indicator analyses.

The level of shoreline microbiological sampling and analysis effort was not evenly distributed throughout southern California (Table 1). The greatest number of monitoring programs (n = 7) were found in San Diego County. The greatest number of shoreline sites were sampled in Orange County (n = 145). The most microbiological analyses were conducted in Los Angeles County (n = 26,814 per year). Beach and bay sampling and analyses were roughly 10-fold less in Santa Barbara County (2 programs; 21 sites; 3,276 analyses per year) and Ventura County (2 programs; 29 sites; 2,054 analyses per year).

Sampling frequency also differed among counties (Table D-2). Only in Los Angeles and Orange Counties was daily monitoring conducted on any beach or bay; more than 65% of the effort in Los Angeles County was allocated toward daily monitoring. The difference in sampling frequency

between winter and summer was small, except in Ventura County where the effort in summer nearly quadrupled. Santa Barbara and Los Angeles County maintained the same level of effort throughout the year.

TABLE D-1. Agencies which conduct routine microbiological monitoring in southern California. * indicates NPDES permittee.

	No. of Sites	No. of Analyses Per Year	No. of Sites	No. of Analysis per Year	No. of Sites	No. of Analyses per Year
- Santa Barbara County -						
Santa Barbara County Department of Health Services	14	2,184	-	-	14	2,184
Goleta Sanitation District*	7	1,092	13	468	20	1,560
- Ventura County -						
City of Ventura*	16	884	-	-	16	884
City of Oxnard*	13	1,170	13	3,408	26	4,578
- Los Angeles County -						
Los Angeles County Department of Health Services	33	5,148	-	-	33	5,148
City of Los Angeles, Hyperion Wastewater Treatment Plant*	18	14,220	33	9,000	51	23,220
City of Los Angeles, Terminal Isl. Wastewater Treatment Plant*	20	3,414	-	-	20	3,414
Los Angeles County Department of Beaches and Harbors	18	648	-	-	18	648
Los Angeles County Sanitation Districts*	8	2,916	8	3,020	16	5,936
City of Long Beach, Dept. of Health and Human Services	39	468	-	-	39	468
- Orange County -						
Orange County Sanitation District*	17	3,840	4	624	21	4,464
Aliso Water Management Agency*	18	6,864	6	648	24	7,512
South East Regional Reclamation Authority*	17	3,978	13	576	30	4,554
Orange County Environmental Health Division	93	6,968	-	-	93	6,968
- San Diego County -						
San Diego County Department of Environmental Health	45	540	-	-	45	540
City of Oceanside*	10	1,170	12	432	22	1,602
Encina Wastewater Authority*	5	780	10	1,080	15	1,860
San Elijo Wastewater Authority*	7	819	14	504	21	1,323
City of San Diego, Point Loma Wastewater Treatment Plant*	16	1,872	8	4,320	24	6,192
City of San Diego, Mission Bay*	20	3,120	-	-	20	3,120
International Boundary Water Commission*		8832	-	-	8	832
Total	442	62,927	134	24,080	576	87,007

TABLE D-2. Number of shoreline/bay samples analyzed each year in southern California during summer season (April 1 - September 30) and winter season (October 1 - March 31) as a function of monitoring frequency.

County	Summer Season			Winter Season			
	M, W, F M thru F or 7 d/wk	1/wk or 5/mo	Biweekly to Monthly	M, W, F M thru F or 7 d/wk	1/wk or 5/mo	Biweekly to Monthly	Total
Santa Barbara	-	1,638	-	-	1,638	-	3,276
Ventura	-	1,612	-	-	442	-	2,054
Los Angeles	8,763	4,014	630	8,763	4,014	630	26,814
Orange	8,124	3,484	-	5,232	4,810	-	21,650
San Diego	-	4,940	540	-	2,366	1,287	9,133
Total	16,887	15,688	1,170	13,995	13,270	1,917	62,927

The bacterial indicators and their testing methods varied, with the distinction most pronounced between health agencies and NPDES permittees (Table D-3). Public health departments focused on total coliforms measurements, measuring them at almost twice the frequency of fecal coliforms and three times the frequency of enterococcus. In contrast, most NPDES dischargers measured all three indicators at most sites. Additionally, health departments primarily tested for bacteria using the multiple tube fermentation method or Idexx kits (Colilert® and Enterolert®). In contrast, NPDES permittees relied primarily on the membrane filtration method.

TABLE D-3. Number of shoreline/bay analyses per year as a function of indicators studied and type of monitoring agency.

	Public Health Agencies	NPDES Permittees
Total coliforms		
Multiple tube fermentation	7,090	6,141
Membrane filtration	468	16,074
Colilert®	728	-
Fecal coliforms		
Multiple tube fermentation	4,282	1,417
Membrane filtration	-	13,734
Colilert®	728	-
Enterococci		
Multiple tube fermentation	1,932	1,417
Membrane filtration	-	8,188
Enterolert®	728	-
Total	15,956	46,971

Spatial Allocation of Shoreline Monitoring

Microbiological sampling occurred in all of the shoreline habitats we delineated, but the allocation of effort among them was not equal. The majority of effort was allocated towards high use sandy beaches (55%), where human water contact is most likely (Table D-4). Perennial and ephemeral stormwater outlets, which are a frequent source of bacterial contamination, received nearly 20% of the sampling effort while accounting for less than 2% of the shoreline. This category represented the greatest proportional allocation of effort among habitats. Restricted access areas received the least proportional allocation of effort.

Although a large amount of effort was conducted throughout southern California, most of it was allocated towards revisiting a selected set of sites. For example, high use sandy beaches received the greatest amount of sampling effort, yet only 11% of the high use sandy beach shoreline was monitored (Table 5). Perennial freshwater inputs, which are potential sources of chronic indicator bacteria contamination, were the most extensively monitored, with 31% of the storm drain areas sampled. Roughly 7% of the southern California shoreline as a whole was monitored.

Monitoring coverage of the coastline varied among counties (Table D-5). The greatest coverage occurred in Orange County (10% of county total), followed by Los Angeles, San Diego, Ventura, and Santa Barbara Counties. Likewise, the coverage among different beach types was not consistent within or between counties. Up to 50% of beaches adjacent to freshwater inputs were monitored in Santa Barbara, Orange, and San Diego Counties; 20% or less of these beaches were monitored in Los Angeles and Ventura Counties. Roughly one-fifth of the high use sandy beaches in Los Angeles County and Orange County were sampled, the highest of the five counties. Less than one-tenth of the high use sandy beach miles in Ventura and San Diego Counties were monitored. Only a single high use sandy beach was targeted for monitoring in Santa Barbara County.

Monitoring Costs

It was estimated that about $3M is spent annually on marine microbiological monitoring in southern California (Table D-6). About 70% of that expenditure was for shoreline and bay monitoring. Los Angeles County monitoring cost estimates were highest, approximately 10-fold higher than Santa Barbara County. When expressed as cost per mile of recreational shoreline, similar differences among counties were also apparent. When expressed as per capita expenditure, Ventura County, which had no routine health department monitoring and collected the smallest number of samples, had the second highest expenditure, and Los Angles County the least. When expressed as a fraction of tourism dollars, Orange County had the greatest expenditure on monitoring and San Diego County the least.

TABLE D-4. Relative allocation of monitoring effort in southern California by shoreline type.

Shoreline Type	Percent of Shoreline miles	Percent Allocation of Sampling Effort
Sandy		
High Use	25.9	54.5
Low Use	9.3	7.8
Rocky		
High Use	2.5	3.9
Low Use	2.9	1.8
Freshwater Inputs		
Perennial	1.0	14.2
Ephemeral	0.7	4.6
Embayments	27.5	11.0
Restricted Access	30.2	2.2
Total	100.0	100

TABLE D-5. Percent of shoreline miles sampled in southern California by county.

Beach Type	Percent Shoreline Monitoring Coverage by County					
	Santa Barbara	Ventura	Los Angeles	Orange	San Diego	All of Southern California
Sandy						
High Use	2.0	5.1	21.9	17.7	8.9	11.2
Low Use	2.1	31.9	17.2	< 0.1	12.9	9.9
Rocky						
High Use	< 0.1	< 0.1	7.7	18.6	9.5	8.7
Low Use	< 0.1	< 0.1	3.8	6.2	3.6	4.0
Freshwater Inputs						
Perennial	49.7	18.9	15.8	35.4	28.0	31.4
Ephemeral	< 0.1	< 0.1	4.5	20.1	21.0	13.2
Embayments	< 0.1	4.3	15.0	9.8	4.1	8.8
Restricted Access	< 0.1	0.6	0.4	1.1	1.1	0.6
Total	1.7	4.3	9.6	10.2	6.4	7.2

TABLE D-6. Costs per county for microbiological monitoring in southern California. Costs per capita, per mile, and per tourist dollar are for shoreline and bay monitoring only.

County	Estimated Cost (in $1,000)					
	Shoreline/Bay Monitoring	Offshore Monitoring	Total Monitoring	Per capita	Per Mile	Per Million Tourism Dollars
Santa Barbara	111.4	17.2	128.5	$0.27	$1,593	$125
Ventura	76.3	125.5	201.8	$0.28	$1,047	$99
Los Angeles	946.7	535.4	1,482.1	$0.15	$6,721	$78
Orange	794.5	72.0	866.5	$0.32	$6,336	$203
San Diego	313.3	223.2	536.5	$0.19	$1,824	$59
Total	2,242.1	973.3	3,215.4	$0.20	$3,861	$97

DISCUSSION

The amount of marine microbiological monitoring conducted in southern California appears to exceed that in the rest of California or in any other part of the country. Less than $0.5M is spent annually on monitoring in the rest of California, and the rest of the country combined spends less than $2M (NRDC 1998). Our estimates of nearly $3M annually for microbiological monitoring in southern California is a conservative estimate in that it only includes cost of routine monitoring. Most of the agencies we surveyed also sample in response to sewage spills, overflows and beach closures in addition to what the inventory included. The higher expenditures we estimated for southern California reflect the large contributions from NPDES permittee monitoring efforts, which is uncommon in shoreline monitoring programs in other parts of the country. Southern California's beach monitoring programs are still among the largest in the country even without the NPDES effort, but the local coordination between the NPDES and health agencies makes it that much larger.

While the amount spent on microbiological monitoring in southern California is large, the expenditure reflects the high population density and extensive tourism industry in the area. Southern California has the highest coastal population density of any area in the country (Culliton *et al.* 1988). Coastal tourism in California is estimated double that of any other state in the country and lifeguarding statistics indicate that there are more beach visit-days in southern California than in the rest of the country combined (Table D-7).

We found considerable difference in how effort was allocated by different organizations and across different counties. For instance, the Orange County Environmental Health Division collects data from more sites than any other organization, yet collects less than 25% of the number of analyses as Los Angeles City Environmental Monitoring Division. This results because Los Angeles City typically measures three indicators at each site daily, whereas Orange County does not measure enterococcus and measures most sites weekly. No studies have been conducted to assess if the public's interest is best served by allocating effort primarily to more sites, more temporal coverage at these sites, or more indicators at each site. What is clear is that the monitoring organizations throughout southern California have not developed a unified strategy to select the most appropriate effort allocation.

Table D-7. Beach usage statistics throughout the United States (Data courtesy of R. Gould, U.S. Lifesaving Association).

Region	1997 Beach Usage	
	No. Beach Visits (in thousands)	Percent of total
New England	2,643	0.9
Mid-Atlantic	11,020	3.9
South Atlantic	14,949	5.3
Southeast	45,848	16.3
Great Lakes	22,860	8.1
Gulf Coast	2,500	0.9
Northwest	5,831	2.1
Hawaii	20,659	7.4
Northern California	9,073	3.2
Southern California	146,264	51.9
Total	281,648	100.0

One factor that leads to inconsistencies in effort allocation is the different monitoring mandates for health departments and NPDES permittees. In southern California, the NPDES permittees and health departments coordinate their efforts to address management needs, but the EPA, State and Regional Water Quality Control Boards, not the health departments, define the NPDES permittee monitoring requirements. EPA presently endorses the use of enterococcus as a primary bacterial indicator, which may be the reason we found that enterococcus, is typically measured by NPDES permittees. However, the recreational water quality objectives for enterococcus in California are only preliminary, so it is rarely measured by health departments. Similarly, methodological inconsistencies follow from different mandates. The State of California Environmental Laboratory Accreditation Program (ELAP) certifies all NPDES and private laboratories for microbiological analyses of marine recreational waters. ELAP does not, however, certify laboratories using the Colilert® or Enterolert® Idexx kit methods since EPA has not approved them for marine recreational water testing. This accounts for the fact that no NPDES laboratories utilize this method. Public health departments, who do not report to EPA, have traditionally focused on multiple tube fermentation methods, but are increasingly relying upon the premanufactured Idexx kits.

A similar issue that results from the division between NPDES dischargers and health departments is the allocation of nearly $1M in southern California towards monitoring of offshore areas where few people swim and shellfish standards are not an issue. Moreover, many of these samples are collected at depths up to 100 meters, far below typical diving depths. NPDES permittees use this monitoring data to track their wastewater plume and ensure that it remains submerged and far from shore. It is not clear whether the public interest is best served by such a large effort distant from the beaches where people swim. It is also interesting that while NPDES permittees accounted for more than 75% of monitoring effort, all the NPDES monitoring was conducted by sewage dischargers, even though most POTWs have consistently demon-

strated that their outfalls are sufficiently offshore to avoid beach exposure. In southern California, stormwater dischargers also hold NPDES permits yet none of the stormwater permittees presently conduct microbiological monitoring in receiving waters even though 19% of present monitoring effort is allocated towards stormwater outlets and most of the public warnings about beach safety in southern California have been associated with stormwater outlets (NRDC 1998).

We found that more than half of the shoreline effort was focused on freshwater outlets and selected high use beaches. Perhaps it is appropriate that effort be targeted towards those areas most likely to have a problem and those areas where the public is most likely to be exposed. However, these areas represent a small portion of the total shoreline, which presents a challenge in ensuring that the public gets a complete perspective on the quality of their shoreline. Many groups summarize beach monitoring data on the basis of beach closures, rather than on the amount of shoreline that is safe (or unsafe) to swim. Organizations that monitor more extensively, and focus their monitoring towards high risk areas, are more likely to produce beach warnings or closures. Thus, southern California beaches have developed a reputation as more unsafe than others in the country in part due to their greater monitoring activity (NY Times January 5, 1997). One of the reasons that closures and warnings are frequently used as the primary measure of beach quality is that the information is accessible; the raw bacterial concentration data, which are collected by many organizations that have historically maintained their data independently, is less accessible. Some local organizations, such as the Santa Monica Bay Restoration Project and Heal the Bay in Los Angeles County, and San Diego County Environmental Health Department in San Diego County have already taken steps to provide the public with more complete information through the use of report cards and web sites that characterize conditions across several monitoring organizations within a county.

The inconsistencies and unresolved policy issues that we observed in southern California appear to be a microcosm of issues faced nationally. NRDC (1998) found the same kind of differences in temporal, spatial and indicator allocation among states as we found among counties. California also appears to be a microcosm for a solution. California recently passed legislation (AB 411) requiring the State Health Department to develop a consistent beach monitoring program to be implemented throughout the state. The federal EPA also recently initiated its Beach Environmental Assessment, Closure and Health (BEACH) program with the goal of increasing consistency in monitoring and reporting. Legislation similar to AB 411 is also pending at the national level.

Resolving inconsistencies among programs requires identifying a common question(s) as a focal point for partnership among monitoring organizations. While cooperation between NPDES discharge monitoring agencies and health departments is probably higher in southern California than in most parts of the country, the allocation of effort indicates there are still differences in focus between them. Public health agencies focus on elevated shoreline bacterial counts relative to water quality standards, whereas NPDES permittees monitor movement of offshore effluent plumes and possible encroachment into inshore recreational waters. The common element of both program types, the public health related to water contact, should provide a common ground for even greater coordination.

One aspect that seems to be serving as a focal point for increasing cooperation is the effect of storm drains on ocean quality (Schiff 1997). Health departments have focused effort in these locations because they are the area in which closures most frequently occur. Many municipal sewage dischargers focus on these areas because their offshore outfalls occur adjacent to areas of stormwater plumes and they have a

need to demonstrate that shoreline closures result from the storm drain plume, not from their outfall. In addition, sewage lines can overflow during heavy rains and the storm drain systems become the transport system for these spills to enter the ocean. Stormwater agencies, while not presently conducting monitoring, are NPDES permittees who may have such responsibilities in the future. Some sewage and stormwater agencies are beginning to merge administratively in southern California for these reasons, with the City of Los Angeles recently reorganizing their Stormwater Management Division into the Bureau of Sanitation and the San Diego County Environmental Health Department seeking leadership status on the San Diego County stormwater NPDES permit. Regardless of whether stormwater is the unifying issue, partnership between public health and NPDES permitted agencies in data collection and assessments would be an important component of cost-effectively ensuring that coastal water contact safety information is effectively communicated to the public.

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