Winter Shoreline Microbiology

SOUTHERN CALIFORNIA BIGHT 1998 REGIONAL MONITORING PROGRAM
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Southern California Bight 1998 Regional Monitoring Program: II. Winter Shoreline Microbiology

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FOREWORD

This study was coordinated by the Southern California Coastal Water Research Project (SCCWRP) as a winter complement to the Summer Shoreline Microbiology Study performed in August of 1998. The same approach and format was used for both reports to facilitate comparison between the winter and summer shoreline microbiology components. Copies of this and other shoreline microbiology reports are available for download at www.sccwrp.org.

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Definition of Terms:

Adenovirus - Genus (subset) of the human enteric virus family, other genera include reovirus and enterovirus.
Coliphage - Virus that infects bacteria in the coliform group.
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 (urban, rural, agricultural, 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-4.
Perennial freshwater outlet - Natural or constructed freshwater source that typically produces measurable and observable flows year round.
Point zero sample – For the purposes of this study, a sample that was taken at the mouth of a freshwater outlet, at the location of surfzone-freshwater mixing.
Random sample - In this study, a sample that was taken at a random location within 100 yards of the mouth of a freshwater outlet.
Reverse transcriptase polymerase chain reaction (RT-PCR) - Molecular biology primer-based technique for the detection of RNA.
Standard - Level of water quality measurement (characteristic) for ocean waters set by State of California statute and regulations, e.g., Assembly Bill 411 which refers to bacteriological indicator levels. See Table II-4.
Storm drain – Constructed subset of the freshwater outlets that generally do not have a main source from riverine or creek 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-4.
Urban runoff - Runoff from a freshwater outlet or storm drain whose watershed is primarily an urban land use area.
Viral genome - The complete set of genes contained in a virus particle (can be either RNA or DNA). Used to infer the presence of human fecal contamination, but cannot be used to infer health risk as genetic material is not always evidence of an intact, infectious virus particle.
Water quality – For the purposes of this report, refers to water quality of a microbiological nature.

EXECUTIVE SUMMARY

Beaches along the Southern California Bight (SCB) are the most frequently and extensively monitored areas in the country for bacteriological water quality. Because most of the effort is focused in known problem areas, traditional sampling methods do not provide an assessment of overall shoreline water quality. To address this limitation, all of the organizations that routinely monitor bacteriological water quality along the southern California shoreline coordinated their efforts to conduct an integrated survey of the coastline in the winter of 1999. This survey complements a similar regional survey that was conducted in the summer of 1998. The primary goals for the survey were to:

- Determine the percentage of shoreline mile-days in the SCB that exceeded bacterial indicator thresholds during February and early March 1999.

- Compare the responses among the three bacterial indicators used to assess beach water quality in California.

- Determine how well these bacterial indicator measurements correlated with detection of human pathogenic viral genetic material and coliphage.

Samples were collected weekly from 240 sites between Point Conception, California, and Punta Banda, Mexico, beginning February 2, 1999, and continuing for five weeks. Sampling sites were selected using a stratified random design, with separate strata for sandy beaches, rocky shoreline, and areas adjacent to freshwater outlets. Total coliforms, fecal coliforms (or *E. coli*), and enterococci were measured at each site using standardized protocols. Molecular analyses to measure the presence of human pathogenic viral genetic material and coliphage were performed on samples from 18 randomly selected perennial freshwater outlet locations.

Over the course of our study, 90% of the shoreline mile-days along the southern California shoreline met all three California bacterial indicator standards. Ninety-eight percent of the samples that exceeded a California standard did so for a single bacterial indicator, while other bacterial indicators measured at the site on the same day were within State standards. Less than one percent 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. These findings are consistent with those from the summer survey. The similarity in response between the findings of the winter and summer surveys may largely reflect the dry winter conditions in 1999, as less than one inch of rain fell during the winter study period.

Areas adjacent to freshwater outlets, which constitute only a small fraction of the southern California coastline, exhibited the worst microbiological water quality. About half of the shoreline mile-days in these areas failed State of California standards. Most

of these failures were for multiple indicators and occurred repetitively throughout the five-week study period. Enterovirus genetic material was detected in 50% and adenovirus genetic material was detected in 18% of the samples taken from freshwater outlet locations. Coliphage were detected, sometimes at high concentrations, at all of the sites tested, but the level of coliphage was not significantly correlated to levels of any of the bacterial indicators. Human enteric viral genetic material infers the presence of human fecal contamination, but does not necessarily infer health risk, as genetic material is not always evidence of an intact, infectious virus particle.

The State of California maintains water quality standards for three bacterial indicators and the probability of exceeding a standard differed significantly among these indicators. The enterococci standard was exceeded three times as often as any other standard. In areas away from freshwater outlets, 78% of the standards failures were for enterococci alone. No sample away from a freshwater outlet failed all standards. The correlation with viral concentrations was poor for all three bacterial indicators.

The cooperative Winter Shoreline Microbiology Study, combined with the summer 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 83% of the beach samples in Mexico met the State of California's bacteriological water quality standards, the standards were exceeded two to five times more often along Mexican than United States beaches, depending on the indicator examined. The magnitude of the indicator exceedances along most of the beach areas was not substantially different between Mexico and the United States, except for beaches adjacent to freshwater outlets, where the average bacterial concentration in Mexico was about one hundred times higher than in the United States. This information provides valuable baseline information that can be used to assess progress in efforts by Mexican authorities to improve their shoreline bacteriological water quality.

I. INTRODUCTION

The Southern California Bight (SCB) is noted for its shoreline and beaches, which attract an estimated 175 million visitors annually (USLA 1998). Areas of the southern California shoreline are impacted by human activities, particularly stretches lying adjacent to densely populated urban centers. A major source of stress to the environment resulting from human activity in this area is non-point source runoff. Runoff enters the recreational waters from many freshwater outlets ranging in size from rivers having year-round flows to small ephemeral drains that flow only during wet weather.

Recreational waters affected by urban runoff have demonstrated elevated levels of indicator bacteria and human enteric virus (Gold *et al.* 1990, 1991, 1992) that can result in increased risks of illness to swimmers (Haile *et al.* 1999). Due to the potential threat to the human population inherent in urban runoff, extensive bacteriological shoreline monitoring has been performed by a variety of southern California agencies. Although the scope of this bacteriological monitoring is impressive (Schiff *et al.* in press), the data collected cannot be integrated easily to provide a regional assessment of recreational water quality because most monitoring is spatially focused on a small set of high-use beaches or other areas of concern.

To address this concern and provide the public with an integrated assessment of beach quality, all of the agencies that routinely monitor bacteriological water quality along the southern California shoreline, as well as several university and volunteer organizations, coordinated their efforts for the purpose of conducting a regional survey to assess the overall condition of the southern California and northern Baja, Mexico, shoreline in the summer of 1998 (Noble *et al.* in press). The study found that 94% of the shoreline in the study area met State of California water quality standards. Although the study found that the majority of the shoreline had good water quality, it was conducted in the summer when the influence from these outlets would be least. Areas near freshwater outlets had the poorest water quality, with 60% of the shoreline immediately adjacent to freshwater outlets failing at least one of the State standards. In southern California, the majority of rainfall occurs from November through March, with average precipitation of 9.7 inches in Los Angeles in these months out of a total annual average of 11.3 (National Weather Service: www.nwsla.noaa.gov). Increased rainfall during the winter has the potential to result in increased contamination of shoreline waters adjacent the freshwater outlets.

To address the concern that beach water quality is substantially different during the winter wet season, the same organizations that conducted the summer study joined forces to conduct a similar regional survey in the winter of 1999. The primary goals of the winter survey were similar to those of the summer survey. The goals and their rationale included:

- To determine the percentage of shoreline mile-days in the SCB that met bacterial indicator thresholds during the winter;

The number of shoreline mile-days provides a space-time integrated assessment of the likelihood that a beachgoer electing to swim on a southern California beach in the winter will do so in waters that meet all of the State's water quality standards. While the focus of the effort of this study was on the shoreline in the United States, the project also included a coordinated effort by Mexican scientists to assess water quality between Tijuana and Ensenada. The international participation provides the first opportunity for cross-border comparison of bacteriological water quality using comparable methods.

- To assess the comparability of the responses of the three bacterial indicators measured during winter conditions;

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

- To determine how well bacterial indicators correlate with detection of human viral genetic material and coliphage;

  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 1983a, Kay *et al.* 1994, USEPA 1986). One part of this survey assesses the presence of waterborne human pathogenic viral genetic material and coliphage at the point zero site of freshwater outlets (where the outlet meets the ocean) along the coast of the SCB, and to determine whether the presence of the genetic material of these viruses is correlated with levels of indicator bacteria. Detection of human pathogenic 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 analyses 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 Winter Shoreline Microbiology Study involved sampling at 240 sites along the SCB coastline between February 2 and March 5, 1999. Each site was sampled once per week during the 5-wk study period. A 5-week study period was selected to meet the minimum of 5 samples required for calculation of the 30-day geometric mean threshold detailed in the California Ocean Plan and AB411 regulations. The study was conducted during winter to coincide with the average period of rainfall, although actual rainfall during this period was minimal.

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

Sampling sites were selected using a stratified random approach, with the strata corresponding to the three 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. The high-use and low-use divisions that were used in the summer survey were combined to form one sandy beach stratum and one rocky shoreline stratum. A total of 81 freshwater outlets were identified and differentiated as perennial or ephemeral based upon whether water flowed year-round or seasonally, respectively. The freshwater outlets selected are those outlets that are typically responsible for 99% of the total freshwater/stormwater 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
Sandy beaches	23	19	30
Rocky shoreline	24		
Freshwater outlets			
Ephemeral	36		
Ephemeral point zero	29		
Perennial	39		
Perennial point zero	30	10	
	181	29	30

The number of samples allocated to each stratum was that necessary to achieve a 95% confidence interval of approximately +/- 5% around estimates of areal extent. The

site selection process was implemented separately by county, with the number of sites within a stratum and within a county in proportion to the percentage of southern California shoreline of that stratum type within the county. A county-specific selection process was implemented to accommodate the availability of additional effort in some counties, beyond that necessary to achieve the program's precision goals.

Although the basic sample allocation scheme was stratified random, a systematic component was added to minimize clustering of sample sites along the shore. This approach was accomplished using an extension of the National Stream Survey sampling design (Messer *et al.* 1986, Overton 1987), whereby each stratum was divided into a series of linear sections of coastline, with each section identified by a count variable. The sections were joined 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 and ephemeral water outlet strata were selected using two methods. First, sites were selected at a random distance within 100 yards from the mouth of the outlet, using the systematic random approach described above. Second, a site was placed at the mouth of the outlet (referred to as the point zero site). Random sites were placed around 39 of the 40 perennial water outlets in southern California. Point zero sites were placed at the mouths of 30 of the 40 systems, which were selected by availability of effort. Eighteen of these 30 point zero sites were randomly selected to also receive virus samples. At the ephemeral outlets, 36 random sites were sampled of the possible 47 systems. Twenty-nine of the 47 ephemeral outlets also received point zero samples.

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

Volunteer Monitoring

Volunteer organizations enhanced the sampling effort with a total of 30 sampling sites, 11 of which ranged in location from the Talbert Marsh area of Huntington Beach northward to the Long Beach Harbor region of San Pedro Bay, and 19 sampling sites in southern Santa Monica Bay (between Ballona Creek and the Palos Verdes peninsula). Volunteer sites were limited to the high-use sandy beach stratum. Volunteer sites were selected as a supplement, rather than as an integrated part of the program. This supplemental overlay of sites was selected using the same statistical design approach described above in numbers that would not have affected the integrity of the base sample

design had the volunteer effort been unsuccessful. Since the volunteers were successful in collecting all of their assigned samples and meeting all of the quality assurance requirements, their results were integrated directly into the base program.

B. Field and Laboratory Methods

Bacteria

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, fecal coliforms or *E. coli*, and enterococci were measured for all sites.

Three methods were used to detect and enumerate bacteria: membrane filtration (MF); multiple tube fermentation (MTF); and defined substrate technology tests. 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 Laboratories, Inc. The Idexx kits use either multiple tubes or multiple wells, with an MPN approach, to detect the presence or absence of total coliforms 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 metabolism of a substrate specifically by *E. coli*. This assay is read within 18-22 hours. In this study, *E. coli*, which typically constitute the majority of fecal coliforms, were treated as fecal coliforms for data analysis. However, it should be mentioned that the percent of *E. coli* that make up the fecal coliform group varies depending upon the sample matrix and location. With Enterolert®, the detection of enterococci is based upon the release of a fluorogen by metabolism of a nutrient-indicator substrate by members of the enterococci group.

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

A subset of laboratories also performed side-by-side analyses. Total coliforms and *E. coli* were analyzed using Colilert® and enterococci were analyzed using Enterolert® and/or EPA 1600. The methods and number of samples analyzed for the method comparison are outlined in Table II-3. The results of this method comparison are presented in Appendix B.

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

	Total Coliforms	Fecal Coliforms	Enterococci
Santa Barbara County			
Santa Barbara Health Care Services	22[c]	22[c]	22[d]
City of Santa Barbara	4[b]	4[b]	4[d]
Goleta Sanitation District	4[b]	4[b]	4[b]
Ventura County			
Ventura WWTP	5[b]	5[b]	5[b]
City of Oxnard	5[b]	5[b]	5[b]
Aquatic Bioassay Labs	3[b]	3[b]	3[b]
Ventura Co. EHD	10[c]	10[c]	10[d]
Los Angeles County			
City of Los Angeles	17[a]	17[a]	17[a]
Los Angeles Co. Sanitation Districts	6[a]	6[a]	6[a]
Los Angeles Co. DHS	13[c]	13[c]	13[d]
City of Long Beach	2[a]	2[a]	2[b]
Southern California Marine Institute	30[c]	30[c]	30[d]
Orange County			
Orange Co. Sanitation District	15[c]	15[c]	15[d]
Orange Co. Environmental Health Division	24[b]	24[b]	24[d]
AWMA/SERRA	14[a]	14[a]	14[a]
San Diego County			
Encina Wastewater Authority	7[a]	7[a]	7[a]
City of Oceanside	1[b]	1[b]	1[a]
City of San Diego	31[a]	31[a]	31[a]
San Diego Co. Department of Env. Health	6[b]	6[b]	6[a]
San Elijo Joint Powers Authority	2[b]	2[b]	2[b]
Mexico			
Instituto de Investigaciones Oceanologicas	29[b]	29[b]	29[d]

[a]MF
[b]MTF
[c]Colilert®
[d]Enterolert®
[f]Analyses performed by Orange County Sanitation District.

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

	Total Coliforms	Fecal Coliforms	Enterococci
Santa Barbara County			
City of Santa Barbara	4[b,c]	4[b,c]	
Ventura County			
Ventura WWTP	5[b,c]	5[b,c]	
Ventura Co. EHD	4[b,c]	4[b,c]	4[b,d]
Los Angeles County			
City of Los Angeles	14[a,c]	14[a,c]	14[a,d]
City of Long Beach	2[a,c]	2[a,c]	2[b,d]
Orange County			
Orange Co. Sanitation District	1[b,c]	1[b,c]	1[d,e]
San Diego County			
City of Oceanside	1[b,c]	1[b,c]	1[a,d]
City of San Diego	31[a,c]	31[a,c]	31[a,d,e]
San Diego Co. Department of Env. Health	6[b,c]	6[b,c]	6[a,d,e]
San Elijo Joint Powers Authority	2[b,c]		2[b,d]

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

TABLE II-4. Indicator thresholds for the Winter Shoreline Microbiology Study.

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

GM = geometric mean

[a]From California Ocean Plan.
[b]Regulations developed in response to California Assembly Bill 411.
[c]Mexican Criterios Ecológicos de Calidad del Agua (CE-CCA-001/89).

Human Virus and Coliphage Methods

The presence of viral genetic material from enteroviruses (RNA virus) and adenoviruses (DNA virus) was measured from samples taken at the mouths (point zero sites) of 18 randomly selected perennial freshwater outlets using the reverse transcriptase polymerase chain reaction (RT-PCR) technique for the detection of enteroviruses, and the PCR technique for the detection of adenoviruses. Both of these methods are capable of detecting small quantities of viral genetic material in seawater and are a potentially useful tool for discriminating between the presence of human and animal fecal contamination. However, these methods cannot be used to infer the infectivity of the specific types of viruses tested, as viral genetic material is not always associated with a "live" or infectious virus (Sobsey 1998). We also measured coliphage (virus associated with coliform bacteria) levels at 12 of the 18 sites.

The following procedure was used for the concentration of the seawater sample and the detection of enterovirus genomes at the laboratory of Dr. Jed Fuhrman at the University of Southern California (USC). Twenty liters of seawater were collected in a plastic carboy from 12 sites 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 4 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 µL.

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, Noble and Fuhrman, in press). Briefly, a 2 µL 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 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. Amplified DNA was visualized by staining a 2% agarose gel with ethidium bromide and illuminating with UV light. Lane markers of 100 base pair increments were used for size comparison.

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.

Procedures used for detection of adenovirus and coliphage at the University of California at Irvine (UCI) laboratory were slightly different from those outlined above. Twenty liter seawater samples were collected from 12 sites along the southern California coast. Replicate subsamples of 0.1 mL and 1.0 mL were analyzed for the presence of coliphage using the top agar overlay method. The remaining seawater sample was concentrated by a Membrex Vortex Flow Filtration (VFF) system, using a 100 kD filter, to a final volume of 40 to 60 mL as previously described (Jiang *et al.* 1992). The concentration efficiency of this system for MS2 and T2 bacteriophage ranged from 60 to 80%, which was comparably higher than most other viral concentration techniques (Paul *et al.* 1991). This technique does not require prefiltration to remove large particles before concentration, thus retaining both particle-associated viruses as well as free viral particles. The VFF concentrates were sub-divided into two portions. One portion was

used directly for viral nucleic acid extraction as described below; and the other portion was extracted with an equal volume of chloroform to remove lipid containing particles and pigments from phytoplankton and further concentrated by ultracentrifugation. The pellet was suspended in PBS and used for nucleic acid extraction as described below.

Viral nucleic acid extraction and purification were performed using the method originally developed by Boom *et al.* (1990) with minor modifications. This method uses guanidinium thiocyanate (GuSCN) with Triton X-100 to lyse viral particles and silica beads to adsorb nucleic acid, allowing effective purification of DNA and RNA at the same time. In brief, 50 µL of viral concentrate was lysed by 900 µL of GuSCN lysis buffer at room temperature for 10 min. Then 40 µL of silica particles were added and nucleic acids were adsorbed at room temperature for 10 min with gentle shaking. Silica beads were pelleted, washed, and dried. The nucleic acid was eluted from the beads using a 50 µL TE buffer at a temperature of 56° C. The purified nucleic acid was used for detection of human viruses by nested-PCR following the method outlined by Pina *et al.* (1998). The nested PCR produced a 143 base pair product. PCR products were analyzed and visualized on 2% agarose gels. Human adenovirus 40 was used as a positive control (donated by Dr. Charles Gerba).

C. Quality Assurance/ Quality Control

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

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

Positive and negative growth performance and sterility tests were performed on newly prepared batches of media. Broth cultures and plates were read within specified

times. Proper functioning of coliform water baths was demonstrated while analyses were in progress using control cultures of *E. coli* and *Enterobacter aerogenes*.

A performance-based approach was employed to ensure data comparability among laboratories; an intercalibration test using a common sample was performed before the winter survey. Laboratories that had demonstrated proficiency during the summer survey were accepted for the winter study. Laboratories performing an analytical method that they had not used during the summer program were required to analyze one common sample using the new method. All laboratories involved obtained results within the +/- 0.5 median log count comparability goal.

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

Intercalibration exercises were conducted twice during the winter survey. These exercises were performed to assess the variability introduced by the inclusion of multiple laboratories and measurement methods. Standardized samples were prepared using filtered seawater and sewage. Results from this performance exercise are presented in Chapter III Quality Assurance Evaluation.

D. Data Analysis

The assessment of shoreline condition focused on estimating the percent of shoreline mile-days that exceeded a threshold of concern. Data from indicator comparisons (laboratories where multiple methods were performed simultaneously) and Mexican waters were not used for the overall assessment of shoreline condition. Two sets of thresholds were used, one based upon daily measurements and the other based upon monthly averages (Table II-4). Both sets of thresholds were derived from a combination of the State of California beach closure thresholds, one set established in response to the AB411 legislation and primarily applicable to county health departments and the second set from the California Ocean Plan, which proscribes state standards for NPDES-permitted ocean dischargers.

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):

Where:

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

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

Standard error of the response was calculated as:

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

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 range of the confidence interval (Stevens and Kincaid 1997).

The comparison of indicator responses was accomplished primarily through correlation analysis. Indicator comparisons were performed with the entire data set (including data from Mexican waters). Combination tables were also developed to categorically assess the frequency with which individual sites were classified the same by different indicators. Venn diagrams were developed to assess the degree of overlap in threshold exceedances among indicators.

The relationship between bacterial indicators and viral concentrations was assessed by examining the correlation between the presence/absence of human enteric viruses versus the log-transformed bacterial indicator results (logistic regression).

III. QUALITY ASSURANCE EVALUATION

Participants successfully sampled 99% of the sites targeted for study during the survey period. Two stations were not surveyed in one week due to high tide.

Participants successfully analyzed 3,736 of 3,744 (>99%) sample sites targeted for analysis, exceeding the data quality objective of 95%. Three of these analyses were unsuccessful due to improper sample dilution and the remaining five were missed due to 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-summer-survey quality assurance exercises (Noble et al, 1999).

The reference sample analyses showed that the cross-laboratory variability established in the pre-survey intercalibration exercises was generally achieved during the survey, so that all data were included in the final database and the subsequent analyses. Although most laboratories were within the specified quality criteria (within $0.5 * \log_{10}$ of the overall median) for the first sample, there were nine labs that were not. Two laboratories reported having one-time lab errors. Four laboratories had low values only for fecal coliforms using MF. All MF results were significantly lower than both Colilert and MTF (t-test $p<0.01$), and the four labs' results were within $0.5 * \log_{10}$ of the median for MF. Thus, these four laboratories' odd values were considered to be method based deviations and not laboratory errors. The other three laboratories obtained values that were below the criterion level, but were within $1 * \log_{10}$ of the median. Although the results did not meet the study's strict quality criteria, they were not considered extreme discrepancies. All laboratories having problems with the first sample had performed well in the five intercalibration exercises that preceded the summer study and the subsequent quality control sample, so their results were included in the data set. Only two laboratories had low values from the second sample and these were just barely below the cut-off point of $0.5 \log_{10}$ below the overall median. All reported results were included in the database.

During the course of data checking, it was discovered that 1% of the reported samples had fecal coliform levels that were higher than the total coliform levels. Since fecal coliforms represent a subset of the total coliform group, their numbers should not exceed the total coliform numbers. On-site audits conducted by the Project QA Officer confirmed that these anomalies resulted from the lack of analytical precision when different methods were used for measuring total coliforms and fecal coliforms and did not reflect errors in analytical methodology. The median difference between fecal coliforms and total coliforms for these cases was 4.5. Only one of the discrepancies was from a sample that exceeded bacterial indicator standards for fecal coliforms and none exceeded standards for total coliforms.

We met our data quality objective to quantify high counts. Correlation and magnitude questions are difficult to address with values that are represented as greater than some value instead of the actual (or most probable) count. Less than 1% of sample results were reported as greater than some value. All of the eight results from U.S. laboratories which were qualified were > 24,192 (an MPN chart number). The 16 results from the Mexican laboratories ranged from >200 to >16 million.

IV. ASSESSMENT OF THE SOUTHERN CALIFORNIA BIGHT

A. Results

Approximately 90% of the shoreline mile-days in southern California during the five-week study period met daily State bacteriological water quality standards (Figure IV-1, Table IV-1). The frequency of good region-wide bacteriological water quality was even higher (96%) when monthly thresholds were used (Table IV-2, Figure IV-1).

Most of the exceedances we observed were for a single State standard, while other State standards were met at the site; less than one-tenth of the area that exceeded a daily threshold for one bacterial indicator exceeded thresholds for multiple indicators measured at the site (Figure IV-2, Table IV-1). Similarly, only one-fifth of the area that exceeded a monthly threshold for one bacterial indicator exceeded multiple monthly thresholds. Only 0.7% of the shoreline, most of which were freshwater outlet sites, failed all indicators for any particular sample (Table IV-2).

The probability of exceeding a daily bacterial indicator threshold in waters along the United States portion of the SCB shoreline differed among indicators (Figure IV-3, Table IV-3). Based upon daily thresholds, enterococci was the indicator for which thresholds were most frequently exceeded, followed in descending order by fecal coliforms, total:fecal ratios, and total coliforms. The shoreline mile-days for which enterococci exceeded thresholds were more than three times those for fecal coliforms, and five times those for total coliforms. Based upon monthly thresholds, enterococci remained the indicator for which thresholds were most often exceeded, followed by total coliforms and fecal coliforms (Table IV-4)

Few sites exceeded bacterial indicator thresholds for more than one of the five weeks of sampling (Figure IV-4). Less than one percent 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 seven of the sites sampled in this study exceeded bacterial indicator thresholds during every week of the study; four were in Mexico and three were in the United States. Six of the seven 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 and monthly threshold exceedances occurred along rocky shoreline; and the highest frequency of exceedances (of both daily and monthly thresholds) occurred at perennial point zero freshwater outlet sites (Figure IV-5, Table IV-1). Nearly 33% of the shoreline mile-days at perennial point zero freshwater outlet sites failed daily bacterial indicator thresholds for at least one indicator during this study. Also, approximately 24% of the shoreline mile-days at point zero freshwater outlet sites failed daily bacterial indicator thresholds for at least one indicator

during this study, while approximately 5% exceeded monthly thresholds. About a quarter of the point zero samples that exceeded a threshold for a single indicator also exceeded the threshold for multiple indicators. Areas within 100 yards of perennial and ephemeral freshwater outlets failed thresholds approximately 14% and 7% of the time, respectively (Figure IV-5).

Even though 83% of the shoreline in Mexico met bacterial indicator standards, beaches and perennial freshwater outlets in Mexico were 2-5 times more likely to exceed bacterial indicator thresholds than those in the United States, depending on the indicator (Table IV-5). The probability of exceeding indicator thresholds at point zero freshwater outlets in Mexico was roughly three times that in the United States. The most noticeable difference was for total:fecal ratios on sandy beaches, where Mexican beaches exceeded the thresholds seven times more often than beaches in the United States. Exceedances of total:fecal ratios at freshwater outlets in Mexico occurred three times as frequently as in the United States.

The magnitude by which thresholds were exceeded was generally higher near freshwater outlets than for the remaining shoreline (Table IV-6). In the U.S., enterococci and fecal concentrations were about four times higher near outlets, although the pattern was reversed for total coliforms. In Mexican waters, the average concentration near freshwater outlets was more than 100 times higher than on the remaining shoreline for each of the indicators.

The average indicator concentrations were considerably higher in Mexican waters than in the U.S. (Table IV-6), although these differences were more pronounced near freshwater outlets than on the open shoreline. On the open shoreline, only for fecal coliforms did the average differ by a factor of more than 3. For waters near the outlets, however, the average concentration in Mexican waters was more than 50-fold higher for every indicator. An even more striking difference between the two countries was in the average total:fecal ratio. Along the U.S. shoreline, the ratio was 846:1, meaning that fecal coliforms on average were less 1% of total coliforms. Along the Mexican shoreline, the ratio was approximately 5:1.

Figure IV-1: Percent of shoreline-miles that met all indicator thresholds

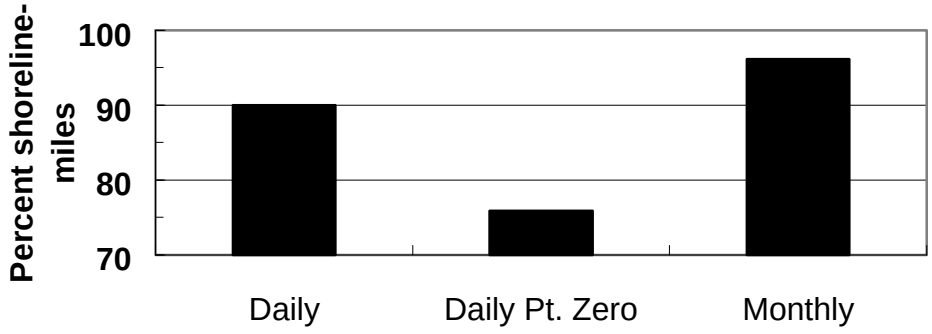

Figure IV-2: Percent shoreline-miles that failed multiple indicator thresholds

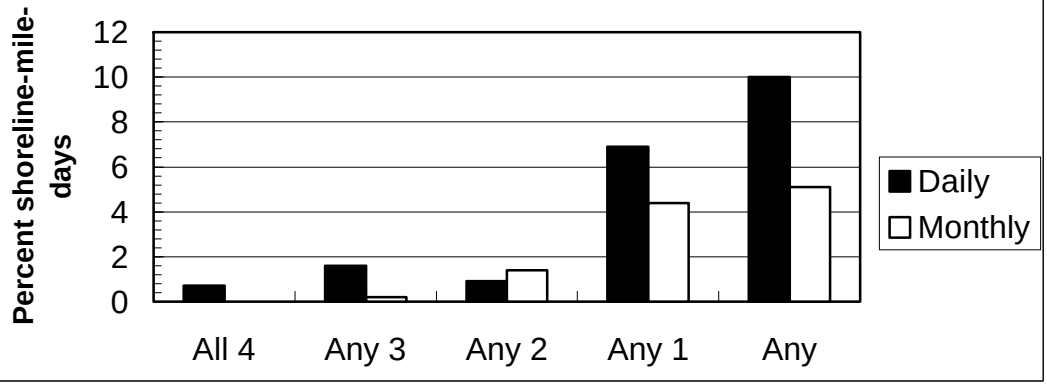

Figure IV-3: Percent shoreline-miles that met indicator thresholds

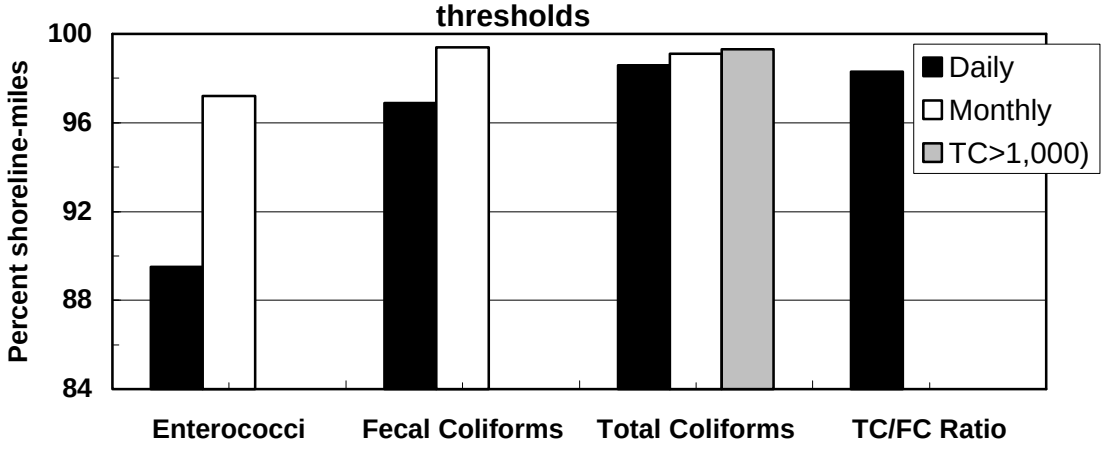

Figure IV-4: Multiple exceedances

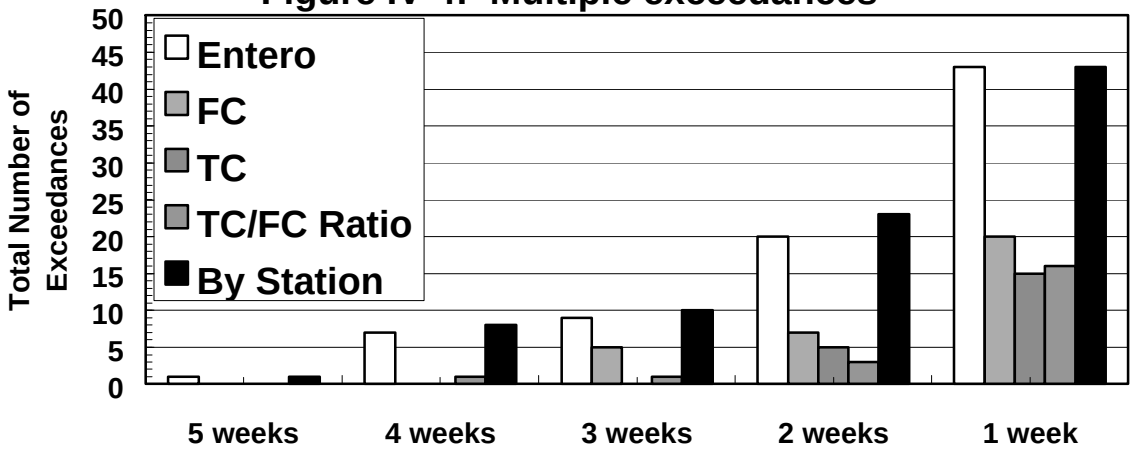

Figure IV-5: Percent shoreline-miles that Met Indicator Thresholds by Shoreline Type

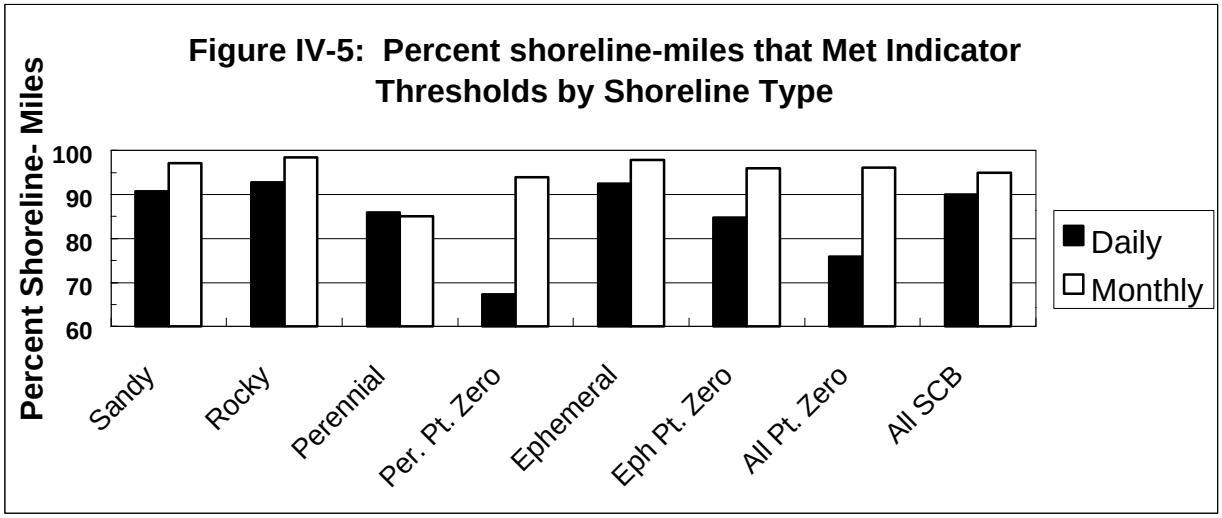

TABLE IV-1. Percent of shoreline mile-days exceeding daily thresholds for all of the indicators, three of the indicators, two of the indicators, any one indicator, and any indicator. Estimates are based upon the subset of sites at which all indicators were measured.

Strata	All 4	Any 3	Any 2	Any 1	Any
Sandy	0.7	1.7	0.2	6.7	9.3
Rocky	0.0	0.0	1.7	5.4	7.2
Perennial	0.5	3.2	2.1	8.3	14.1
Perennial Point Zero	1.3	6.7	10.7	14.0	32.7
Ephemeral	0.5	0.0	1.5	5.6	7.6
Ephemeral Point Zero	2.8	0.7	1.4	10.3	15.2
All Point Zero	2.0	3.7	6.1	12.2	24.1
All SCB	0.7	1.6	0.9	6.9	10.0

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

Strata	All 3	Any 2	Any 1	Any
Sandy	0.0	0.5	2.4	2.9
Rocky	0.0	0.0	1.6	1.6
Perennial	1.1	0.5	13.3	14.9
Perennial Point Zero	2.7	2.0	6.0	6.0
Ephemeral	0.0	0.0	2.2	2.2
Ephemeral Point Zero	0.7	0.7	2.8	4.1
All Point Zero	1.7	1.4	4.4	5.1
All SCB	0.2	0.5	3.4	3.9

TABLE IV-3. Percent of shoreline mile-days exceeding daily bacterial indicator thresholds.

Strata	Enterococci	Fecal Coliforms	Total Coliforms	TC:FC <10	TC:FC <5
Sandy	9.9	3.0	1.4	1.7	1.7
Rocky	7.6	0.0	0.0	0.5	0.5
Perennial	14.9	6.2	2.5	2.1	1.1
Perennial Point Zero	30.7	14.7	7.3	8.0	4.7
Ephemeral	8.2	1.0	0.0	0.0	0.0
Ephemeral Point Zero	12.4	4.1	3.4	6.2	4.1
All Point Zero	21.7	9.5	5.4	10.8	
All SCB	10.5	3.1	1.4	1.7	

TABLE IV-4. Percent of shoreline mile-days exceeding monthly bacterial indicator thresholds.

Strata	Enterococci	Fecal Coliforms	Total Coliforms	Total Coliforms 1999*
Sandy	2.2	0.5	4.6	0.0
Rocky	1.6	0.0	0.5	0.0
Perennial	6.8	2.0	5.7	1.1
Perennial Point Zero	10.7	2.7	10.7	4.7
Ephemeral	2.2	0.0	2.7	0.0
Ephemeral Point Zero	4.1	0.7	6.2	1.4
All Point Zero	7.5	1.7	8.5	3.1
All SCB	2.8	0.6	0.9	0.7

*Using the new 1999 daily threshold of 1,000 cfu or MPN/100 mL for total coliforms.

TABLE IV-5. Percent shoreline mile-days exceeding indicator thresholds in Mexico and the United States.

Strata	Enterococci	Total Coliforms	Fecal Coliforms	TC:FC <10
Sandy Beaches:				
Mexico	17.0	3.2	15.8	13.7
U.S.	10.2	1.6	2.8	2.0
Point Zero:				
Mexico	50.0	16.0	32.0	34.0
U.S.	21.7	5.4	9.5	10.8

TABLE IV-6. Comparison of the mean concentration by indicator in United States shoreline versus Mexico.

Strata	Enterococci	Fecal Coliforms	Total Coliforms	TC:FC <10
U.S. Shoreline	49	68	3,502	846
U.S. Outlets	210	237	1,244	55
Mexican Shoreline	96	1,056	1,360	5
Mexican Outlets	10,275	117,617	369,045	4

B. Discussion

The vast majority (90%) of the southern California shoreline had good bacteriological water quality during February and early March 1999, similar to the results of the summer survey; but rainfall was atypically light, making it difficult to extrapolate our results to all winter conditions. Only about one inch of rain fell during our study period, less than half of the average rainfall for winter season, and little of that fell immediately before our sampling events. As a result, only about two-thirds of the perennial freshwater outlets and one-fourth of the ephemeral freshwater outlets were flowing at the time of sampling. Southern Californians are routinely warned to avoid contact with ocean waters for three days following a rainstorm because of concerns associated with more extensive flow from freshwater outlets. Our study confirmed concerns about the quality of freshwater runoff, but did not allow us to assess the spatial or temporal extent of water quality effects from large storm-related runoff events.

Our finding that the poorest water quality in the SCB occurred near freshwater outlets is consistent with the summer survey and with several previous studies (Gold *et*

al. 1992, Haile *et al*. 1999, Schiff 1997). Storm drains in southern California are independent from sewer systems (with the exception of a few recent dry weather urban runoff diversions) and their flows receive no treatment or disinfection prior to ocean discharge. 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, unauthorized sewage connections, sanitary sewer system leaks, leachate from septic systems, runoff from homeless populations, and/or illegal dumping of waste. 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).

The cooperative Winter Shoreline Microbiology Study, combined with the summer survey, is the first to use consistent sampling approaches to compare the relative quality of United States and Mexican beaches. While Mexican beaches had a higher frequency of threshold exceedances than on U.S. beaches, 83% of Mexican beaches met U.S. standards and the average bacterial concentration was not appreciably different for total coliforms or for enterococci. The more pronounced difference between the two countries was in the condition of water near freshwater outlets. Areas near Mexican outlets were twice as likely to exceed standards and average concentrations were as much as 500-folod higher, depending on indicator. This is consistent with previous studies, which found high total and fecal coliform counts near Mexican outlets (Orozco *et al*. 1994, Segovia *et al.* 1995). The Mexican government has already taken actions to reduce bacteriological pollution of coastal waters by improving existing infrastructure, as well as constructing new facilities to collect, treat, and dispose of sewage from the rapidly growing population in the region. One such facility, the El Naranjo wastewater treatment plant, began operation after this study was completed. One outlet site which had high bacteriological counts in this study, but which subsequently had flow diverted to the new treatment facility, met bacteriological standards in several samplings conducted in late 1999. Hopefully, data from this study will continue to provide a valuable baseline for assessing the effectiveness of future actions.

One of the most striking results of this study was the difference in response among indicators. Beach closure and posting 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. Regulations drafted in 1999 in response to AB411 require measuring three indicators (enterococci, total coliforms, and fecal coliforms) during the summer, although many of the county health departments have extended their sampling of all three indicators to the winter as well. Of these, the enterococci standard was exceeded three times as often as any other standard, and in

areas away from freshwater outlets, 78% of the standards failures were for enterococci alone. Measuring all three indicators was found to increase by 20-fold the number of beach water quality warnings compared to those issued under the historic total coliforms standard.

V. INDICATOR COMPARISONS

A. Results

Correlation Analysis

Total and fecal coliform concentrations were strongly correlated (r = 0.84), with weaker correlations between these indicators and enterococci (r = 0.64 and r=0.70 respectively, Figures V-1 through V-3). The correlation between indicators was largely independent of which laboratory method was used to analyze the samples (Table V-1). Samples analyzed with MTF had marginally better relationships between indicators compared to MF for total to fecal comparisons. This result is likely an artifact of the method. In MTF tests, the fecal coliform positive tubes are nearly always a subset of the total coliform positive tubes; thus, the fecal coliform result rarely exceeds the total coliform result. This is not the case for the other two methods. Correlation coefficients using Idexx kits were nearly identical to those of MF, except for the total coliform to fecal coliform comparison where the Idexx kit had a slightly lower correlation (Table V-1). This may result because the Idexx kits measure *E. coli*, rather than the more inclusive fecal coliform group.

The correlations among indicators were uniformly higher in Mexican waters than in U.S. waters (Table V-2). Within the U.S., the correlations were highest at sites near freshwater outlets, although the higher correlations near outlets were primarily limited to comparisons with enterococci; correlations between total and fecal coliforms were largely independent of strata. There was little difference in the correlations between rocky and sandy shoreline.

Threshold Analysis

Eighty-six percent of the U.S. shoreline samples away from freshwater outlets that failed a daily standard for one indicator bacteria failed for only a single indicator, while no sample failed for all three indicators (Figure V-4 A). Most of the single indicator failures were for enterococci. Only about one-third of the samples that failed for one of the other indicators also failed for enterococci. None of the samples that failed the total coliforms standard also failed the enterococci standard. The concordance of standards exceedances among indicators was slightly higher near freshwater outlets, with 6% of the samples failing for all three indicators (Figure V-4 B). Still, 77% of the failures near freshwater outlets were for a single indicator.

A higher correspondence was found in standards exceedances among indicators in Mexican waters (Figure V-4 C, D). Away from freshwater outlets, 16% of samples exceeding a standard failed for all three indicators. Near outlets, 35% of the samples failed for all three indicators (Figure V-D). Similar to the U.S., most of the samples that failed a single standard failed the enterococci standard.

The concordance among indicators for monthly standards was similar to that for the daily standards. Along all shoreline, 71% of the monthly exceedances were for

enterococci alone, with 16 % for both fecal coliforms and enterococci. Along U.S. shoreline, 71% of the monthly standards exceedances were for enterococci alone, whereas only 13% of the exceedances were for all three indicators (Figure V-5 A). In Mexico, these fractions were 71% and 23%, respectively (Figure V-5 B).

Figure V-1: Comparison of Log Transformed Total Coliforms vs. Fecal Coliforms

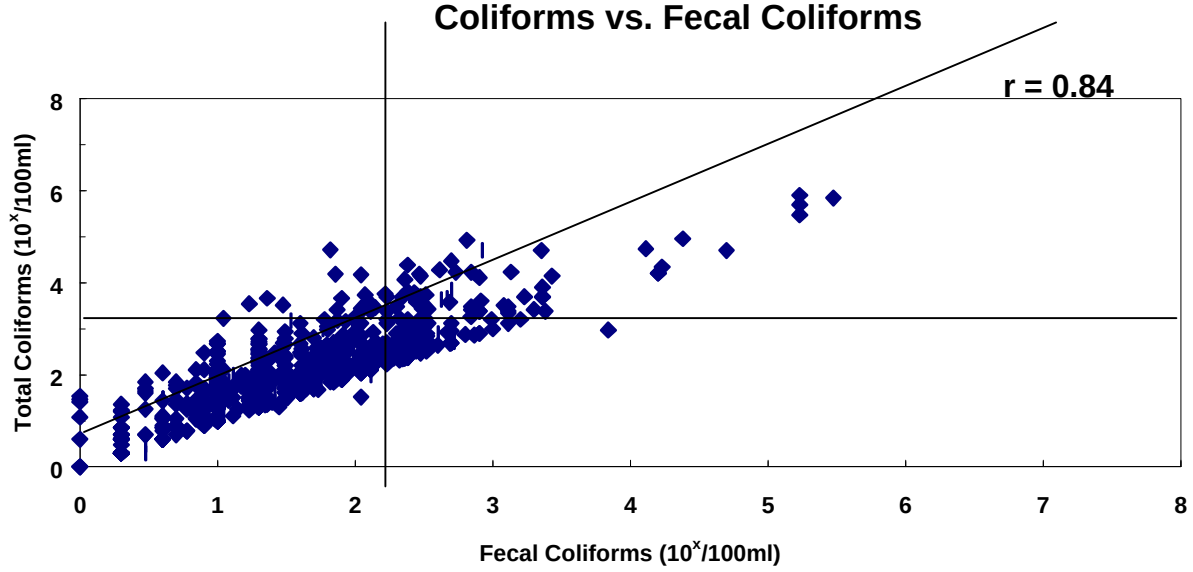

Figure V-2: Comparison of Log Transformed Total Coliforms vs. Enterococci

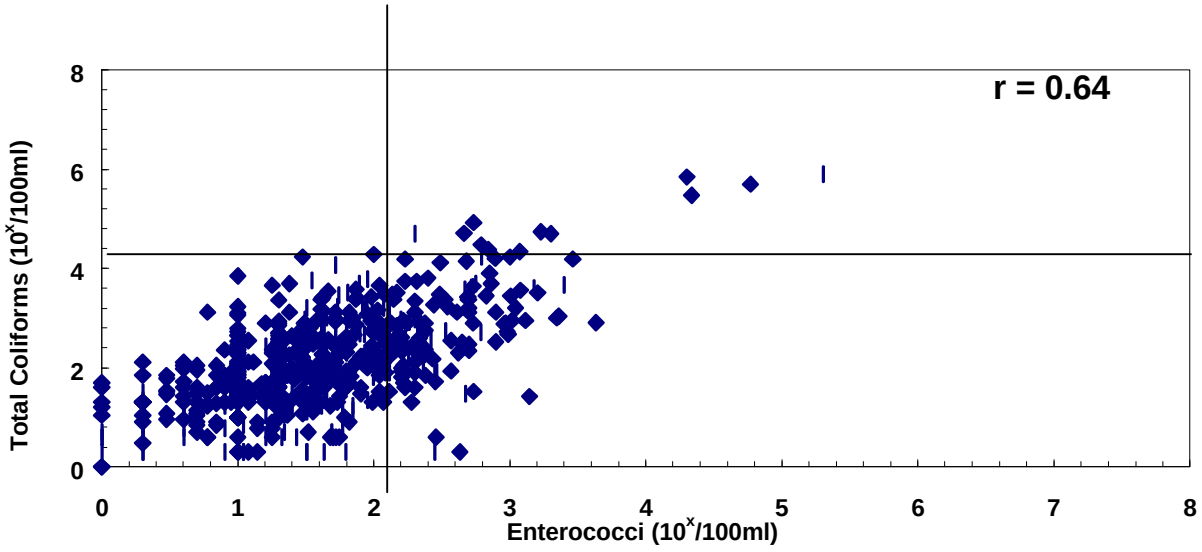

Figure V-3: Comparison of Log Transformed Fecal Coliforms vs. Enterococci

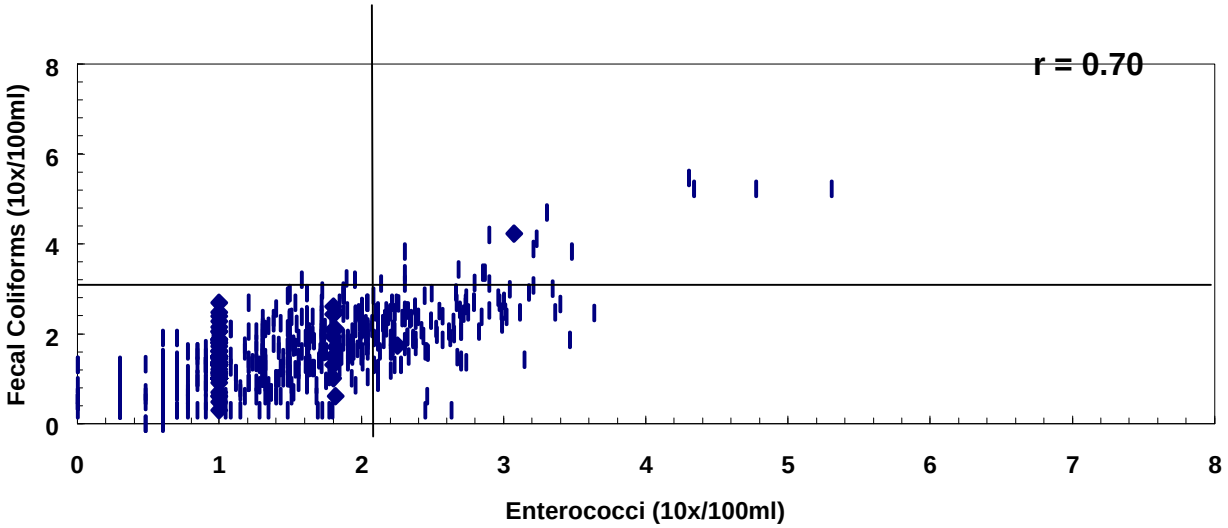

Figure V-4 A:
U.S. Shoreline (Sandy Beaches and Rocky Shoreline)

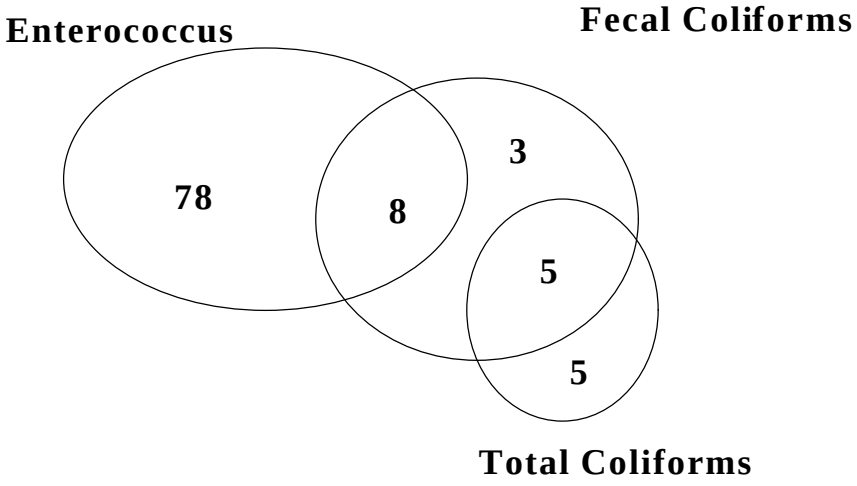

Figure V-4 B: U.S. Freshwater Outlets

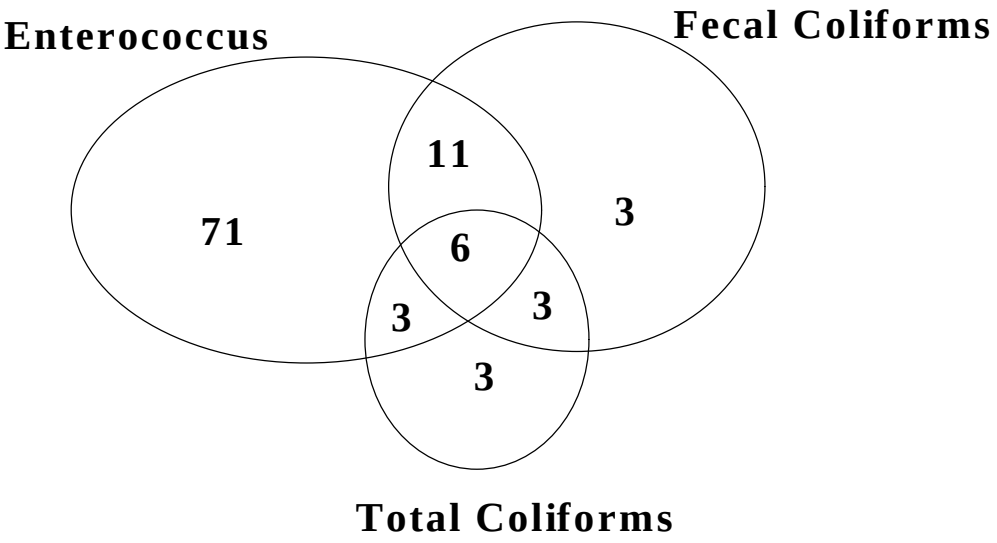

Figure V-4 C: Mexico Shoreline (Sandy Beaches)

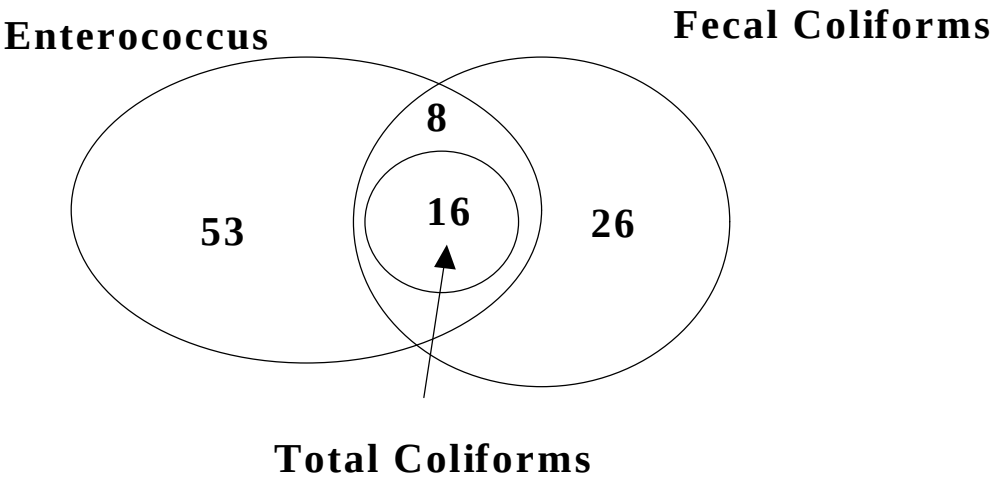

Figure V-4 D: Mexico Freshwater Outlets

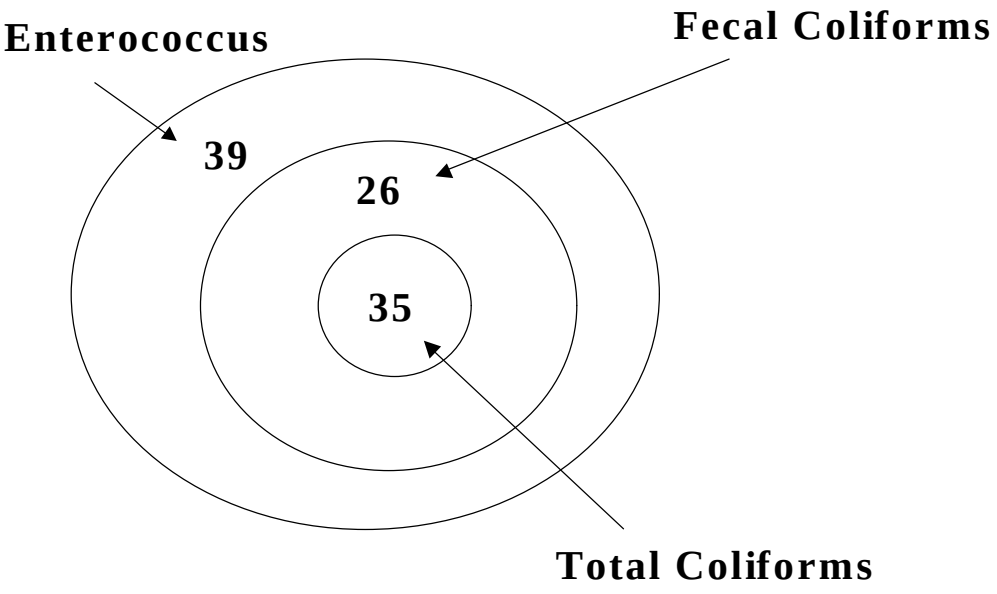

Figure V-5 A: U.S. Shoreline (MonthlyThresholds)

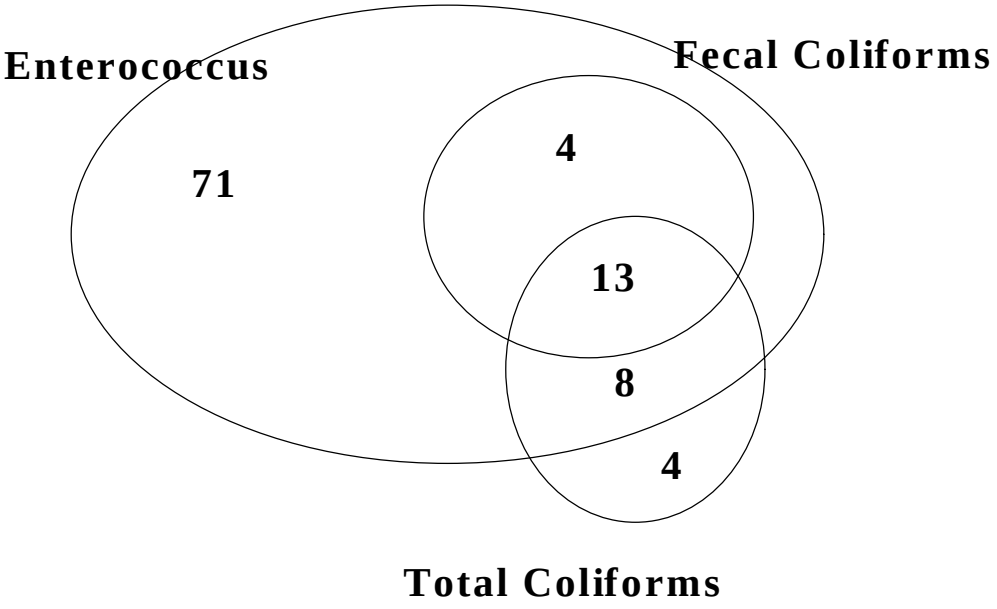

Figure V-5 B: Mexico Shoreline (MonthlyThresholds)

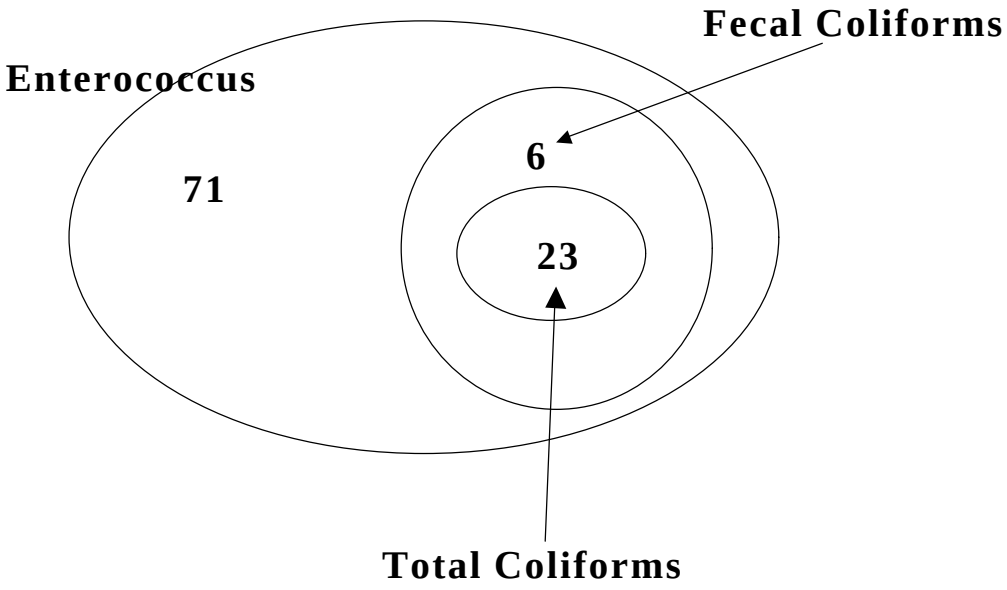

TABLE V-1. Correlation between enterococci, fecal coliforms, and total coliforms in the Bight'98 Shoreline Microbiology survey, for all results, and for all methods.

	Total Coliforms: Fecal Coliforms	Fecal Coliforms: Enterococci	Total Coliforms: Enterococci
Entire data set	0.85	0.70	0.64
Entire data set with qualified values	0.83	0.71	0.65
Membrane filtration	0.84	0.81	0.74
Multiple tube fermentation	0.92	0.70	0.69
Idexx Kits	0.75	0.80	0.69

TABLE V-2. Correlation between enterococci, fecal coliforms, and total coliforms in the Bight'98 Shoreline Microbiology survey, along different strata.

	Total Coliforms: Fecal Coliforms	Fecal Coliforms: Enterococci	Total Coliforms: Enterococci
All sandy beaches	0.85	0.66	0.61
All rocky shoreline	0.79	0.71	0.71
Mexico only	0.95	0.76	0.75
Perennial outlets	0.81	0.76	0.70
Ephemeral outlets	0.51	0.66	0.42
Perennial point zero only	0.89	0.81	0.77
Perennial point zero and ephemeral point zero	0.86	0.83	0.78
All freshwater outlets	0.84	0.79	0.73

TABLE V-3. Percent of samples which failed a state standard for a particular indicator and also had a total:fecal ratio of less than 10.

	U.S. Waters	Mexican Waters
Total coliforms	35	100
Fecal coliforms	45	77
Enterococci	20	49

B. Discussion

The higher correlation found between total coliform and fecal coliform bacterial densities than that found between either total or fecal coliforms and enterococci was similar to the results of the summer study and is consistent with previous experience of the study participants. This is largely attributable to both coliform tests being designed to detect the same organism, *E. coli*, with different sensitivities and specificities, while the enterococci test measures a different bacterial species. The ratio of these species groups differs depending on the source of the bacterial material.

Our finding of more samples failing the enterococci standard than any other standard is also consistent with results from the summer survey and does not appear 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 higher rate of enterococci threshold exceedances is that the thresholds may be set at different levels of sensitivity, which is consistent with our observation of high correlation among raw indicator values. This could have resulted from the different approaches that were used to generate the thresholds. 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 (1983b) established that enterococci densities correlated with numbers of highly credible gastroenteritis (HCGI) in swimmers at beaches influenced by wastewater in New York, New Orleans, and Boston. Similarly, Haile *et al.* (1999) 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 1983c).

A second possible explanation 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. The higher correspondence among indicators in Mexican waters and near freshwater outlets supports the degradation hypothesis, as these waters are closer to the source material and would have undergone less degradation than waters on the open coast.

A third possible explanation is that the indicators are display different sensitivities to different source material. Our results are also consistent with this hypothesis. Haile *et al.* (1999) have suggested that waters with bacterial inputs of recent human origin will have a low total:fecal coliforms ratio (i.e., fecal coliforms make up most of the total coliforms count), while a high ratio would be expected for non-human or degraded material. Only 20% of the U.S. samples and 49% of the Mexican samples in which enterococci exceeded state standards had a total:fecal ratio of less than 10 (Table V-3). In contrast, 100% of the Mexican total coliform samples had a total:fecal ratio of less than 10.

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 for recreational waters based upon enterococci densities (USEPA 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.* (1999) 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 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.

VI. ENTERIC VIRUSES AND COLIPHAGE

A. Results

Eighteen perennial point zero freshwater outlet sites were analyzed for the presence of enterovirus and adenovirus genomes, and coliphage. Six of the samples were analyzed by at both the USC and the UCI laboratories. At the USC laboratory, prefiltration combined with tangential flow filtration (TFF) was used to concentrate the seawater samples. At UCI, vortex flow filtration (VFF) was used to concentrate the seawater sample. For the other 12 sites, 6 samples were concentrated at USC, with concentrates sent to UCI for adenovirus analysis, and 6 samples were concentrated at UCI, with concentrates sent to the USC laboratory for enterovirus analysis. Samples were analyzed by either PCR (for adenovirus) or RT-PCR.

Nine samples were positive for enterovirus genomes and three were positive for adenovirus (Table VI-1). Only one sample was positive for adenovirus in the TFF concentrate as determined by Nested PCR, with three of them being positive in the VFF. In all of the RT PCR analyses, it was difficult to estimate the number of human enterovirus genomes using the MPN approach (as performed for the Summer Shoreline Microbiology Study). Positive detection occurred in the most dilute samples, probably due to the presence of inhibitory substances, making the MPN approach ineffective for this purpose. Coliphage were present in every sample tested, with coliphage concentrations ranging from 5.3 to 3332 PFU/l. Some analyses, denoted in Table VI-1 by ?, yielded positive results but with inhibition of the positive control, indicating inconclusive results that require further analyses.

Logistical correlation analyses between the presence of adenovirus and enterovirus genomes and each of the bacterial indicators (total coliforms, fecal coliforms, and enterococci) demonstrated no statistically significant relation. In addition, no correlation was found between any of the bacterial indicators and coliphage concentrations.

B. Discussion

Human enteric viruses, unlike most bacterial indicators, are direct indicators of the presence of human fecal contamination. In this study, we focused upon the detection of the genetic material (genome) of enteroviruses and adenoviruses. Enteroviruses are a subgroup of the entire human enteric virus family, and 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 genomes, although not a public health risk because it is an attenuated version of the virus, are also detected using our RT-PCR technique, and are 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. Adenoviruses are DNA viruses, a member of the family aviadenoviridae, and are viruses with one linear DNA strand. They are found in human fecal contamination, and can be responsible for symptoms similar to those of the

"common cold". Adenoviruses cause childhood pneumonia, acute respiratory disease, ill-defined syndrome of fever, pharyngitis, cough, hoarseness, chest pain, nosocomial respiratory infections and heart problems.

The results of this study were similar to those in the Winter Shoreline Microbiology Study, with enteroviruses observed in nearly half of the freshwater outlets in both studies. However, when analyzed using other concentration and amplification methods, such as the VFF and Nested PCR, respectively, fewer freshwater outlets demonstrated the presence of either enteroviruses or adenoviruses. Both VFF and TFF are used for concentrating viruses in seawater, but the concentrates produced are quite different for the purposes of PCR. With TFF, the sample is prefiltered to remove any material larger than 1.0 μm. With VFF, the whole seawater sample is concentrated. Because of the prefiltration and the nature of the TFF method, the recovery is lower than with the VFF method. However, inhibitory products (humic and fulvic acids, polysaccharides, enzymes, salt concentration) are also present in increased quantities in the VFF concentrate, making inhibition of the PCR reaction more likely. Currently, both laboratories are performing research to further develop and standardize methods for concentrating/separating human pathogenic viruses from seawater.

While enteroviruses and adenoviruses are responsible for a variety of illnesses or symptoms, the measurement techniques used in this study do not provide direct information about infectivity of the observed virus particles. The 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 and adenoviruses, 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 was combined with other measures, such as direct plating of coliphage, to assess the relationship between infectivity present in viruses from fecal contamination and the presence of human pathogenic viruses; however, no relationship was found.

Additional research is needed to understand the poor correlation between bacterial and viral indicators. The importance of several factors in the analysis of bacterial and viral contaminants (survival of pathogens in seawater, sedimentation, mixing, ionic effects, effects of sunlight, indirect effects of UV light, turbulence, sunlight intensity, temperature, and predation) are poorly understood. 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 (USEPA 1985). Our studies are consistent with previous work by LeGuyader (1993, 1994), Goyal (1993) and Yamashita (1992) that demonstrated no relation between the presence of human pathogenic viruses and bacterial indicators in both seawater and shellfish. Understanding the factors that affect bacterial and viral pathogens, and how they relate to the presence of bacterial and viral indicators that are currently being used to infer microbiological water quality, is essential to predicting human health threats in marine waters

TABLE VI-1. Detection of enteroviruses, adenoviruses, and coliphage in coastal waters directly adjacent to southern California freshwater outlets. "+" indicates positive detection, "–" indicates either negative detection, or no indicator exceedances.

Freshwater Outlet	Sampling Date	Temp. (°C)	Salinity (ppt)	Coliphage (PFU/L)	Nested-PCR Adenovirus in TFF* Concentrate	Nested-PCR Adenovirus in VFF** Concentrate	RT-PCR for Enterovirus in TFF* Concentrate	RT-PCR for Enterovirus in VFF** Concentrate	Any indicator exceedances?
Malibu Lagoon	2/8/99	ND	10	192±40.7	-	-	+	-	All
Santa Monica Canyon	2/8/99	ND	9	96±30.2	-	-	-	-	Entero
Los Angeles River	2/9/99	ND	28	472.5±111.8	+	+	+	+	All
San Gabriel River	2/16/99	15.5	28	106.2±30.7	-	+	+	+	-
Santa Ana River	2/16/99	15	33	9.5±1.5	-	+	+	+	-
San Juan Creek	3/1/99	13	24	20.5±6	NA	-	NA	-	Entero
Aliso Creek	3/1/99	15.5	26	NS	NA	NS	+	-	-
San Luis Rey River	3/1/99	14	27	20±13.9	NA	-	NA	-	-
Moonlight Creek	3/1/99	15	34	37.5±4.4	NA	-	NA	-	All
Los Penosquitos Lagoon	2/22/99	14	32.5	5.3±2.5	NA	-	NA	?	-
San Diego River	2/22/99	14	31	367.5±22.7	NA	-	NA	?	-
Tijuana River	2/22/99	16	29.5	3332±80.9	NA	-	NA	?	-
Ballona Creek	2/16/99	19.7	NS	NS	-	NA	-	NA	Fecal, Entero
Calleguas Creek	2/24/99	22	NS	NS	-	NA	-	NA	-
Goleta Creek	2/24/99	21.3	NS	NS	-	NA	+	NA	-
Carpinteria Creek	3/1/99	21.4	NS	NS	-	NA	+	NA	-
Mission Creek	3/1/99	21.4	NS	NS	-	NA	+	NA	Entero
Arroyo Burro	3/1/99	21.6	NS	NS	-	NA	+	NA	-

* Tangential Flow Filtration (performed at USC).
** Vortex Flow Filtration (performed at UCI).
NS - Not sampled.
NA - Not analyzed.
? – Inconclusive results.

VII. CONCLUSIONS

This study represents the first regional assessment of winter microbiological water quality along the Southern California Bight shoreline and complements a similar study conducted last summer. 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 bacteriological water quality along Mexican and United States shorelines in the winter 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 along the southern California shoreline during the winter of 1999 was not much different than that in the summer of 1998.**

Approximately 90% of the shoreline mile-days from Santa Barbara to San Diego during the winter (February 1999) met all of the State of California's bacterial water quality standards for human body contact, as compared to 95% for August of 1998. 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 one percent 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.

These results are similar to those observed in the summer survey, in which 94% of the shoreline met water quality standards. The present study was conducted during a dry winter season in southern California that resulted in one inch of rain, or less than half the average. Little rain fell immediately before sampling events, which prevents us from extending our results to periods after large rainfall events. However, we can conclude that widespread water quality problems are not experienced during non-rain periods in the winter.

- **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 consistently poor microbiological water quality. About half of the shoreline mile-days in these areas failed State standards. Most of these exceedances were for multiple indicators and occurred repetitively throughout the five-week study period. Human enteric viral genetic material was detected in samples taken from most of these freshwater outlet locations.

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

Although 83% of the beach samples in Mexico met California's bacteriological water quality standards, the standards were exceeded two to five times more often along Mexican than United States beaches, depending on the indicator. Differences between the two countries were even more pronounced near freshwater outlets, where average bacterial concentrations in Mexico were as much as 500-fold higher. This study provides valuable base-line information that is already being used to assess the effectiveness of Mexican authorities to improve water quality.

- **Beach quality decisions in southern California are sensitive to which indicators are measured.**

Beach closure and posting 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. Regulations drafted in response to AB411 require measuring three indicators (enterococci, total coliforms, and fecal coliforms) during the summer, although many of the health departments have extended their sampling of all three indicators to the winter as well. Of these, the enterococci standard was exceeded three times as often as any other standard. In areas away from freshwater outlets, 78% of the standards failures were for enterococci alone. No sample away from a freshwater outlet failed all standards.

- **Bacterial indicators were poorly correlated with the presence of viruses.**

Point zero freshwater outlet samples were found to contain enteroviral genetic material in 50% of the samples, adenoviral genetic material in 18% of the samples, and coliphage (viruses that infect coliform bacteria) in 100% of the samples tested. None of these viral measures were correlated with any of the bacterial indicators tested.

VIII. RECOMMENDATIONS

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

As in the summer study, ocean waters immediately adjacent to freshwater outlets were found to be the areas of poorest water quality along southern California beaches. Virtually all of the routine monitoring in ocean waters near freshwater outlets is conducted presently by county health departments or by ocean-discharging sewage treatment organizations, both of which have limited jurisdiction to address problems observed near freshwater outlets. This dissociation between the organizations that conduct coastal microbiology monitoring programs and the organizations that bear most of the management responsibility for correcting observed problems is inefficient for protecting the public. 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 the Santa Monica Bay, and the stormwater agencies for Orange, Riverside, and San Bernardino Counties were co-sponsors of this regional monitoring program. It is recommended that this cooperative interaction be expanded.

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

This study found a high degree of inconsistency among the three bacterial indicators used for beach posting/closure decisions. Furthermore, there was little agreement between these indicators and concentration of either viral RNA or viral infectivity. Because of such uncertainties, all health agencies in the state now measure multiple bacterial indicators and provide warnings to the public if any of the indicators are exceeded. The California State Department of Health Services and the U.S. Environmental Protection Agency have independently embarked upon research efforts to understand the relationship between indicators, pathogens, and public health risk. Microbiology studies have identified factors such as salt concentrations, sedimentation, mixing, sunlight, adsorption, turbulence, temperature, and predation that affect the survivability and persistence of both indicator bacteria and pathogenic bacteria and viruses. We need to conduct further research to understand how bacterial and viral indicators relate to one another, and how and when the presence of these indicators indicates the presence of dangerous pathogens and direct public health concerns. 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 more closely relate existing measures to health risk.

- **Conduct additional studies to assess the effect of rainfall events on beach water quality.**

While this study was effective in assessing the water quality under background conditions in the winter, it was ineffective at describing beach water quality following a winter rainstorm. Health agencies routinely issue warnings to prohibit human contact with ocean water for 72 hours following a rainstorm. Little information exists as to whether those warnings should be limited to areas near freshwater outlets or apply to all shoreline areas. An additional study, targeted to assess the spatial extent of beach quality in a period immediately following a rainstorm, is still warranted.

- **Conduct additional studies to compare bacterial measurement methods.**

Chromogenic substrate kits have become increasingly popular in southern California because they are less expensive and, in some cases, more rapid than either of the historically used membrane filtration or multiple tube fermentation testing procedures. Laboratory intercalibration studies conducted during the summer regional survey have suggested that these new methods provide comparable and more precise results than the historically used methods. The field intercomparison tests conducted as part of this study, however, were inconclusive, largely due to the limited range of bacterial concentrations observed. Although limited testing suggests that the chromogenic substrate tests were more accurate than the standard USEPA methodologies for enterococci, many agents interfere with bacterial indicator assays that cannot be mimicked effectively in laboratory tests. Given the desirability of the chromogenic substrate techniques as a potential mainstay of health testing laboratories in southern California, additional field studies that compare this method with historically used methods under field conditions is desirable.

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APPENDIX A: LIST OF PARTICIPANTS IN THE SOUTHERN CALIFORNIA BIGHT 1998 REGIONAL MONITORING PROGRAM (BIGHT'98)

[a] Denotes participants in the Winter Shoreline Microbiology component.

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

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

APPENDIX B: METHODS COMPARISON

Thousands of marine water samples are analyzed annually for indicator bacteria in southern California, with laboratory methods varying among the different organizations that conduct the monitoring (Schiff *et al.* in press). The number of methods used by these labs is increasing. Two of the new methods are chromogenic substrate kits (Colilert® and Enterolert®). While these methods are not yet approved by the U.S. EPA for use in marine waters, they have gained favor because they are cheaper, easier to use, and yield results more quickly than standard methods. Another new method is the EPA 1600 method for enterococci, which provides results in 24 hours, rather than the 48 hours required by the other traditional membrane filtration (MF) and multiple-tube-fermentation (MTF) methods.

Numerous studies have assessed comparability of the chromogenic substrate methods and standard methods within individual laboratories (Abbott, *et al* 1998, Budnick, *et al* 1996, Eckner 1998, Palmer, *et al* 1993), but no study has ever simultaneously compared traditional methods, chromogenic substrate methods and the EPA 1600 method. Here, seven laboratories participated in a study to assess the comparability of these new methods and place them in context of natural variability among laboratories.

METHODS

Seven labs performed side-by-side analyses on approximately 280 samples for three indicator bacteria, total coliforms, fecal coliforms (or *E. coli* in the case of Colilert®), and enterococci, as part of the Bight'98 Winter Microbiology Survey. Seawater samples, from randomly selected sites were split and analyzed using at least two different methods by each laboratory. The site selection, collection methods, and laboratory procedures are described in detail in the Methods section of the Bight'98 Winter Microbiology Report.

Four analysis methods were used in the comparisons. Standard methods for the isolation of bacteria from environmental samples include MF and MTF (APHA, 1995). With Colilert®, total coliforms are detected by a color change in a chromogenic substrate. In the same medium, *E. coli* release a fluorogen which causes the well to fluoresce. While total coliforms are indicated by a color change (to yellow) in the culture medium, samples are considered positive for *E. coli* only when the medium both changes color and develops a blue fluorescence. Enterolert®, is a similar chromogenic substrate method which detects enterococci by its ability to enzymatically hydrolyze the substrate, causing fluorescence. There are 18 and 24-hour incubation formulations for Colilert® reagent, but only a 24-hour formulation for Enterolert®. Both media are proprietary of Idexx Corporation. The EPA 1600 is a modification of the original MF method developed by the EPA for the detection of enterococci. The original method is a 48-hour analysis requiring a two-step incubation using two culture media. The EPA 1600 method combines the properties of the two media into one and reduces the incubation time to 24 hours.

The data were analyzed in three ways. First, results were tabulated in contingency tables, using the State Health Department daily thresholds recently enacted in response to AB 411. Second, correlations between methods were calculated for all indicators to determine the degree of comparability throughout the range of values. Lastly, pairs of values obtained from each sample were compared using a Wilcoxon signed rank test.

RESULTS

Comparable results were generally found using the threshold analysis (Table B-1). For enterococci, the correspondence was highest (99%) between the EPA 1600 method and standard methods. The correspondence between Enterolert® and the EPA 1600 method was 97%, with generally good agreement both above and below the 104 cfr or MPN/100 mL threshold. The correspondence between Enterolert® and standard methods was 88%, though all of the samples for which there were contradictory results were ones that Enterolert® produced concentrations above the State threshold while standard methods produced results below. For total coliforms, there was 96% agreement between Colilert® and standard methods, although in all cases of disagreement Colilert® values were below the threshold while standard methods were above. Similarly, 94% of the fecal coliform results agreed between Colilert® and standard methods, but all of the samples in disagreement were ones for which standard method were above the threshold while Colilert® results were not.

Correlations between the Idexx methods and standard methods were generally low (Table B-2). For enterococci, the two methods were not significantly correlated. For total and fecal coliforms, the methods were significantly correlated, but the correlations explained less than half of the variability. In contrast, there were high correlations between both the EPA 1600 method and standard methods, and between the EPA 1600 method and Enterolert® for the determination of enterococci ($r=0.9$ and $r=0.89$, respectively).

The paired measurements were significantly different using the Wilcoxon test when comparing Idexx methods to standard methods, and when comparing the EPA 1600 method to Enterolert®, but not when comparing the EPA 1600 method to standard methods (Table B-3). Idexx results were significantly higher for enterococci and significantly lower for fecal and total coliforms.

DISCUSSION

The EPA 1600 method produced comparable results to the standard methods in our comparison with a random set of California shoreline seawater samples. The EPA 1600 method produces results in 24 hours, instead of the 48 hours required for the standard methods. Since results were comparable, the newer test procedure seems an

appropriate replacement for standard enterococci MF methods in order to expedite public notification of beach water quality, especially during episodic events (storms, sewage spills, etc.). In addition, the EPA 1600 method produced comparable results to the Enterolert® method. Even with the high correlation between these two methods and their threshold-based agreement, there was a significant difference between the magnitude of each set of paired results (Table B-3). It is apparent that further studies need to be done to both examine a wider range of enterococcus concentrations, and to determine the exact types of organisms responsible for positive reactions by each method.

The much poorer comparison between the Idexx methods and standard methods suggests the need for more extensive comparative testing before the newer methods are adopted for widespread use in California. Of particular concern is the lack of significant correlation between the Enterolert® and standard methods. Some of the poor correlation reflects the low range of response we observed in our testing. The highest enterococci value observed using either method was only about twice the State threshold and higher correlations might be expected if the testing extended over a wider range of bacterial concentration. Still, the samples were from California beaches and the public warning process would have differed for 12% of the sites depending on which method was used to quantify the samples. Of even more concern, though, was that two laboratories did species-specific confirmation testing of the positive wells in the Enterolert® test and found the Enterolert® results to be accurate. This suggests that standard methods may be underestimating enterococci concentrations, at least over the limited range of our testing. Further comparative testing should focus on time periods or locations in which a larger range of bacterial concentrations are likely to be encountered and for which all participating laboratories conduct confirmation testing of samples which disagree among methods.

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TABLE B-1: Contingency tables based on daily thresholds.

Enterococci

		Enterolert®	
		< 104	> 104
Standard Method	< 104	265	30
	> 104	0	0

		EPA 1600	
		< 104	> 104
Standard Method	< 104	167	1
	> 104	1	16

		EPA 1600	
		< 104	> 104
Enterolert®	< 104	158	1
	> 104	4	16

Total Coliforms

		Colilert®	
		< 10,000	> 10,000
Standard Method	< 10,000	332	0
	> 10,000	11	0

Fecal Coliforms

		Colilert®	
		< 400	> 400
Standard Method	< 400	315	0
	> 400	20	0

TABLE B-2: Correlation between methods.

Method Comparison	Enterococci	Total Coliform	Fecal Coliform
Idexx vs. Standard Method	0.1	0.4	0.6
EPA 1600 vs. Standard Method	0.9		
EPA 1600 vs. Enterolert®	0.89		

TABLE B-3: P-value from Wilcoxon signed rank test for differences between paired values between methods.

Method Comparison	Enterococcus	Total Coliform	Fecal Coliform
Idexx vs. Standard Method	0.0	0.0	0.0033
EPA 1600 vs. Standard Method	0.5		
EPA 1600 vs. Enterolert®	0.0126		