Relationships among bacterial indicators during a regional survey of microbiological water quality along the shoreline of the Southern California Bight

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ABSTRACT

A regional shoreline microbiology study was conducted in order to examine microbiological water quality along the entire shoreline of the Southern California Bight (SCB). A major goal of the study was to compare the responses among indicator bacteria. Samples were collected weekly at 307 sites between Point Conception, California, and Punta Banda, Mexico, beginning August 2, 1998, and continuing for five weeks. Three analytical methods, multiple tube fermentation (MTF), membrane filtration (MF), and chromogenic substrate tests, were used to test for the three bacterial indicators currently used in southern California for human water-contact health standards: total and fecal coliforms, and enterococci. Samples were collected using standardized protocols. Total and fecal coliforms were analyzed for all samples, and enterococci were measured in approximately 70% of the samples. Sampling sites were selected using a stratified random design, with six sampling strata: high- and low-use sandy beaches and rocky shoreline, and ephemeral and perennial freshwater outlets. Results demonstrated that total and fecal coliforms correlated strongly with one another (r = 0.93). The correlation between enterococci and either total or fecal coliforms was much lower, but still significant (r = 0.29). However, these correlation coefficients were produced by including 564 values that had qualifiers; i.e., < or > symbols qualified the discreet value. Analysis of the data without the qualified results demonstrated stronger correlations between enterococci and fecal coliforms (r = 0.40) and enterococci and total coliforms (r = 0.77). No indication was found that the relationship between one bacterial indicator and another was dependent upon strata. However, the probability of exceeding a bacterial indicator threshold differed substantially between the indicators. The allocation of threshold exceedences among indicators was very dependent upon the indicator examined. Enterococci alone exceeded the bacterial indicator thresholds most often; i.e., one out of every three bacterial indicator exceedences was for enterococci alone. This finding demonstrated that enterococci was by far the most "conservative" indicator of bacteriological water quality in this study. Less than one-half of the enterococci threshold exceedences paired with threshold exceedences of another indicator, while nearly 90% of the total and fecal coliform threshold exceedences were partnered with exceedences of another indicator. These results shed new light on the quantitative assessments of bacteriological water quality and the interpretion of the results of bacteriological water quality analyses.

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INTRODUCTION

With its world-renowned recreational waters, explosive population growth, and high-intensity development, southern California has become one of the nation's most critical coastal environments, and protection of its beach water quality is a high priority. The potential for pathogenic microorganisms such as viruses, bacteria, and protozoa to enter the coastal environment has increased exponentially, especially from non-point sources. These pathogenic microorganisms impact microbiological water quality and potentially pose health risks to beachgoers. Bacterial indicators have been used to infer microbiological water quality and safety for drinking water, bathing waters, and shellfish harvesting areas. Total coliforms have been used as the sole bacterial indicator of microbiological water quality for years.

Some of the most common indicators of fecal contamination used today are total coliforms, fecal coliforms (of which *E. coli* is the major component), and enterococci. In the late 1990s, the agencies involved in developing regulations for protecting the recreational water quality in the state of California began to move toward including all three major bacterial indicator groups (total coliforms, fecal coliforms, and enterococci) in the testing of water quality. Currently, more than 80,000 shoreline bacteriological samples are collected annually in southern California from more than 500 sites. This monitoring effort represents approximately one-half of the total yearly bacteriological monitoring conducted in the United States (Schiff *et al.* in press). Despite this impressive amount of bacteriological monitoring, the resulting data are difficult to integrate because the over 22 different organizations that conduct monitoring use different sampling strategies and different data management systems. To address this issue, the California State Legislature recently passed Assembly Bill 411 (AB411) requiring the State Department of Health Services (SDHS) to adopt regulations that provide consistency in monitoring indicators and standards. AB411 requires that all three indicators be used as testing standards, and that warnings be posted at beaches if one or more of the indicators exceeds a threshold.

Unfortunately, no single bacterial indicator has been identified to fill all three of the following criteria: (1) that it is applicable to all types of water, (2) that it is present whenever pathogens are present, and (3) that the indicator does not reproduce in contaminated waters, thereby producing overestimates of risk (Scarpino 1974). It has been suggested that the use of all three bacterial indicators will provide a more complete picture for the determination of recreational water quality. One of the problems associated with using a single type of bacteria to indicate water quality is that, once released into the environment, unfavorable physical and chemical conditions can affect the relative survival rate of fecal and non-fecal bacteriological components. Fecal coliforms may not survive as well as total coliforms in the unfavorable environment outside of the gut of warm-blooded animals (Hanes and Fragala 1967, Sieracki 1980). With this in mind, our goal was to compare the responses of these indicators under the differing conditions of the study strata. This may provide information about the responses of each type of indicator organism (or group of organisms) at specific types of locations (e.g., high-use sandy beaches as opposed to those close to freshwater outlets). These results can be used to understand which indicator organisms are most "conservative" at each of several shoreline types, and to assess potential redundancy among indicators. We have provided a quantitative examination of how the indicators compare to one another, as well as a "threshold exceedence" comparison. Other goals of this study include a region-wide assessment of coastal water quality, the results of which can be found in Noble *et al.* (2000).

METHODS

Sampling Design

The Shoreline Microbiology component of Bight'98 involved sampling at 307 sites along the SCB coastline between August 2 and September 5, 1998. Each site was sampled once per week during the five-week study period. A five-week study period was selected to meet the minimum of five weekly samples for calculation of the 30-d geometric means required under the California Ocean Plan and proposed AB411 regulations. The study was conducted during summer to coincide with the period of maximum beach bathing usage. The study area extended from Point Conception in Santa Barbara County, California, to Punta Banda, Baja California, just south of Ensenada, Mexico. Sampling sites were selected using a stratified random design, with the strata corresponding to six shoreline types of interest: high- and low-use sandy beaches, high- and low-use rocky shoreline, and perennial and ephemeral freshwater outlets. Sample sites within the perennial water outlet stratum were either random sites (sites selected at a random distance within 100 yards from the mouth of the outlet), or point zero sites (sites placed at the mouth of the outlet).

Field and Laboratory Methods

Each participating laboratory used its standard method for sample processing, with a performance-based approach employed to ensure data comparability among laboratories; intercalibration tests using common samples were performed before the start of the sampling period (McGee 1997-1998). More detailed information on the methods used by all participants can be found in Standard Methods for the Examination of Water and Wastewater, 18th Edition, 1995 (APHA 1995).

In brief, 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 capped tightly and stored on ice in the dark. All samples were returned to the laboratory in time to begin analysis within 6 h of sample collection.

Total coliforms and fecal coliforms were measured for all sites. Enterococci were measured at approximately 70% of the sites, depending upon the capability and capacity of the participating organization responsible for the site. Three methods were used to measure bacteria: membrane filtration (MF), multiple tube fermentation (MTF), and substrate technology tests. The first method, MF, is a direct plating method for the detection and enumeration of bacteria in water. The second method, MTF, involves inoculating multiple tubes of broth with dilutions of the sample. Organism density is based upon the number of tubes with acid and gas production at the various dilutions and is reported in terms of the most probable number (MPN) as determined by a series of probability formulas. The third method used defined substrate technology tests, Colilert® and Enterolert®, manufactured by Idexx, Inc. The Idexx kits use either multiple tubes or multiple wells, with an MPN approach, to detect the presence or absence of total coliforms and *E. coli*, or enterococci. With Colilert®, the detection of coliforms is based upon a color change for total coliforms and the release of a fluorogen by an enzyme produced only by *E. coli*. This assay is read within 18-22 h. In this study, *E. coli*, which typically constitute the overwhelming majority of fecal coliforms, were treated as fecal coliforms for the purpose of data analysis.

RESULTS

A total of 1,293 samples were included in our base correlation analyses. This result includes samples taken from the sites where which multiple analysis methods were used.

Total and fecal coliforms correlated strongly with one another (r = 0.93, Figure 1). The correlation between enterococci and either total or fecal coliforms was much lower, but still significant (r = 0.29, Figure 2). However, the data analysis that produced the correlation coefficients included 564 values that had qualifiers; i.e., < or > symbols qualified the discreet value. Analysis of the data without the qualified results demonstrated stronger correlations between enterococci and fecal coliforms (r = 0.40) and enterococci and total coliforms (r = 0.77, Table 1). No significant difference was found in the relationship between bacterial indicators based upon the method used for analysis, supporting the intercalibration studies that were conducted previously (McGee 1997-1998). In addition, no indication was found that the relationship between bacterial indicators was dependent upon strata. The probability of exceeding a bacterial indicator threshold differed substantially among indicators. The allocation of threshold exceedences among indicators, however, was very dependent upon the indicator examined. Enterococci alone exceeded bacterial indicator thresholds one-third of the time that an exceedence was observed, demonstrating that enterococci was the most "conservative" indicator of bacteriological water quality. Less than one-half of the enterococci threshold exceedences paired with threshold exceedences by another indicator, while nearly 90% of the total and fecal coliforms threshold exceedences were partnered with exceedences of another indicator. These results shed new light on quantitative assessments of

FIGURE 1. Comparison of log-transformed total coliforms versus fecal coliforms.

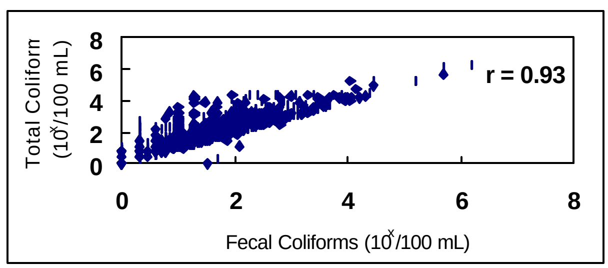


FIGURE 2. Comparison of log-transformed total coliforms versus enterococci.

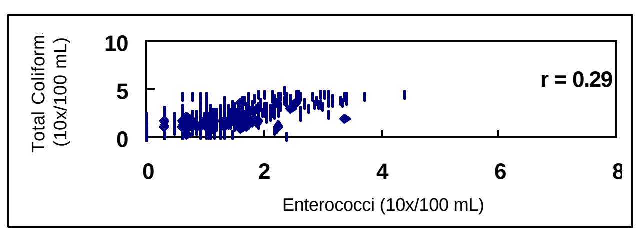
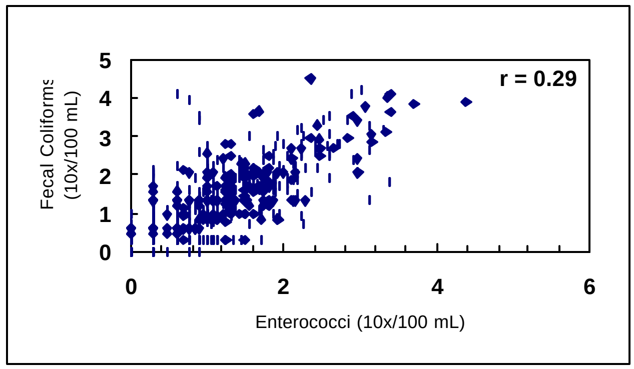


TABLE 1. Correlation between total coliforms, fecal coliforms, and enterococci in the Bight'98 Summer Shoreline Microbiology Study.

	Total Coliforms: Fecal Coliforms	Fecal Coliforms: Enterococci	Total Coliforms: Enterococci
Entire Data Set	0.93	0.29	0.29
Membrane Filtration Alone	0.89	0.38	0.29
Multiple Tube Fermentation Alone	0.93	0.47	0.42
Idexx Alone	0.93	0.38	0.3
High-use Sandy Beaches Alone	0.88	0.25	0.25
Water Outlets Alone	0.93	0.3	0.28
Without Qualified Values	0.91	0.4	0.77

FIGURE 3. Comparison of log-transformed fecal coliforms versus enterococcus.



bacteriological water quality and the interpretation of results of bacteriological water quality analyses.

A number of samples from the survey were not quantified because they were below the detection limit for the MTF and MF methods, or they exceeded the capacity of the dilution series performed; instead they were reported as "<" or ">" values. For the overall analyses, these values were truncated to the lower or upper end of their quantification range (i.e., converting <2 to 2, and >16,000 to 16,000). Removing these data points, rather than truncating the number, had little effect on the correlation between fecal coliforms and enterococci or total coliforms. The correlation between total coliforms and enterococci more than doubled with the reduced data set (Table 1). A strong correlation was found between total and fecal coliforms (r = 0.93), while the correlation between enterococci and both total and fecal coliforms was weak (r = 0.29, Table 1, Figures 2 and 3).

The correlation between indicators was largely independent of laboratory method used to analyze the samples; for example, the correlation between total coliforms and fecal coliforms analyzed by MF was 0.89, whereas the correlation between the two using MTF analysis was 0.93 (Table 1). Samples analyzed with MTF had marginally improved relationships between indicators compared to MF. Correlation coefficients were nearly identical when comparing the MTF and MF methods to analyses using the Idexx kits (Table 1).

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

Threshold Analysis

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

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

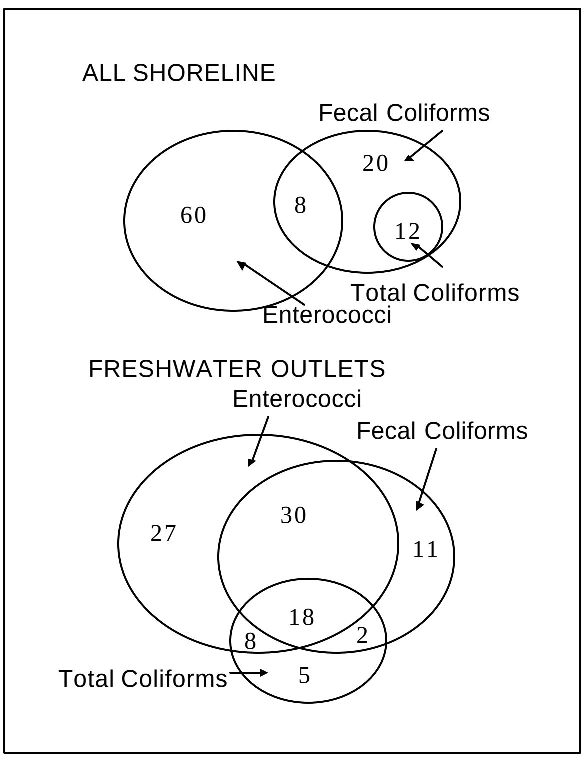
DISCUSSION

Our finding that different indicators do not equally reflect whether a site exceeds thresholds, as well as the higher incidence of enterococci threshold exceedences during summer, is consistent with the observations of the project participants from their routine monitoring programs. This conclusion does not appear to be limited to southern California. Nuzzi and Burhans (1997) compared the responses among total coliforms, fecal coliforms, and enterococci at 143 New York beach sites and found that

TABLE 2. Allocation of all observed threshold exceedences (by percent) organized by combinations of indicator bacteria.

	Total Coliforms	Fecal Coliforms	Total: Fecal Coliform Ratio	Enterococci
Alone	3.1	6.3	5.2	32.3
Total Coliforms				
Fecal Coliforms	1			
Total:Fecal Ratio	0	6.3		
Enterococci	5.2	3.1	2.1	
Fecal Coliforms and Total:Fecal Ratio	3.1			
Fecal Coliforms and Enterococci	5.2			
Total:Fecal Ratio and Enterococci	0	19.8		
All 4 Indicators	7.3			

FIGURE 4. Percent correspondence of indicator threshold exceedences at sites along the shoreline of the Southern California Bight during August 1998.



while indicator values were correlated, the likelihood of exceeding an enterococci threshold was more than twice that for either of the coliform measures.

One possible explanation for the disparity among indicator threshold exceedences 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 d while enterococci survival was 2.4 d. Sieracki (1980) demonstrated that the rate of enterococci die-off did not increase as the intensity of sunlight increased while *E. coli* die-off was dependent upon sunlight levels. Both of these factors could increase the likelihood of enterococci threshold exceedences relative to coliforms.

The applicability of bacterial indicators, and their thresholds, for influencing decisions about beach closures is dependent upon their relationship to the pathogenic organisms that cause illness. Investigators have shown that enterococci and coliphage have similar survival characteristics in receiving lake waters (Rajala and Heinonen-Tanski 1998). If the etiology of swimming-associated gastroenteritis is viral, and if coliphage react to physical and environmental stressors in a manner similar to human enteric viruses, then enterococci alone might be a better predictor of adverse health outcomes from exposure to fecal contamination. Cabelli (1982) and Dufour (1984) showed that enterococci correlated better with swimming-associated gastroenteritis at marine and freshwater bathing beaches with wastewater influences, resulting in the development of water quality guidelines by the United States Environmental Protection Agency (U.S. EPA) for recreational waters based upon enterococci densities (U.S. EPA 1986). This relationship between enterococci and swimming-associated gastroenteritis has been more recently examined by Kay *et al.* (1994), who demonstrated a significant dose-response relationship between gastroenteritis and fecal streptococci (of which enterococci are a subgroup) concentrations. Conversely, 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 highly credible gastroenteritis (HCGI) and diarrhea were observed when enterococci thresholds were exceeded. These results are also supported by Fleisher *et al.* (1996), who showed that fecal streptococci were predictive of upper respiratory tract illness, while fecal coliform exposure was predictive of ear ailments.

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

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

LITERATURE CITED

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

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

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

Cabelli, V.J. 1983c. Health Effects Criteria for Marine Recreational Waters. EPA-600/1-80-031. U.S. Environmental Protection Agency, Health Effects Laboratory. Research Triangle Park, NC.

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

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

Fleisher, J.M. 1990. The effects of measurement error on previously reported mathematical relationships between indicator organism density and swimming-associated illness: A quantitative estimate of the resulting bias. *International Journal of Epidemiology* 19:1100-1106.

Fleisher, J.M., D. Kay, R.L. Salmon, F. Jones, M.D. Wyer and A.F. Godfree. 1996. Marine waters contaminated with domestic sewage: Nonenteric illnesses associated with bather exposure in the United Kingdom. *American Journal of Public Health* 86:1228-1234.

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

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

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

McGee, C.M., M.K. Leecaster, P.M. Vainik, R.T. Noble, K.O. Walker and S.B. Weisberg. 1999. Comparison of bacterial indicator measurements amoung Southern California marine monitoring. pp. 187-198 *in:* S.B. Weisberg and C. Francisco (eds.), Southern California Coastal Water Research Project Annual Report 1997-1998. Westminster, CA.

McNeill, A.R. 1992. Recreational water quality. pp. 193-216 *in:* D.W. Connell and D.W. Hawker (eds.), Pollution in Tropical Aquatic Systems. CRC Press Inc. Boca Raton, FL.

Messer, J.W. and A.P. Dufour. 1998. A rapid, specific membrane filtration procedure for enumeration of enterococci in recreational water. *Applied and Environmental Microbiology* 64:678-680.

Moore, A.C., B.L. Herwaldt, G.F. Craun, R.L. Calderon, A.K. Highsmith and D.D. Juranek. 1994. Waterborne disease in the United States, 1991 and 1992. *American Water Works Association Journal* 86:87-99.

Noble, R.T., J. Dorsey, M. Leecaster, D. Reid, K. Schiff and S.B. Weisberg. 2000. A regional survey of the microbiological water quality along Southern California Bight shoreline. *Environmental Monitoring and Assessment* 64:435-447.

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

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

Scarpino, D. 1974. Human enteric viruses and bacteriophages as indicators of sewage pollution. International Symposium from Sea Outfalls. Pergamon Press.

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

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

United States Environmental Protection Agency (U.S. EPA). 1986. Bacteriological ambient water quality criteria for marine and freshwater recreational waters. PB86-158-045. U.S. EPA, National Technical Information Service. Springfield, VA.

Weiskel, P.K., B.L. Howes and G.R. Heufelder. 1996. Coliforms contamination of a coastal embayment: Sources and transport pathways. *Environmental Science and Technology* 30:1872-1881.

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