Shoreline Microbiology

BIGHT'03

Southern California Bight
2003 Regional Monitoring
Program
Vol. VI

Southern California Bight 2003 Regional Monitoring Program: VI. Shoreline Microbiology

August 2007

J.F. Griffith[1], K.C. Schiff[l], S.B. Weisberg[1], C. McGee[2] and C. Clifton[3]

[1]Southern California Coastal Water Research Project, 3535 Harbor Blvd., Suite 110, Costa Mesa, CA 92663
[2]Orange County Sanitation District, 10844 Euclid Avenue, Fountain Valley, CA 90601
[3]County of San Diego Department of Environmental Health, PO BOX 129261, San Diego, CA 92112-9261

MEMBERS OF THE MICROBIOLOGY COMMITTEE

Amador, Ric	City of San Diego
Aumand, Larissa	MEC Analytical/Weston Solutions
Bera, Angie	Santa Monica Baykeeper
Bahariance, Zora	City of Los Angeles, Environmental Monitoring Division
Branch, Nicki	San Elijo Joint Powers Authority
Clifton, Clay	County of San Diego Department of Environmental Health
DeShazo, Renee	Regional Water Quality Control Board - Los Angeles
Dorsey, John	Loyola Marymount University
Edwards, Eric	Los Angeles Department of Public Health
Ferguson, Donna	Orange County Public Health Laboratory
Flaherty, Kerry	Southern California Marine Institute
Griffith, John	Southern California Coastal Water Research Project
Harley, Ann	South Orange County Wastewater Authority
Hauge, Richard	Ventura County Environmental Health
Helyer, Karen	Port of San Diego
Jay, Florence	City of Ventura
Kolb, Ruth	City of San Diego Stormwater
Lee, Ioannice	City of Los Angeles, Environmental Monitoring Division
Lyons, Michael	Los Angeles Regional Water Quality Control Board
Marcelino, Phil	Aquatic Bioassay and Consulting
Mazur, Monica	County of Orange Environmental Health
McGee, Charles (Co-Chair)	Orange County Sanitation District
Meehan, John	City of Oxnard
Nikaido, Mai	City of Long Beach
O'Connell, Linda	State Water Resources Control Board
Othman, Laila	City of San Diego
Padilla, Michelle	Los Angeles County Sanitation Districts
Parks, Jeff	Encina Joint Powers Authority
Petralia, Jack	Consultant to the State Water Resources Control Board
Plana, Jennifer	City of Santa Barbara
Schiff, Ken	Southern California Coastal Water Research Project
Schulz, Don	Surfrider Foundation
Tierney, Valerie	City of Oceanside
Walker, Kathy	Los Angeles County Sanitation Districts
Weisberg, Steve (Chair)	Southern California Coastal Water Research Project
Wu, Frank	Los Angeles County Department of Public Works
Yahya, Moy	CRG Labs

FOREWORD

This study was a cooperative effort in which 58 organizations (Appendix A) joined to assess the overall condition of the southern California near-coastal ecosystem. This study was coordinated by the Southern California Coastal Water Research Project (SCCWRP) as one component of the Southern California Bight 2003 Regional Monitoring Program (Bight '03), and builds upon the success of a similar SCCWRP-coordinated regional monitoring programs conducted in 1994 and 1998. Copies of this and other Bight '03 reports are available for download at www.sccwrp.org.

The proper citation for this report is:
Griffith, J.F., K.C. Schiff, S.B. Weisberg, C. McGee and C. Clifton. 2007. Southern California Bight 2003 Regional Monitoring Program: VI. Shoreline Microbiology. Southern California Coastal Water Research Project. Costa Mesa, CA.

ACKNOWLEDGEMENTS

This report is the culmination of many hours of hard work by the dedicated individuals at the 36 organizations that participated in the Microbiology component of Bight '03. Although the report was prepared by a subset of the Microbiology Committee, we would like to thank all members of the Committee, who represented their organizations admirably and displayed a spirit of cooperation throughout the planning and implementation of this effort.

EXECUTIVE SUMMARY

Beach water quality monitoring in southern California is extensive, but samples are collected in shallow water (0.3 m) because breaking waves make sampling in deeper water inconvenient and potentially dangerous. To assess how well shallow water sampling characterizes conditions in deeper waters, we collected paired *Enterococcus* spp. samples at the shallow depth where sampling typically occurs, and outside the breaking surf offshore, where surfers typically line up to catch surfable waves. Sampling was conducted at 12 beaches in the summer dry season and 9 beaches following winter rainstorms. Beaches selected for study all had a flowing freshwater creek, surfers present at the site and a history of microbial water quality standards exceedences. Seven pairs of samples at different distances from the freshwater outlets were collected at all beaches. The nearshore and offshore samples were correlated during both the wet and dry sampling periods, but the correlation was higher following rainstorms. Concentrations of enterococci were typically higher in shoreline samples than offshore samples, with the difference being nearly three-fold under dry conditions and only 25% higher under wet conditions. For only one sample pair in dry weather and three sample pairs during wet weather, constituting less than 1% of total samples, did shoreline samples meet water quality standards when a corresponding offshore sample failed the standard.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

I. INTRODUCTION

Beach water quality in Southern California is the most intensively monitored water quality in the nation. Over 185,000 water samples are collected and analyzed each year during routine shoreline monitoring at a yearly cost of more than $3 million (Schiff *et al*. 2001). This investment reflects the importance of beaches to the local economy and to the more than 175 million beachgoers that visit southern California beaches each year (Schiff *et al.* 2001).

All shoreline water quality monitoring occurs at a depth of about 0.3 m, as breaking waves make sampling in deeper water inconvenient and potentially dangerous. This is also the depth of exposure for small children, who are the most immunologically susceptible swimmers. The 0.3-m depth is also sampled because it is where sampling was conducted during the primary epidemiology study on which California's water quality standards are based (Haile *et al*. 1999).

It is unclear how well sampling at this depth protects surfers, who receive much of their exposure at locations typically 20 m or more offshore. Surfers are often avid users of recreational waters and among the most vulnerable to waterborne illnesses because of repeated, substantial (head to toe), and sudden exposure. Compounding this exposure, prime surfing locations are often found at the mouths of creeks and rivers where sand bars formed by sediment deposition cause waves to break farther offshore, offering surfers a longer ride. These channels serve as drains that carry urban runoff to the ocean, causing the waters at their outlets to be some of the most contaminated along the beach (Noble *et al.* 2000, Noble *et al*. 2003, Schiff *et al*. 2003, Jiang *et al*. 2001).

Here we examine the relationship between the microbial water quality of samples taken at the 0.3-m depth and water quality of samples obtained offshore, where surfers typically line up to catch surfable waves. The goal of the study was to determine if samples taken along the shoreline adequately characterize microbiological water quality in deeper waters where surfers receive the majority of their exposure.

II. METHODS

The study involved paired sampling at shoreline (ankle to knee depth) and offshore (just below the surface in the surfer setup zone) sites near 12 different freshwater outlets (Figure 1). Beaches included in the study were selected based on the following criteria: a) presence of a river or creek flowing across the beach; b) presence of surfers at the site; and c) a significant number (>20% of samples) of microbial water quality standards exceedences in the historical shoreline monitoring.

The first set of sampling events occurred in September 2003, southern California's dry season. One hundred and fourteen pairs of shoreline/offshore samples were collected. Shoreline samples were collected directly in front of each freshwater outlet, from a bridge overlooking the center of the outlet, and from locations at distances of 25, 50, 75, and 225 meters up coast and down coast of the channel. Offshore samples at these same distances were collected either by swimmers, from kayaks, or from personal watercraft directly offshore at the point where surfers were waiting or would typically wait for a surfable wave. Sampling took place at high tide and was repeated at nine of the beaches (Table 1) on the subsequent low tide. Visual cues were used so that both shoreline and offshore samples were collected simultaneously.

A second set of samplings occurred between February 24 and March 26, 2004, following storm events (>0.1" rain). One hundred and forty two pairs of wet weather samples were collected at nine of the twelve beaches for up to three days following storm events (Table 2). As in dry weather, wet weather samples were collected from the wavewash in front of each creek mouth or from a bridge overlooking the channel, and at specific intervals upcoast and downcoast of the discharge. Unlike dry weather, however, the location of upcoast and downcoast sampling was not fixed. Instead, gradient distances surrounding each freshwater outlet were dictated by the geographic characteristics of each site, the volume of stormwater discharged, and the extent of the discharge plume.

All samples were collected in duplicate in sterile 120 ml polystyrene bottles and transported to local laboratories on ice. Enterococci were enumerated using Enterolert™ (IDEXX Westbrook, ME) defined substrate kits following the manufacturers instructions, or using membrane filtration and EPA Method 1600 (Messer and Dufour 1998). Several agencies also analyzed samples for total coliform, fecal coliform (ENT), or *Escheria coli;* however, *Enterococcus* spp. was the only indicator analyzed in all samples at all locations.

Ten local laboratories participated in sample collection and analysis (Table 3), which was necessary to ensure sample holding time requirements were met. Prior to sampling, all laboratories participated in an intercalibration exercise to ensure comparability (Griffith *et al*. 2006; Appendix B). The among laboratory variability was not significantly different from within laboratory variability.

Enterococci data were analyzed in two ways. First, regression was conducted to assess the relationship between paired samples collected along the shore with those collected at the surfer line-up depth offshore. Second, contingency tables were constructed to determine the relative frequency with which pairs of samples produced the same results with respect to the California single-sample water quality standard of 104/100 ml for enterococci.

III. RESULTS

The relationship between shoreline and offshore enterococcus concentrations during dry weather period was significant, but accounted for only 21% of the variability (Figure 2). Nearly 95% of samples collected at the shoreline during dry weather met water quality standards, with average concentrations along the shoreline more than five times greater than concentrations directly offshore at some sites (Figure 3). This was also reflected in the concordance analysis, where five samples collected at the shoreline exceeded water quality standards, compared to only a single sample exceeding at the offshore sites (Table 4).

The relationship between shoreline and offshore samples was stronger for wet weather than dry weather samples, with shallow samples accounting for 52% of the variability observed in offshore samples (Figure 3). As in dry weather, shoreline samples were higher than offshore, but differences were more extreme in winter (Figure 4). Forty-five percent of wet weather samples exceeded water quality standards and about two-thirds of those simultaneously exceeded standards in shoreline and offshore pairs (Table 5). There were only three samples in which the offshore sample exceeded standards when the shallow sample did not, whereas there were 21 that exceeded onshore but not offshore.

IV. DISCUSSION

Water samples taken in shallow shoreline waters were found to be protective of health risk to swimmers and surfers who are exposed to water quality offshore of the sampling site. In only a few cases (<1% in dry weather, <2% in wet weather) did shoreline samples meet water quality standards when a corresponding offshore sample failed standards. In contrast, about 7% of the offshore samples met standards when the corresponding shoreline samples failed standards. This suggests that shallow water sampling may be overprotective. However, surfers are also exposed to water closer to shore when they paddle out from shore or finish their ride by either intentionally dismounting or unintentionally falling from their boards.

This is not the first study to compare density of fecal indicator bacteria in waters of different depth, though this is the first to specifically examine the depths and locales populated by surfers. Wymer *et al*. (2005) observed that indicator bacteria densities declined with distance from shore up to chest depth. Similarly, in a study of two Lake Erie beaches, Francy *et al*. (2006) found that concentrations of *E. coli* were highest at one and two-foot depths, mid-range at waist depth, and lowest in samples collected 150 feet offshore.

The higher bacterial concentrations observed inshore probably reflects the greater proximity to the urban runoff drainage systems that are the principal fecal source for southern California beaches (Schiff *et al.* 2003). Flows from these creeks during dry weather are generally small relative to longshore transport. Grant *et al*. (2005) found that along-shore flux is about 100 times greater than cross-shelf flux. Several dye studies have shown that the creek water typically remains entrained in a narrow band parallel to shore, with only occasional offshore excursions associated with riptides (Kim *et al*. 2004, Grant *et al*. 2005, Rosenfeld *et al*. 2006, Clarke *et al*. 2007). Moreover, Taggart (2002) found that bacterial concentrations generally diluted an order of magnitude within 100 m from a freshwater outlet during low flow. This contrasts, however,

with wet weather conditions, when freshwater flows increase substantially and the predominant transport of stormwater plumes is cross-shelf (Warrick *et al*. In press). This would account for the much smaller onshore-offshore differences in bacterial concentrations that we observed in wet weather.

Despite our finding that indicator bacteria levels are typically higher in shallow water than offshore, it is unclear whether surfers receive a higher level of protection than do shallow-water bathers. The water quality standards and associated warning system are based on epidemiology studies which focused on individuals who swam only once during a potential incubation period (approximately two weeks). Surfers often surf multiple times a week and their exposure is more substantial than the typical bather due to pushing under waves to paddle out and occasional wipeouts (falling off a surfboard). This higher exposure than has been quantified in epidemiology studies may require a lower bacterial concentration threshold for issuing warnings to achieve the same level of protection for surfers. However, additional epidemiology studies that focus on multiple exposure swimmers would be necessary to establish an appropriate bacterial level that is protective of health risks to surfers.

V. CONCLUSION

- Sampling in shallow water is adequate to determine whether offshore waters comply with California's bacterial standards

Concentrations of enterococci were typically higher in shoreline samples than offshore, with the difference being nearly three-fold under dry conditions and 25% higher under wet conditions. For only one sample pair in dry weather and three sample pairs during wet weather, constituting less than 1% of total samples, did shoreline samples meet water quality standards when a corresponding offshore sample failed the standard. In contrast, there were 26 samples that failed standards on the shoreline but met standards offshore. It is unclear, though, whether this bias towards higher concentrations in the shoreline samples leads to a higher level of protection for surfers, as the frequency and magnitude of exposure for surfers is typically greater than for bathers.

VI. LITERATURE CITED

Clarke, L.B., D. Ackerman and J. Largier. 2007. Dye dispersion in the surf zone: measurements and simple models. *Continental Shelf Research* 27:650-669.

Francy, D.S., E.E. Bertke, D.P. Finnegan, C.M. Kephart, R.A. Sheets, J. Rhoades and L. Stumpe. 2006. Use of spatial sampling and microbial source-tracking tools for understanding fecal contamination at two Lake Erie beaches. U.S. Geological Survey Scientific Investigations Report 2006–5298. USGS. Columbus, OH.

Grant S.B., J.H. Kim, B.H. Jones, S.A. Jenkins, J. Wasyl and C. Cudaback. 2005. Surf zone entrainment, along-shore transport, and human health implications of pollution from tidal outlets. *Journal of Geophysical Research* 110, C10025, doi:10.1029/2004JC002401.

Griffith, J.F., L.A. Aumand, I.M. Lee, C.D. McGee, L.L. Othman, K.J. Ritter, K.O. Walker and S.B. Weisberg. 2006. Comparison and verification of bacterial water quality indicator measurement methods using ambient coastal water samples. *Environmental Monitoring and Assessment* 116:335-344.

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

Jiang, S. C., R. Noble, W. Chu. 2001. Human adenoviruses and coliphage in urban runoff-impacted coastal waters of southern California. *Applied and Environmental Microbiology*. 67: 179-184.

Kim, J.A., S.B. Grant, C.D. McGee, B.F. Sanders and J.L. Largier. 2004. Locating sources of surf zone pollution: A mass budget analysis of fecal indicator bacteria at Huntington Beach, California. *Environmental Science and Technology* 38:2626-2636.

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

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

Noble, Rachel T., S.B. Weisberg, M.K. Leecaster, C.D. McGee, J.H. Dorsey, P. Vainik and V. Orozco-Borbon. 2003. Storm effects on regional beach water quality along the southern California shoreline. *Journal of Water and Health* 1:23-31.

Rosenfeld, L.K., C.D. McGee, G.L. Robertson, M.A. Noble and B.H. Jones. 2006. Temporal and spatial variability of fecal indicator bacteria in the surf zone off Huntington Beach, CA. *Marine Environmental Research* 61:494-510.

Schiff, K.C., J. Morton and S.B. Weisberg. 2003. Retrospective evaluation of shoreline water quality along Santa Monica Bay beaches. *Marine Environmental Research* 56:245-253.

Schiff K., J. Dorsey, and S. Weisberg. 2001. Marine microbiological monitoring in the southern California Bight. *Environmental Management* 27:149-157.

Taggart, M. 2002. Oceanographic and Discharge Factors affecting Shoreline Fecal Bacteria Densities around Storm Drains and Freshwater Outlets at Marine Beaches. School of Public Health, University of California, Los Angeles. Los Angeles, CA.

Warrick, J.A., P. DiGiacomo, S.B. Weisberg, N. Nezlin, M. Mengel, B. Jones, C. Ohlmann, L. Washburn and E. Terrill. In press. River plume patterns and dynamics within the Southern California Bight. *Continental Shelf Research*.

Wymer, L.J., A.P. Dufour, K.P. Brenner, J.W. Martinson, W.R. Stutts and S.A.Schaub. 2005. The EMPACT Beaches Project; Results from a study on microbiological monitoring in recreational Waters. EPA 600/R-04/023. United States Environmental Protection Agency. Washington, D.C.

Table 1. Dry weather sampling sites. Check marks denote when samples where taken in relation to the tidal cycle.

	Ventura River	Santa Clara River	Malibu Creek	Ballona Creek	San Gabriel River	Santa Ana River	Talbert Marsh	Aliso Creek	San Elijo Creek	San Diego River	Kellogg Beach	Tijuana River
High Tide	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑
Low Tide	☑	☑	☑			☑	☑	☑		☑	☑	☑

Table 2. Wet weather sampling sites and dates sampled.

	Ballona Creek	Aliso Creek	Santa Ana River	Talbert Marsh	San Gabriel River	Kellogg Beach	San Elijo	San Diego River	Tijuana River
2/24/2004						☑	☑	☑	☑
2/27/2004	☑	☑	☑	☑	☑				
2/28/2004	☑	☑	☑	☑	☑	☑	☑	☑	☑
2/29/2004						☑		☑	☑
3/1/2004	☑	☑	☑	☑	☑		☑		
2/13/2005									☑
2/14/2005									☑
2/15/2005									☑
3/24/2005	☑		☑	☑	☑				
3/25/2005			☑	☑	☑				
3/26/2006	☑								

Table 3. Laboratories that participated in sample collection and analysis.

Sample Location	Analysis Laboratory
Ventura River	City of Oxnard, Ventura County Environmental Health
Santa Clara River	City of Oxnard, Ventura County Environmental Health
Malibu Creek	City of Los Angeles Environmental Monitoring Division
Ballona Creek	Loyola Marymount University
San Gabriel River	Orange County Sanitation District
Santa Ana River	Orange County Sanitation District
Talbert Marsh	Orange County Sanitation District
Aliso Creek	South Orange County Water Authority, County of Orange Public Health Laboratory
San Elijo Creek	Encina Waste Water Authority, San Elijo Joint Powers Authority*
San Diego River	Marine Environmental Consulting Analytical Systems Inc.
Kellogg Beach	Marine Environmental Consulting Analytical Systems Inc.
Tijuana River	City of San Diego

Table 4. Percent agreement regarding the California single-sample water quality standard of 104/100 ml for enterococci between samples taken simultaneously during dry weather at shoreline depth and in the surfzone where surfers line-up.

		Offshore	
		Above Standard	Above Standard
Shoreline	Below Standard	94.7%	0.9%
	Above Standard	4.4%	0.0%

Table 5. Percent agreement regarding the California single-sample water quality standard of 104/100 ml for enterococci between samples taken simultaneously during wet weather at shoreline depth and in the surfzone where surfers line-up.

		Offshore	
		Above Standard	Above Standard
Shoreline	Below Standard	54.9%	2.1%
	Above Standard	14.8%	28.2%

Figure 1. Map of sampling locations.

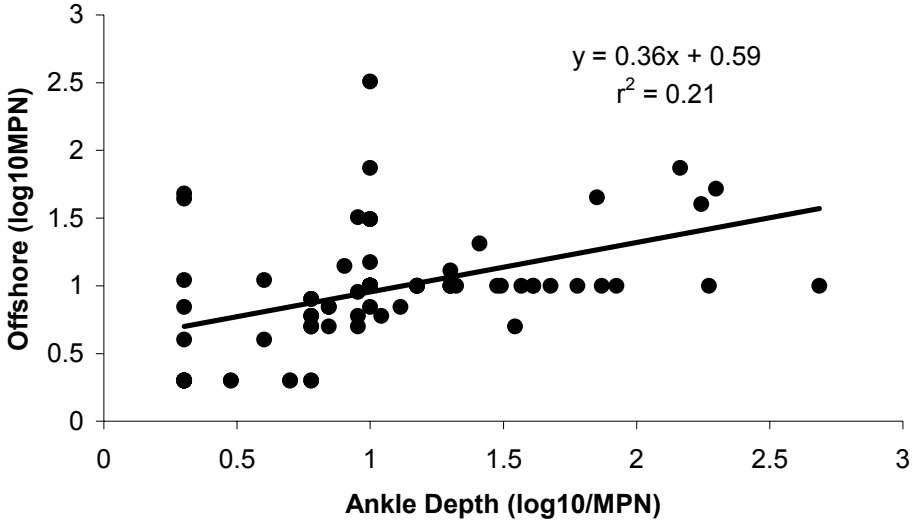


Figure 2. Regression plot for dry weather samples.

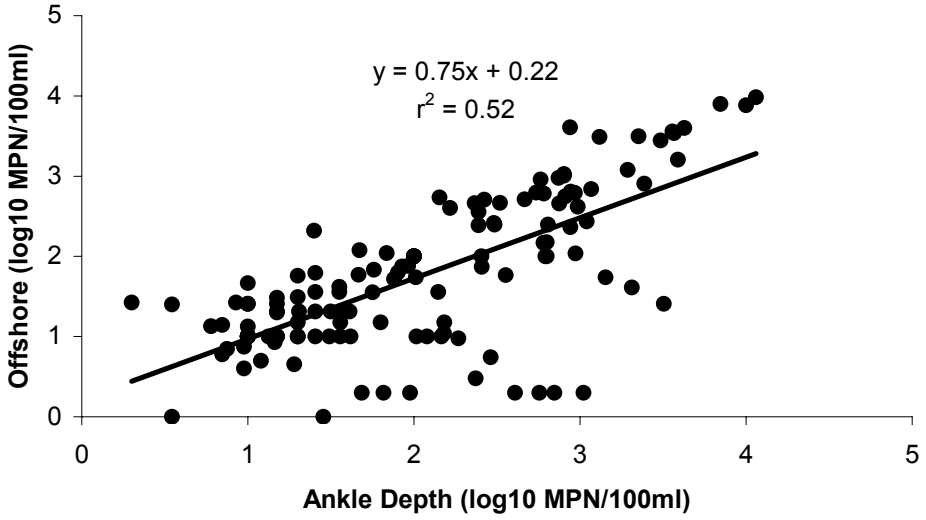


Figure 3. Regression plot for wet weather samples.

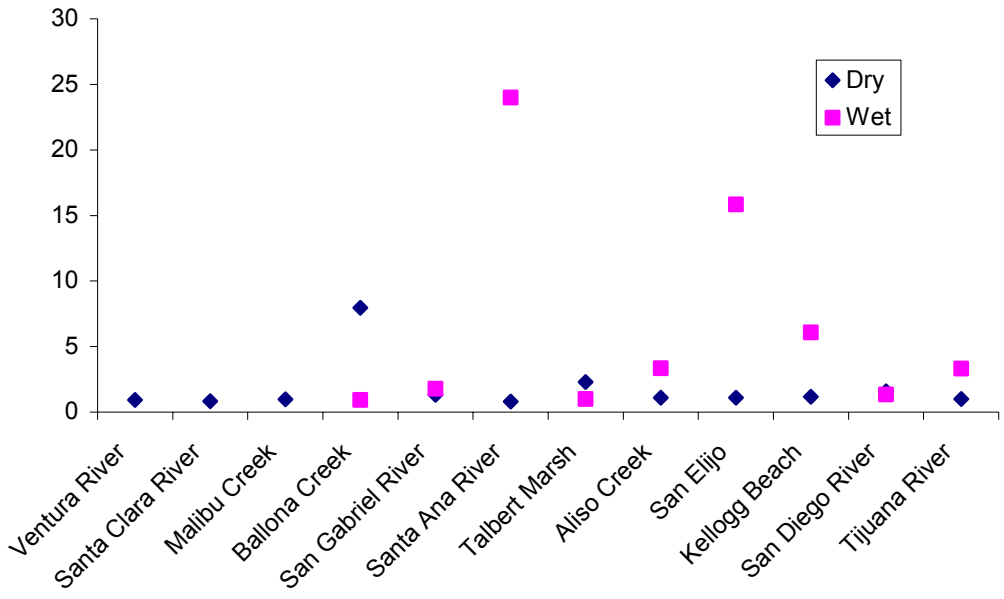


Figure 4. Ratio of average enterococci concentrations in shoreline samples relative to those offshore in wet and dry weather.

APPENDIX A: BIGHT '03 REGIONAL MONITORING PROGRAM PARTICIPANTS

AMEC Incorporated
Aquatic Bioassay and Consulting Laboratories (ABCL)
Channel Islands National Marine Sanctuary (CINMS)
Chevron USA Products Company
City of Long Beach
City of Los Angeles Environmental Monitoring Division (CLAEMD)
City of Oceanside
City of Oxnard
City of San Diego
City of Santa Barbara
City of Ventura
CRG Marine Laboratories
Encina Wastewater Authority
Granite Canyon Marine Pollution Studies Lab
Jet Propulsion Laboratory
Los Angeles Department of Water and Power (LADWP)
Los Angeles County Department of Beaches & Harbors
Los Angeles County Dept. of Health Services
Los Angeles County Dept. of Public Works
Los Angeles Regional Water Quality Control Board
Los Angeles County Sanitation Districts (LACSD)
Loyola Marymount University
Marine Biological Consultants
Minerals Management Service
NES Energy, Inc.
NRG Energy, Inc.
Orange County CoastKeeper
Orange County Environmental Health Division
Orange County Public Facilities and Resources (OCPFRD)
Orange County Sanitation District (OCSD)
Port of Long Beach
Port of Los Angeles
Port of San Diego
Reliant Corporation
San Diego Baykeeper
San Diego County Dept. of Environmental Health
San Diego Regional Water Quality Control Board (SDRWQCB)
San Elijo Joint Powers Authority
Santa Ana Regional Water Quality Control Board
Santa Barbara Health Care Services
Santa Monica Baykeeper
South Orange County Water Authority (SOCWA)
Southern California Coastal Water Research Project (SCCWRP)
Southern California Marine Institute (SCMI)
State Water Resources Control Board (SWRCB)
Surfrider Foundation
University of California, Los Angeles
University of California, Irvine
University of California, Riverside
University of California, San Diego
University of California, Santa Barbara
US EPA Region IX
US EPA Office of Research and Development
US Geological Survey
Vantuna Research Group
Ventura County Environmental Health Division
Ventura County Watershed Protection Division
Weston Solutions

APPENDIX B: COMPARISON AND VERIFICATION OF BACTERIAL WATER QUALITY INDICATOR MEASUREMENT METHODS

COMPARISON AND VERIFICATION OF BACTERIAL WATER QUALITY INDICATOR MEASUREMENT METHODS USING AMBIENT COASTAL WATER SAMPLES

John F. Griffith[1**], Larissa A. Aumand[2], Ioannice M. Lee[3], Charles D. McGee[4], Laila L. Othman[5], Kerry J. Ritter[1], Kathy O. Walker[6], and Stephen B. Weisberg[1]

[1]Southern California Coastal Water Research Project (SCCWRP), Westminster, California
[2]MEC Analytical Systems, Inc., Carlsbad, California
[3]City of Los Angeles, Department of Sanitation, Los Angeles, California
[4]Orange County Sanitation District, Fountain Valley, California
[5]City of San Diego, Metropolitan Waste Water Department, San Diego, California
[6]Los Angeles County Sanitation District, Whittier, California

ABSTRACT

More than 30 laboratories routinely monitor water along southern California's beaches for bacterial indicators of fecal contamination. Data from these efforts frequently are combined and compared even though three different methods (membrane filtration (MF), multiple tube fermentation (MTF), and chromogenic substrate (CS) methods) are used. To assess data comparability and quantify variability within method and across laboratories, 26 laboratories participated in an intercalibration exercise. Each laboratory processed three replicates from eight ambient water samples employing the method or methods they routinely use for water quality monitoring. Verification analyses also were conducted on a subset of wells from the CS analysis to confirm or exclude the presence of the target organism. Enterococci results were generally comparable across methods. Confirmation revealed a 9% false positive rate and a 4% false negative rate in the CS method for enterococci, though these errors were small in the context of within- and among-laboratory variability. Fecal coliforms also were comparable across all methods, though CS underestimated the other methods by about 10%, probably because it measures only *E. coli*, rather than the larger fecal coliform group measured by MF and MTF. CS overestimated total coliforms relative to the other methods by several fold and was found to have a 40% false positive rate in verification. Across-laboratory variability was small relative to within- and among-method variability, but only after data entry errors were corrected. One fifth of the laboratories committed data entry errors that were much larger than any method-related errors. These errors are particularly significant because these data were submitted in a test situation where laboratories were aware they would be under increased scrutiny. Under normal circumstances, it is unlikely that these errors would have been detected and managers would have been obliged to issue beach water quality warnings.

INTRODUCTION

Southern California's beaches are monitored extensively to screen for fecal contamination from human activities, such as wastewater discharges, industrial input, and surface runoff (Schiff *et al.* 2002). More than 30 groups are involved in this monitoring, including city and county health departments, treated wastewater dischargers, stormwater permittees, and non-profit environmental organizations. These groups all measure the same parameters enterococci (ENT), fecal coliforms (FC) and total coliforms (TC), but have the option of choosing from a number of different measurement methods. Wastewater dischargers primarily rely on membrane filtration (MF). Stormwater agencies and environmental groups primarily use the IDEXX® chromogenic substrate (CS) method. Health departments historically have relied on multiple-tube fermentation (MTF) and MF, but have begun to use CS more frequently in the last several years.

Data from these multiple providers are collated and used collectively in several ways. On a daily basis, they are used to assess beach water quality and as the basis for issuance of beach water quality warnings. On a long-term basis, they are integrated to identify chronically contaminated beaches for Section 303(d) listing under the federal Clean Water Act and for development of environmental report cards that compare water quality among locations and over time. Using these data interchangeably assumes that results

from multiple laboratories using different measurement methods are comparable, even though the laboratories may have varying levels of proficiency and may employ detection methods that rely on widely different products of bacterial growth.

A number of studies have compared the response of MF and MTF, and a few studies have compared these methods to CS (Kinzelman et al. 2003, Francy and Darner 2000, Abbott et al. 1998, Eckner 1998, Budnick et al. 1996, Palmer et al. 1993, Bej et al. 1991, Edberg et al. 1990, Covert et al. 1989). Noble et al. (2003a) was the first to compare results among all three methods and place differences among methods into the context of variability among laboratories that use the same method. However, Noble *et al.* used fabricated samples created primarily from laboratory strains of bacteria seeded into clean matrices. Natural ambient water samples often contain contaminants, particularly suspended solids, which have the potential to interfere with these methods. Natural samples also contain native bacteria, such as *Aeromonas*, *Vibrio, Pseudomonas*, and *Flavobacteria spp.*, which have been shown to produce positive reactions in substrates containing 4-methylumbelliferyl-β-glucuronide (MUG) and can lead to false positives in the CS test (Pisciotta et al. 2002, Landre et al. 1998, Davies et al. 1995, Hidalgo *et al.* 1977).

Here, we present an intercalibration study modeled after Noble et al. (2003a), but based on measurement of ambient water samples collected from sites known to have complex matrices. The study assessed comparability of results among 26 southern California laboratories that conduct routine bacterial monitoring using three bacterial indicator measurement methods and identified common causes of error in determining bacterial concentrations for water quality monitoring purposes. In addition, the study evaluated the reliability of CS methods through verification of target organisms. Reliability of the CS method is particularly important in southern California because results from this method are increasingly the basis for decisions regarding the issuance of beach water quality warnings.

Methods

Ambient water samples were collected from eight sites throughout southern California, including open marine beaches, estuaries, and flowing creeks carrying dry-weather urban runoff (Table 1). All samples were collected in sterile, 20-L carboys following Standard Methods 1060 protocol for aseptic sampling techniques (APHA 1995). Samples were then transported on ice to the Orange County Sanitation District (OCSD) laboratory in Fountain Valley, California. Upon arrival, carboys were placed on magnetic stir-plates, a sterile stir bar was added, and samples were stirred continuously for a minimum of 20 minutes to ensure homogeneity. Water from each carboy was dispensed into 26 sets of sterile, pre-labeled 100 ml bottles, which were transported on ice to participating laboratories. Sample processing began simultaneously at all laboratories at a pre-arranged time to eliminate differences due to holding time.

Samples were analyzed for TC, FC, *Escherichia coli* (EC), and ENT, using the method or methods routinely performed by each laboratory. Three classes of methods were used:

CS, MTF, and MF. Each laboratory analyzed multiple dilutions of each sample to minimize the number of samples occurring outside of a quantifiable range. All analyses were performed in triplicate.

Laboratories performing CS used IDEXX media and the Quanti-Tray/2000® system for all samples, following the manufacturer's instructions. Colilert®-18 media were used for enumeration of TC and EC, while Enterolert® media were used for ENT.

Multiple-tube fermentation for TC recovery used APHA 9221B (LTB/BGB). FC recovery was by either APHA Method 9221E.1 (EC) or APHA Method 9221E.2 (A-1). The ENT group was enumerated using the APHA Method 9230B (azide dextrose/PSE; APHA 1995).

Membrane filtration for TC recovery was by APHA Method 9222B (M-Endo), both single-step and enrichment-step procedures. FC enumeration using MF was by APHA Method 9222D (M-FC). The 24-h EPA Method 1600 (M-EI) procedure (APHA 1995) was used for ENT.

Five laboratories conducted confirmation testing on CS. When available, 10 positive wells were selected randomly for confirmation from trays with 80% or more positive wells. In certain instances, when a low number of positive wells was present, a smaller number of wells was selected from the tray. This resulted in confirmation testing for 71 ENT wells, 35 *E. coli* wells, and 153 TC wells. In addition, 55 non-fluorescing Enterolert® wells and 21 weakly fluorescing wells (scored as not containing ENT following manufacturer protocols) were subjected to verification analysis to test for false negatives.

Bacterial isolates for confirmation testing were obtained by wiping the back of the Quanti-Tray™ with 70% isopropanol, puncturing the well with either a sterile syringe or sterile scalpel, and withdrawing the well contents. TC bacteria were confirmed by transferring well contents to either Tryptic Soy (TSB) or Brain Heart Infusion (BHI) broths, incubating at 35ºC, transferring again to Brilliant Green Bile broth (BGB), and subsequently plating to MacConkey agar. *E. coli* was confirmed by transferring well contents to either EC or A-1 broths, incubating at 44.5ºC and plating to either MacConkey or LES Endo agars. ENT was confirmed by inoculating either TSB or BHI broth with well contents, incubating at 35ºC, then filtering the broth, plating to mEI agar and incubating at 41ºC. Secondary confirmations were performed on each isolate that tested positive using a second EPA- or APHA-approved biochemical testing method (MF or MTF, as described previously) or by submitting isolates to the Vitek microbial identification system (bioMérieux, Hazelwood, MO).

Statistical differences in median concentrations among methods and within-lab variability among methods were assessed using ANOVA on ranks (Conover and Iman 1981). Median values from replicate samples then were used to compute ranks across methods separately for each station. Where statistical differences among methods were detected ($p > 0.05$), individual stations were examined for possible station effects using

Bonferroni-adjusted significance levels. Within-lab variability among methods was examined by taking the standard deviations of log counts across replicates for each laboratory sample and then ranking them within station. Bonferroni-adjusted significance levels were employed to control overall error rates at 0.05 when testing for station effects.

Estimates of variability for each method, based on the median standard deviation of log counts and the average median log ratios of these counts, were used to estimate the reported bacterial concentrations at which one would be 95% or 99% confident that a single sample analyzed would fall above or below California's standards at which public health warnings are issued.

To assess the importance of individual laboratory error, data were examined to determine how well individual laboratory results agreed with the results from the entire group of laboratories. This was done by identifying the number of samples produced by each laboratory that were more than a half log unit above or below the overall median. This criterion was chosen because it is roughly equal to the average within-lab method variability observed in previous studies (Noble *et al.* 2003a).

RESULTS

Median concentrations of ENT exceeded California's single sample standard (104/100 ml) at five of the eight sample sites (Table 1). State standards for fCs (400/100 ml) and TCs (10,000/100 ml) were exceeded at two stations and one station, respectively.

There was no statistical difference in median concentration between MF and MTF for any of the bacterial indicators, though there was a difference between CS and MF for all three indicators (Table 2). For ENT, CS produced lower concentrations than MF, but most of the difference was attributable to a single station (Figure 1a). The median CS value at Doheny Beach was several-fold lower than that for either MF or MTF, but measured concentrations at that site were low for all methods. The median concentration was only 22/100 ml, with more than three-quarters of the CS values and almost half of the MF and MTF values reported as non-detectable values. When the Doheny Beach station data was removed from the analysis, there was no statistical difference among methods for ENT (Table 2).

The median CS values for FCs were similar to those produced by MTF, but about 30% lower than those produced using MF. Though CS methods are known to underestimate FC levels because they detect only *E.* coli, the majority of the difference was attributable to low bacterial concentrations at two stations, Doheny Beach and MBW10 (Figure 1b). Median concentrations at the Doheny Beach and MBW10 stations were only 20/100 ml and 41/100 ml, respectively. When these stations were removed from the calculation, the difference between results from CS and MF was only 9%.

For TC, median concentrations from CS were several-fold higher than from either MF or MTF. CS produced the highest median concentration among the three methods at five of the eight stations (Figure 1c).

Verification analyses of CS results revealed a large percentage of false positive wells for TC. Only 93 of 153 positive wells (61%) from IDEXX Quanti-Trays™ were confirmed to contain bacteria from the TC group, a finding consistent with the higher median concentrations measured using CS. In contrast, *E. coli* was successfully isolated from 100% of the positive wells tested.

For Enterolert, 67 of 71 (94.3%) fluorescing wells and 5 of 55 (9.1%) empty wells were confirmed to contain ENT. Only 3 of the 21 tested wells exhibiting weak fluorescence contained ENT.

MTF exhibited the highest within-method variability of the three methods across all three classes of bacterial indicators (Table 3). In contrast, MF exhibited the lowest variability among the three methods. When method variability was expressed in terms of a confidence interval surrounding existing California water quality standards, values from MTF measurements needed to be three times the numerical single sample standard to be 95% confident that the true number of indicator bacteria in the sample had exceeded the standard. For MF, a single measured value only needed to be 50% above the standard to be 95% confident that the true value exceeded the standard.

There appeared to be little effect associated with individual laboratories. Only two laboratories had more than one ENT sample for which the result differed by greater than a half log unit from the group median. One of those laboratories later was found to have a defective incubator that did not hold the proper temperature. For FC, two laboratories performing MF and one lab performing MTF reported values above the target range, but this reflected the comparatively large number of CS observations, which measure only *E. coli* and reduced the grand median. When comparisons were limited to within-method median, no results differed by more than half a log unit from the interlaboratory median. Comparisons were limited to within-method for TC because of CS bias for this indicator. In that comparison, no lab results differed by more than a half log unit from the group median.

DISCUSSION

All three methods produced similar results for ENT. Verifications confirmed both false positives and false negatives using CS, but both rates were small relative to the within-laboratory measurement variability. This is consistent with Fleischer (1990) and Noble *et al.* (2003a), but differs from Kinzelman *et al*. (2003), who found nearly 50% false positives for ENT verifications with CS. However, Kinzelman et al. suggested that their false positives occurred primarily for weakly fluorescing wells, which is consistent with our results that 86% of weakly fluorescing wells did not contain ENT. The CS manufacturer presently recommends that poorly fluorescing wells not be counted, which was the protocol used by laboratories in this study.

One shortcoming of our study was that we did not have the resources to perform confirmation testing on all three methods. The very nature of this study, in which laboratories donated their time and supplies, necessarily constrained confirmation tests to CS. Each of the methods has characteristics that may affect the reliability of results depending on the type of water sample analyzed and the number and type of cells in the water. For example, a recent study in southern California using ambient marine water samples found false-positive rates for ENT using MF similar to and in some cases greater than those observed for CS in our study (Ferguson *et al*. In Press).

The only large difference among methods observed was the severe overestimation of TC density using CS relative to the other two methods. The high rate of false positives likely results from interference by non-coliform organisms, such as *Aeromonas*, *Vibrio, Pseudomonas*, and *Flavobacteria spp.*, which are known to metabolize MUG (Pisciotta *et al*. 2002, Landre *et al*. 1998, Davies *et al.* 1995, Hidalgo *et al*. 1977). In practical terms, though, this overestimation seems to have little effect on beach warning systems in California, as the TC standard is so high that the standard is almost never exceeded without fCs or ENT also exceeding the standard (Noble *et al*. 2003b).

While such a systematic methodological error is of concern, we found that the largest source of error was attributable to data processing. These data entry errors occurred for samples that the laboratories were aware were part of an intercalibration exercise, in which their results would come under greater scrutiny. Prior to the data analysis presented in this article, preliminary screening indicated that results from four of the labs differed by an order of magnitude from those of other labs. Upon inspection of original laboratory data sheets, we discovered that these labs failed to correct for dilution before data submission (which we subsequently corrected before conducting the analysis in this article). We also found that a fifth lab misaligned the sample numbers on the bottle with their internal tracking numbers, leading to values being submitted with the wrong sample number (again confirmed by examination of the original laboratory data sheets and corrected before our data analysis). These labs produced comparable data to all other labs after correcting for data submission errors. However, in typical applications, data from other labs is not available for comparison. These errors would have gone undetected and errant results would have been reported to managers for use in regulatory or public health decisions. These data management errors were far larger and more prevalent than any variability introduced by method or commingling of data across laboratories.

ACKNOWLEDGEMENTS

The authors thank the many individuals and agencies that provided the time and resources to make this study possible.

LITERATURE CITED

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

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

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

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

Conover, W.J. and R.L. Iman. 1981. Rank Transform as a Bridge Between Parametric and Nonparametric Statistics. *The American Statistician* 35:125-141.

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

Davies, C.M., S.C. Apte, S.M. Peterson and J.L. Stauber. 1995. Possible interference of lactose-fermenting marine vibrios in coliform *B*-D-galactosidase assays. *Journal of Applied Bacteriology* 78:287-393.

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

Edberg, S.C., M.J. Allen, D.B. Smith and N.J. Kriz. 1990. Enumeration of total coliforms and *Escherichia coli* from source water by defined substrate technology. *Applied and Environmental Microbiology* 8:366-369.

Ferguson, D.M., D.F. Moore, M.A. Getrich and M.H. Zhowandai. In Press. Enumeration and speciation of enterococci found in marine and intertidal sediments and coastal water in southern California. *Journal of Applied Microbiology.*

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 bias. *International Journal of Epidemiology* 19:1100-1106.

Francy, D.S. and R.A. Darner. 2000. Comparison of methods for determining *Escherichia coli* concentrations in recreational waters. *Water Research* 24:2770-2778.

Hidalgo, C., J. Reyes and R. Goldschmidt. 1977. Induction and properties of B-galactosidase and B-galactosidase permease in Pseudomonas BAL-31. *Journal of Bacteriology* 129:821-829.

Kinzelman, J., C, Ng, E, Jackson, S. Gradus. and R. Bagley. 2003. Enterococci as indicators of Lake Michigan recreational water quality: Comparison of two methodologies and their impacts on public health regulatory events. *Applied and Environmental Microbiology* 69:92-96.

Landre, J.P., A.A. Gavriel and A.J. Lamb. 1998. False-positive coliform reaction mediated by *Aeromonas* in the Colilert defined substrate technology system. *Letters in Applied Microbiology* 26:352-354.

Noble, R.T., S.B. Weisberg, M.K. Leecaster, C.D. McGee, K. Ritter, K.O. Walker and P.M. Vainik. 2003a. Comparison of beach bacterial water quality indicator measurement methods. *Environmental Monitoring and Assessment* 81:301-312.

Noble, R.T., D.F. Moore, M. Leecaster, C.D. McGee and S.B. Weisberg. 2003b. Comparison of total coliforms, fecal coliforms, and enterococci bacterial indicator response for ocean recreational water quality testing. *Water Research* 37:1637-1643.

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

Pisciotta, J.M., D.F. Rath, P.A. Stanek, D.M. Flanery and V.J. Harwood. 2002. Marine bacteria cause false-positive results in the Colilert-18 rapid identification test for *Escherichia coli* in Florida waters. *Applied and Environmental Microbiology* 68:539-544.

Schiff, K.C., S.B. Weisberg and V.E. Raco-Rands. 2002. Inventory of ocean monitoring in the Southern California Bight. *Environmental Management* 29:871-876.

Table 1. Median concentrations of fecal indicator bacteria per 100 ml reported in each sample across all methods.

Location	Site Description	Enterococci	Fecal Coliforms *(E. coli)*	Total Coliforms
Ballona Wetlands	Estuary	80	130	2,400
Cabrillo Beach	Embayment	285	500	820
Doheny Beach	Open Beach	22	20	98
MBW 6	Urban Creek	130	100	12,033
MBW 10	Urban Creek	199	41	2,000
Malibu Beach	Open Beach	10	488	1,194
Malibu Lagoon	Estuary	122	300	5,191
Mission Bay	Embayment	120	285	800

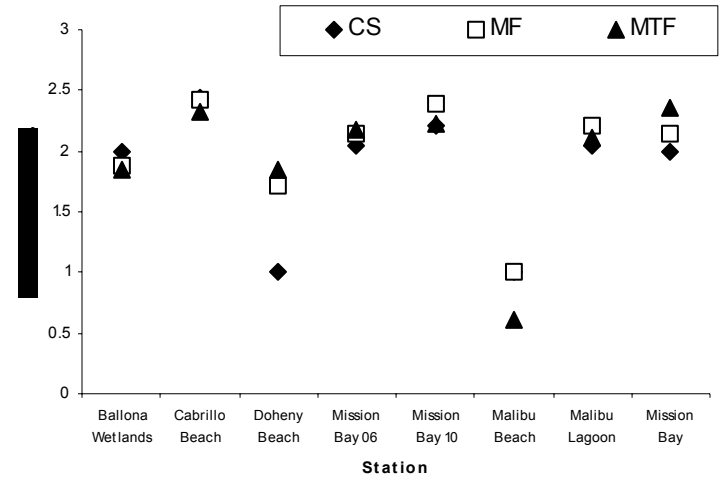
Table 2. Estimated median ratios of log counts between methods, for each indicator. Asterisk indicates statistically different than 1.

	Enterococci		**Fecal Coliform/*E.coli***		Total Coliform
Comparison	All Sites	Without Doheny	All Sites	Without Doheny or MBW10	All Sites
CS/MF	0.71*	0.86	0.69*	0.91	2.54*
CS/MTF	0.80	1.03	1.03	0.95	3.80*
MF/MTF	1.13	1.20	1.46	1.04	1.49

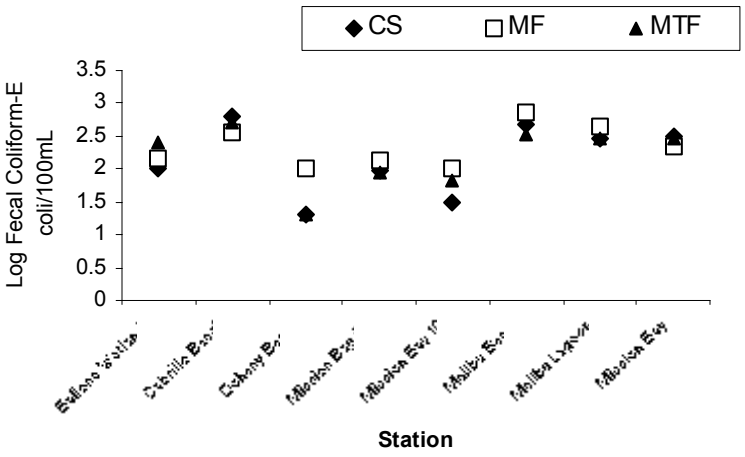
Table 3. Confidence intervals for each method applied to concentrations at California's single sample standards values.

Method	Enterococci (Std = 104)				Fecal Coliform/*E.coli* (Std = 400)				Total Coliform (Std = 10,000)			
	Above		Below		Above		Below		Above		Below	
	95%	99%	95%	99%	95%	99%	95%	99%	95%	99%	95%	99%
CS	166	217	65	50	624	809	256	197	14,955	18,880	5,297	6,681
MF	146	178	73	61	767	1,120	208	143	14,696	18,365	5,445	6,800
MTF	209	314	52	34	856	1,331	186	120	21,409	33,266	3,000	4,670

a)



b)



c)

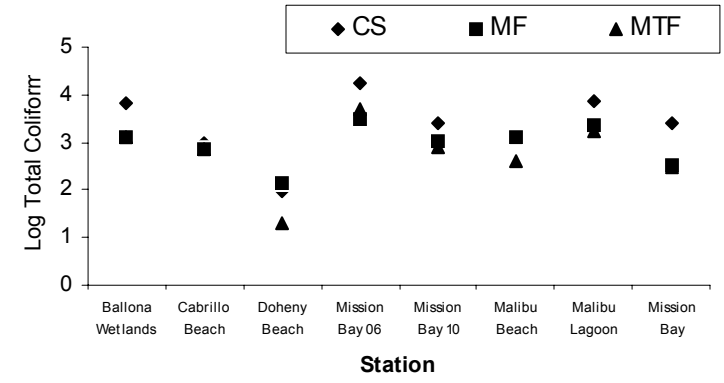


Figure 1. Median log counts vs. station for ENT (a), FC/*E.coli* (b), and TC (c).