Swimmer illness associated with marine water exposure and water quality indicators: Impact of widely used assumptions

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Abstract

Studies of health risks associated with recreational water exposure require investigators to make choices about water quality indicator averaging techniques, exposure definitions, follow-up periods, and model specifications; however, investigators seldom describe the impact of these choices on reported results. Our objectives were to report illness risk from swimming at a marine beach affected by nonpoint sources of urban runoff, measure associations between fecal indicator bacteria levels and subsequent illness among swimmers, and investigate the sensitivity of results to a range of exposure and outcome definitions. In 2009, we enrolled 5,674 people in a prospective cohort at Malibu Beach, a coastal marine beach in California, USA, with health symptoms measured daily for 10 to 19 days after enrollment. We analyzed concurrent water quality samples for indicator bacteria using culture and molecular methods. We compared illness risk between nonswimmers and swimmers, and among swimmers exposed to various levels of fecal indicator bacteria. We found diarrhea to be more common among swimmers than nonswimmers within three days of the beach visit, and sensitivity analyses demonstrated that overall inference was not substantially affected by the choice of exposure and outcome definitions. Our study suggests that the three days following a beach visit may be the most relevant period for health outcome measurement in recreational water studies. Although water quality conditions observed in this study were generally good, fecal indicator bacteria levels were not associated with swimmer illness.

Introduction

Water quality standards for fecal contamination in recreational waters focus on the measurement of fecal indicator bacteria, such as *Enterococcus*,

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which covary with pathogens in sources of human waste but are easier, cheaper, and faster to measure than the pathogens themselves (Griffith *et al.* 2009, USEPA 2012,). Studies that estimate the relationship between fecal indicator bacteria levels and subsequent illness among swimmers often rely on prospective cohort designs. Investigators have used similar designs at a variety of beaches (freshwater and marine), with various sources of pollution (well-defined "point sources," such as sewage treatment discharges, versus "nonpoint sources," such as urban or agricultural runoff), and using indicator bacteria detected by culture (e.g., *Enterococcus* Method 1600) or more rapid molecular methods (e.g., *Enterococcus* qPCR; (Haile *et al.* 1999; Wade *et al.* 2006, 2008, 2010; Colford *et al.* 2007, 2012; Heaney *et al.* 2009, 2012).

Cohort studies require investigators to make many decisions about how to define swimming-related exposure and health outcomes. Studies typically use a small set of exposure definitions and report a limited set of results. For example, investigators often assign daily average indicator bacteria levels to all swimmers at the beach on a given day (Haile *et al.* 1999; Wade *et al.* 2006, 2008, 2010; Colford *et al.* 2007). Cohort studies also follow participants for incident illness over a period of at least 10 days. This follow-up period is long enough to identify a large number of cases and capture relevant pathogen incubation periods, but short enough to avoid excessive problems with errors due to poor recall. Despite the importance of definition choices, only three previous studies have reported any alternative estimates, and then only in supporting materials (Wade *et al.* 2010, Colford *et al.* 2012, Heaney *et al.* 2012). To our knowledge, no study of infections to swimmers has systematically studied the effect of definition choices on the stability of risk estimates.

Our objectives were: 1) to measure the risk of illness that results from swimming at a marine beach affected by dry-weather runoff and nonpoint source contaminants; 2) to determine the association between fecal indicator bacteria, measured with culture and molecular methods, and subsequent illness among swimmers; and 3) to investigate the impact of outcome and exposure measurement choices on the results. We considered daily illness patterns among nonswimmers and swimmers after visiting the beach to determine the most sensitive risk periods. Subsequently, our findings present a simple stability analysis (Rosenbaum 1999) to assess whether parameter estimates calculated with the d*e facto* standard methodology are sufficiently robust to the choices that govern the presentation of primary results.

Methods

Beach Description

Malibu Surfrider State Beach is one of California's premier surfing and swimming beaches. The beach is located at the mouth of the 282 km^2 Malibu Creek watershed. The majority of its 90,000 residents have sewerage connections to the Tapia Water Reclamation facility (8 km from the beach), where wastewater is denitrified, filtered, and chlorine-disinfected. Some residents and commercial facilities, particularly in the lower watershed, use onsite septic and advanced treatment systems. The Tapia facility discharges about 10 million gallons per day of treated effluent into the watershed November through March. Between April and October, the facility does not discharge into the watershed unless the California Department of Fish and Game requests them to discharge water to preserve flows for steelhead fish; instead, the treated effluent is recycled and used for irrigation. There were no discharges during the study period. Currently, large reaches of Malibu Creek and Malibu Surfrider Beach are listed by the State of California and the US Environmental Protection Agency (USEPA) as impaired by bacterial contamination (California State Water Resources Control Board 2006).

Study Design

In a prospective cohort design, we enrolled beach visitors and measured reported daily health outcomes between 10 and 19 days later with a phone interview (median follow-up time [5th, 95th percentile] = 11 [10, 15] days). Interviewers approached beach visitors on 39 days between 23 May and 20 September 2009; 30 of these recruitment days were on weekends. Interviewers enrolled consenting households if they met these eligibility criteria: 1) at least one household member at the beach was aged ≥18 years; 2) home address was in the United States, Canada, or Mexico; 3) no previous study participation in the past 28 days; and 4) ability to speak English or Spanish. Our target enrollment for the summer was 5,000 based on past studies in California (Haile *et al.* 1999; Colford *et al.* 2007, 2012).

Interviewers gave participants an incentive (beach ball) and a short questionnaire to complete, then recorded the closest water-sampling site to each participant. The beach questionnaire asked about water exposure and about exposures or illness experienced in the previous three days. During a follow-up phone call, study staff conducted a 10-to-15-minute interview with each household to measure daily records of acute health outcomes, demographic information, swimming activity, and other exposures since the index beach visit.

Water Quality Sampling and Laboratory Analysis

We collected water quality samples at five sites along the beach; three of the sites were co-located with existing sites monitored by the Los Angeles County Department of Public Health and the City of Los Angeles, Environmental Monitoring Division (Figure 1, Sites A, B, E). On each recruitment day, the field team also collected water quality samples at 8:00 (same as Health Department) and at 13:00 (approximate time of maximum swimmer density). Sampling depth mimicked public monitoring methods: 0.5 m depth on an incoming wave.

We used water quality analyses that were identical to those used by Colford *et al.* (2012). We processed all samples for culture and defined-substrate technology methods immediately; filters for three qPCR methods were frozen for later analysis. We analyzed samples for culture-based fecal indicator bacteria: *Enterococcus* using EPA Method 1600 (USEPA 2006), fecal coliforms on the m-FC media, and total coliforms on m-Endo media (APHA 2009). We measured *Escherichia coli* using Colilert® (IDEXX; Westbrook, ME; APHA 2009). We measured *Enterococcus* using Enterolert® (IDEXX; Westbrook, ME; APHA 2009) and three qPCR methods. We used TaqMan and Scorpion-1 qPCR methods that targeted the same broad species range of the genus *Enterococcus*, but differed in their primer-probe chemistries and final calculation of quantitative results (Haugland *et al.* 2005, Noble *et al.* 2010). The third qPCR method, Scorpion-2, was identical to Scorpion-1 except that it included a primer-probe complex that amplified only *E. faecium* and *E. faecalis*, which are common *Enterococcus* species found in human fecal contamination (Layton *et al.* 2010). We recorded Taqman qPCR results as calibrator cell equivalents per 100 ml for both the delta-Ct and the delta-delta-Ct method (Layton *et*

Figure 1. Overview of the water quality sampling sites (A through E) in the Malibu Beach study, 2009. The surfers-only section of the beach is not open to nonsurfers.

al. 2010, Wade *et al.* 2010); we recorded Scorpion-1 and Scorpion-2 results in cell equivalents per 100 ml using the delta-Ct method (Noble *et al.* 2010).

Swim Exposure Definitions

Individual water exposure was measured using self-reported water activity. Consistent with prior studies (Wade *et al.* 2006, 2008, 2010; Colford *et al.* 2007, 2012), we created a graded classification scheme based on the person's minimum exposure: 1) any water contact, 2) body immersion, 3) head immersion, and 4) swallowed water. We defined body immersion as water contact above the waist, head immersion as head below the water line, and swallowed water as ingestion of any ocean water; those who reported no contact with ocean water were classified as nonswimmers.

We measured water quality exposure by combining swimmer exposure with indicator bacteria levels. We considered nine methods for averaging and assigning indicators (Figure 2). Consistent with two companion studies at Doheny (Colford *et al.* 2012) and Avalon beaches, we used a site-specific daily-average method as our primary exposure definition (Figure 2, Method 5).

We measured two primary outcomes: 1) diarrhea defined as 3 or more loose or watery stools in 24 hours (Baqui *et al.* 1991); and 2) gastrointestinal illness, defined as diarrhea, vomiting, nausea, and stomach cramps; nausea and missed daily activities due to gastrointestinal illness; or stomach cramps and missed daily activities due to gastrointestinal illness (Wade *et al.* 2008, 2010; Heaney *et al.* 2009, 2012; Colford *et al.* 2012). We measured cumulative incident illness in the 10 days following the beach visit (the period for which we had complete follow-up for all participants); we excluded anyone with illness at enrollment. In addition to our primary outcomes, we measured skin rash, eye infection, earache, fever, urinary tract infections, and upper respiratory illness (defined as any two of the following: sore throat, cough, runny nose, common cold, or fever; Wade *et al.* 2008, 2010; Heaney *et al.* 2009; Colford *et al.* 2012).

Descriptive Analysis of Illness

In our primary analysis, we examined incident diarrhea and gastrointestinal illness in the 10 days following the beach visit. Three recently published studies have suggested swimmer illness is elevated in the two to three days following the beach visit (Soller, 2010, Colford *et al.* 2012, Dorevitch *et al.* 2012,). We complemented our primary analysis with a descriptive analysis of time-to-illness onset and daily incidence to identify whether swimmers and nonswimmers had different illness patterns, and, if so, to identify the most relevant period over which to measure swimming exposure risk and associations between indicator levels and illness. The descriptive analysis was not pre-specified. We defined time-to-illness as the number of days between the beach visit and the first symptom, and compared time-to-illness distributions among nonswimmers and swimmers. We calculated the differences and 95% confidence intervals (CIs) in daily incidence between nonswimmers and the graded water-exposure definitions by bootstrapping the dataset (re-sampling observations clustered at the household level) and recalculating differences for each day of follow-up.

We considered nine indicator averaging and assignment methods:

1. Daily average
2. Morning average
3. Afternoon average
4. Daily maximum
5. Site-specific daily average
6. Site-specific morning average
7. Site-specific afternoon average
8. Site-specific daily maximum
9. Site- and time-specific average

Averages were calculated as the geometric mean across samples. Methods 1 through 4 summarize indicator values used for all sampling points at the beach for each day. The indicator value was then matched to all swimmers at the beach on that day. Morning averages include samples collected at 8:00 a.m., and afternoon averages include samples collected at 1:00 p.m. Site-specific methods 5 through 8 summarize indicator values used for each sampling site, then match site-specific indicator levels to respective swimmers based on each swimmer's swim location. Method 9 matched indicator levels to respective swimmers based on sampling points and times closest to each swim event. If a swimmer reported swimming around both sampling times (8:00 a.m. and 1:00 p.m.), the swim event was assigned the average of the indicator levels for each sampling time.

Figure 2. Water quality indicator averaging and assignment methods.

Illness Risk Associated with Swimming and Indicator Bacteria Levels

In swim exposure analyses (comparing swimmers to nonswimmers), our parameter of interest was the relative risk associated with exposure to ocean water. The comparison group for these analyses was nonswimmers. In analyzing the association between indicator bacteria levels and swimmer illness, our parameter of interest was the relative risk associated with a $\log_{10}$ increase in indicator levels among swimmers with a defined level of water contact (body immersion, head immersion, swallowed water). We estimated the relative risk of illness due to swim exposure using the odds ratio (OR) calculated with logistic regression. The Supplemental Information (SI; ftp://ftp.sccwrp.org/pub/download/DOCUMENTS/AnnualReports/2013AnnualReport/ar13_611_624SI.pdf) includes details of the regression models used to estimate the parameters of interest.

Stability Analysis for Indicator Bacteria Levels and Swimmer Illness

We evaluated the stability of our results to the method of indicator assignment (Figure 2), definition of swim exposure (body immersion, head immersion, swallowed water), length of follow-up (10 days, 3 days) and model (unadjusted, adjusted) by repeating the analysis for every combination of these analytic choices. This resulted in 9 x 3 x 2 x 2 = 108 OR estimates for each indicator. We used kernel density distribution plots and forest plots to qualitatively assess the sensitivity of parameter estimates to choice of analysis method.

Results

Study Population

Field interviewers approached 7,231 households; 1,362 did not meet study eligibility criteria. Of the eligible households, 3,469 consented to participate and completed the beach interview, and 2,713 (78%) completed the phone interview. Participants who completed the interview had similar demographics to those lost to follow-up (Table 1). Of the 5,674 participants in the study, 5,091 (90%) were recruited on a Friday, Saturday, or Sunday. Swimmers were more likely to be male and younger than nonswimmers, but all groups had similar ethnicity and household income. Of 2,559 body immersion swimmers, we excluded 83 from the analyses that required site-specific exposure assignment because we had not obtained swim locations for them.

Water Quality

Enterococcus concentrations measured by EPA Method 1600 (USEPA 2006) ranged from <1 to 1,740 colony forming units (CFU)/100 ml in the ocean locations (Table 2). Of the 2,559 body immersion swimmers, 99% swam at Sites A, B, D, and E. *Enterococcus* EPA Method 1600 exceeded (failed to meet) state water quality objectives in 7% (>35 CFU/100 ml) and 4% (>104 CFU/100 ml) of the samples at these four sites; 95% of the samples collected from Site C (in the lagoon) exceeded 104 CFU/100 ml (Figure 1). The exceedance percentages during this study were lower than historic water quality collected by Los Angeles County and City agencies from late May to September between 2004 and 2008. Historic exceedance percentages at Sites A, B, and E were 6.2% (A), 9.1% (B), and 9.7% (E) for *Enterococcus* >104 CFU/100 ml and 12.4% (A), 26.9% (B), and 14.0% (E) for any of the three indicators: total coliforms >10,000 CFU/100 ml, fecal coliforms >400 CFU/100 ml, and *Enterococcus* >104 CFU/100 ml (LACDPH 2012, City of Los Angeles 2012). Water quality worsened when the sand berm blocking the lagoon mouth was open and its contents flowed directly into the ocean (Figure SI-1). At Sites A and B (immediately up- and down-current from the Malibu Lagoon mouth), all samples that exceeded water quality standard occurred with an open berm.

Illness Onset during Follow-Up

Time to diarrhea and gastrointestinal illness was shorter for swimmers compared with nonswimmers (Table SI-1, Figure SI-2). Swimmers had elevated incidence of diarrhea and gastrointestinal illness on days 1 to 3 following the beach visit. By days 3 to 4, diarrhea and gastrointestinal illness incidence among swimmers returned to the nonswimmer level (Figure 3). Other symptoms of gastrointestinal illness (nausea, stomach cramps, vomiting), earache, and skin rash also had shorter time to onset and higher incidence among swimmers compared with nonswimmers (Figures SI-3 and SI-4). We therefore repeated the analyses using cumulative incident illness during a 3-day follow-up, expecting to observe stronger associations between water exposure or indicator levels and illness in the 3-day follow-up than in the 10-day follow-up used in the primary analysis.

Table 1. Cohort demographic characteristics in the Malibu Beach study, 2009. N = number of households; households are considered to be in a swim exposure group if any household member was exposed at that level.

	Follow-up Status		Swim Exposure			
	Lost to Follow-up	Completed Follow-up	Nonswimmers	Body Immersion	Head Immersion	Swallowed Water
Number of Subjects						
Persons	1580	5674	1895	2559	1849	571
Households	752	2713	722	1474	1166	410
Individual Characteristics						
Age (years); %						
0-5	11	12	8	12	9	17
5.1-10	11	9	2	17	17	22
10.1-20	14	14	9	19	21	17
20.1-30	23	18	22	17	17	16
30.1-40	22	19	23	15	16	14
40.1-50	13	16	20	12	12	8
>50	7	11	16	8	8	5
Missing	0	0	0	0	0	0
Sex; %						
Male	48	46	36	56	62	59
Female	52	54	64	44	38	41
Racial and ethnic group; %						
White	48	60	60	58	60	58
White, Hispanic	2	5	5	5	5	4
Non-White, Hispanic	7	14	13	16	17	14
Black	9	2	2	2	1	3
Asian	34	7	9	5	4	6
Indian	0	0	0	1	0	0
Multiple Races	0	4	3	4	4	5
Other	0	4	4	4	3	4
Missing	0	4	3	5	5	6
Household Characteristics						
Household Income USD; %						
<10,000 - 25,000		13	15	13	13	12
25,001 - 50,000		15	15	14	14	13
50,001 - 75,000		13	14	13	12	13
75,001 - 100,000		12	12	13	13	15
100,001 - 150,000		14	12	15	15	14
>150,000		17	15	18	19	19
Missing	100	15	18	14	14	12

Illness Risk Associated with Swimming at Malibu Beach

Swimmers had consistently elevated risk of diarrhea and gastrointestinal illness over the 10-day follow-up period; when we restricted the follow-up period to 3 days, associations were stronger between swimming exposure and both diarrhea and gastrointestinal illness (Table 3). Within three days of the beach visit, the adjusted OR (aOR) for swimmers with body immersion versus nonswimmers was 1.88 (95% CI = 1.09 - 3.24) for diarrhea and 1.90 (95% CI = 1.17 - 3.09) for gastrointestinal illness (Table 3). The strength of association between swimming exposure and diarrhea and gastrointestinal illness declined with longer follow-up periods (Figure SI-5). Swimmers had elevated risk for illnesses other

Table 2. Fecal indicator bacteria levels measured in the Malibu Beach study, 2009, at sample sites A, B, D, and E; the table excludes samples from Site C (in the lagoon) because only 1% of swimmers swam at site C. N = number of samples; differences in number of samples is due to some indicators being sampled only in the morning. CFU = Colony Forming Units per 100 ml; CCE = Calibrator Cell Equivalents per 100 ml for the delta CCE_{Δ} and delta-delta $CCE_{\Delta\Delta}$ CT calculations; qPCR = quantitative real time polymerase chain reaction; and MF = membrane filtration.

Indicator	Reporting Unit	N	Min	Max	Geometric Mean	Non-detects
Enterococcus (EPA 1600)	CFU	307	0.5	1,740	3	69
Enterococcus (Enterolert)	CFU	307	5	1,354	9	182
Enterococcus (Taqman qPCR)	$CCE_{\Delta\Delta}$	307	0.2	2,829	17	39
Enterococcus (Taqman qPCR)	CCE_{Δ}	307	0.2	2,001	13	39
Enterococcus (Scorpion-1 qPCR)	CCE_{Δ}	228	2.5	25,064	31	59
Enterococcus (Scorpion-2 qPCR)	CCE_{Δ}	228	2.5	15,778	10	166
E. coli (Colilert)	CFU	156	0.5	1,000	13	10
Fecal Coliform (MF)	CFU	303	0.5	1,000	5	66
Total Coliform (MF)	CFU	303	0.5	1,550	25	22

than diarrhea and gastrointestinal illness, including earache, vomiting, and fever (Table SI-2).

Illness Risk Associated with Indicator Bacteria Levels among Swimmers

There were no consistent associations between indicator bacteria levels and diarrhea or gastrointestinal illness among swimmers with body immersion (Table 4) or head immersion (Table SI-3). The use of a restricted 3-day follow-up period did not strengthen the associations (Table 4). Log_{10} increases of *Enterococcus* measured by qPCR were associated with increased risk of diarrhea among swimmers who swallowed water (Table SI-4), but the findings were not consistent across other culture and qPCR methods. We found no evidence for effect modification of the association between indicator bacteria levels and illness by berm status (Table SI-5).

Stability Analysis

Odds ratios were sensitive to analytic choices (typical OR range = 0.5 to 2.0). Point estimates from the primary analysis were at or near the center of the OR distributions for diarrhea and gastrointestinal illness (Figures SI-6 and SI-7), with confidence intervals that covered the mass of the OR distributions estimated in the stability analysis. Forest plots of the estimated ORs for all analysis combinations showed that only total coliforms consistently had

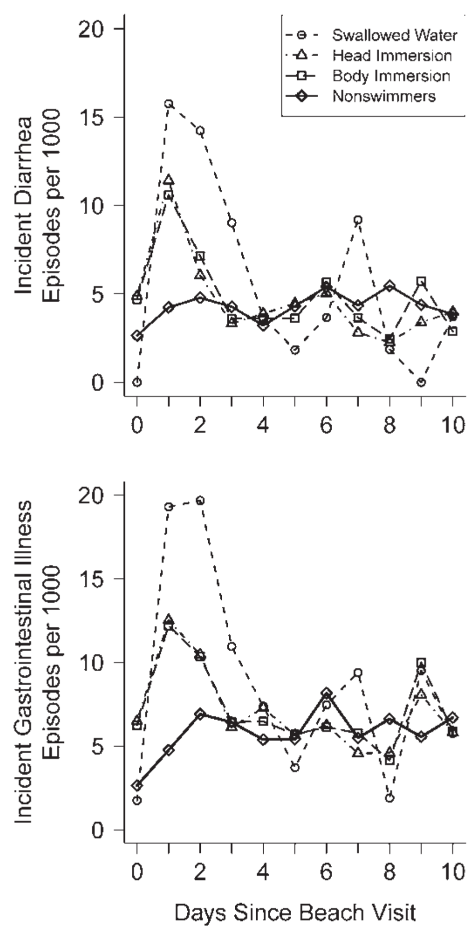

Figure 3. Daily incident episodes of diarrhea (top) and gastrointestinal illness (bottom) by swim exposure definition in the 10 days following the beach visit in the Malibu Beach study, 2009. Incidence calculations exclude individuals who had gastrointestinal illness at enrollment.

Table 3. Incident diarrhea and gastrointestinal illness among swim-exposure groups over two follow-up periods in the Malibu Beach study, 2009. N = number of participants, excluding those with gastrointestinal symptoms at baseline. aOR = adjusted odds ratio, adjusted for age, sex, race, length of follow-up >12 days, swimming on multiple days, allergies, contact with animals, contact with other sick people, frequency of beach visits, digging in the sand, and consumption of raw or undercooked eggs or meat; and CI = confidence interval.

Illness/Swim Exposure	N	Complete Follow-up (10 days)		Restricted Follow-up (3 days)	
		% Ill	aOR (95% CI)	% Ill	aOR (95% CI)
Diarrhea					
Nonswimmers*	1,851	4.21	1.00	1.35	1.00
Body Immersion	2,502	4.92	1.14 (0.80 - 1.61)	2.40	1.88 (1.09 - 3.24)
Head Immersion	1,805	4.65	1.09 (0.75 - 1.58)	2.27	1.78 (1.02 - 3.11)
Swallowed Water	551	5.44	1.25 (0.77 - 2.02)	3.45	2.76 (1.48 - 5.16)
Gastrointestinal Illness**					
Nonswimmers*	1,851	5.89	1.00	1.84	1.00
Body Immersion	2,502	7.35	1.14 (0.85 - 1.54)	3.32	1.90 (1.17 - 3.09)
Head Immersion	1,805	7.15	1.14 (0.83 - 1.56)	3.27	1.91 (1.17 - 3.14)
Swallowed Water	551	8.53	1.31 (0.89 - 1.94)	4.72	2.86 (1.64 - 4.97)

* Reference category.
** As defined in text.

a positive association (OR >1.0) with diarrhea and gastrointestinal illness (Figures SI-8 and SI-9).

Discussion

In this prospective cohort study of 5,674 beachgoers at an urban runoff and nonpoint source contaminated marine beach, swimmers were more likely than nonswimmers to develop diarrhea and gastrointestinal illness; illness risk was greatest in the first 2 to 3 days following the beach visit (Figure 3). Shortening the follow-up period from 10 days to 3 days increased the strength of association between swimming exposure to marine water and subsequent illness (Table 3), but did not increase the strength of association between indicators and swimmer illness (Table 4), under conditions of relatively good water quality (Table 2). Accounting for the berm status of Malibu Creek as a potential effect modifier did not improve the association between indicators and swimmer illness (Table SI-5). The distributions of OR estimates for most indicators were sensitive to analytic choices, but the primary analysis was representative of the broad set of possible results.

Indicator bacteria levels were relatively low during the study period, and beach managers attempted to prevent water contact near Malibu creek (the area with highest contamination levels). Maximum *Enterococcus* concentrations using the EPA 1600 Method exceeded 104 CFU/100 ml on 7 of 39 sampling days. Only 30 swimmers were exposed to *Enterococcus* levels >104 CFU/100 ml based on site-specific averages of EPA 1600 (Figure 2, Method 5); as a point of comparison, 857 swimmers had been exposed to those levels in the 1995 Santa Monica Bay study (Haile *et al*. 1996, Griffith *et al.* 2009). Large variation in indicator bacteria levels would improve the ability to estimate the relationship between a range of indicator levels and illness. It is more difficult to estimate indicator-health associations under conditions similar to those in this study, where indicator levels were generally low. Nevertheless, the indicator levels observed during this study were likely representative of water quality conditions at many beaches in California and the US with nonpoint source contamination.

We asked most swimmers about their illness onset at 10 to 12 days following the beach visit. This long recall period could lead to reporting errors early in the follow-up period. If swimmers misplaced illness days closer to the beach visit differentially compared with nonswimmers, measurement error alone could create the elevated swimmer illness in the three days following the beach visit. However, we do not think differential measurement error led to

Table 4. Association between fecal indicator bacteria and illness for body immersion swimmers in the Malibu beach study, 2009. N = number of participants, excluding those with gastrointestinal symptoms at baseline; aOR = adjusted odds ratio, adjusted for age, sex, race, length of follow-up >12 days, swimming on multiple days, allergies, contact with animals, contact with other sick people, frequency of beach visits, digging in the sand, and consumption of raw or undercooked eggs or meat; CI = confidence interval; qPCR = quantitative real time polymerase chain reaction; and MF = membrane filtration.

Illness/Indicator	N	Complete Follow-up (10 days)		Restricted Follow-up (3 days)	
		aOR	(95% CI)	aOR	(95% CI)
Diarrhea					
Enterococcus (EPA 1600)	2,476	0.74	(0.52 - 1.06)	0.64	(0.38 - 1.07)
Enterococcus (Enterolert)	2,476	0.60	(0.31 - 1.17)	0.49	(0.21 - 1.16)
Enterococcus (Taqman ΔΔ qPCR)	2,476	0.97	(0.74 - 1.25)	0.88	(0.62 - 1.26)
Enterococcus (Taqman Δ qPCR)	2,476	0.96	(0.73 - 1.27)	0.87	(0.59 - 1.28)
Enterococcus (Scorpion-1 qPCR)	2,229	0.88	(0.66 - 1.18)	0.88	(0.58 - 1.33)
Enterococcus (Scorpion-2 qPCR)	2,229	0.86	(0.69 - 1.06)	0.81	(0.59 - 1.12)
E. coli (Colilert)	2,476	0.78	(0.60 - 1.02)	0.70	(0.48 - 1.02)
Fecal Coliform (MF)	2,475	0.97	(0.69 - 1.36)	0.89	(0.54 - 1.47)
Total Coliform (MF)	2,475	1.02	(0.77 - 1.35)	1.22	(0.85 - 1.74)
Gastrointestinal Illness*					
Enterococcus (EPA 1600)	2,476	0.94	(0.70 - 1.25)	0.90	(0.57 - 1.41)
Enterococcus (Enterolert)	2,476	0.84	(0.50 - 1.42)	1.05	(0.55 - 2.01)
Enterococcus (Taqman ΔΔ qPCR)	2,476	1.03	(0.82 - 1.29)	0.93	(0.67 - 1.27)
Enterococcus (Taqman Δ qPCR)	2,476	1.05	(0.83 - 1.34)	0.94	(0.67 - 1.31)
Enterococcus (Scorpion-1 qPCR)	2,229	0.98	(0.78 - 1.24)	0.89	(0.63 - 1.25)
Enterococcus (Scorpion-2 qPCR)	2,229	0.84	(0.70 - 1.00)	0.79	(0.59 - 1.05)
E. coli (Colilert)	2,476	0.90	(0.73 - 1.11)	0.89	(0.65 - 1.23)
Fecal Coliform (MF)	2,475	1.02	(0.77 - 1.35)	1.05	(0.70 - 1.57)
Total Coliform (MF)	2,475	0.98	(0.78 - 1.23)	1.14	(0.84 - 1.55)

* As defined in text.

the observed pattern. First, if swimmers misplaced illness days to earlier in the follow-up period, we would expect their incidence to drop below the level of nonswimmers by Day 10; this did not occur (Figure 3). Second, Dorevitch *et al.* (2012) also observed elevated swimmer diarrhea in the three days following water contact with repeated measurements on Days 2, 5, and 21; their frequent measurements would reduce the chance for reporting errors. Third, we observed a similar pattern for multiple measures of gastrointestinal illness as well as skin rash and earache – all symptoms that we would expect *a priori* to be affected by contact with marine water – which provides internal consistency for the observed patterns. Future recreational water studies could potentially be improved by objective outcome measures, such as the presence of specific pathogens in stool, serological, or salivary specimens of ill swimmers. Studies using self-reported measures should include negative control outcomes to help detect possible reporting bias (Lipsitch *et al.* 2010).

Recent studies of recreational fresh- and marine-water exposure have consistently reported elevated illness among swimmers compared with nonswimmers following a beach visit (Wade *et al.* 2006, 2008, 2010; Colford *et al.* 2007, 2012). These studies have typically used 10 to 14 days as a follow-up period. We found that excess diarrhea, gastrointestinal illness, rash, and earaches among swimmers occurred in the first three days following the beach visit, an observation documented in three recent studies that examined daily illness patterns (Dorevitch *et al.* 2012, Soller *et al.* 2010, Colford *et al.* 2012). This short incubation period following water exposure suggests enteric viruses may be the primary etiologic agents causing swimmer illness, in contrast with typically longer incubation periods required by protozoan infections such as *Cryptosporidium* or *Giardia* (Kay *et al.* 1994, Soller *et al.* 2010). Pathogen-specific outcome measures would be required to make conclusive claims about etiology. Since exposure likely differs across beaches, future studies should measure and report daily illness patterns following the beach visit to determine whether the observed pattern occurs in other settings.

Our use of longer follow-up periods (10 - 14 days) could in some cases under-estimate the swimming associated risk by averaging over a risk period that is partially irrelevant to the exposure of interest. Indeed, we observed this dilution effect of the OR associated with swim exposure as the length of follow-up period increased (Figure SI-5). We hypothesized that the use of a shorter follow-up period might enhance the association between indicator levels and swimmer illness, but found no support for this hypothesis; indicator levels were not associated with swimmer illness under any swim exposure definition or follow-up period (Table 4). The associations we observed between *Enterococcus* measured by qPCR and diarrhea in swimmers who swallowed water (Table SI-4) were broadly consistent with those observed previously (Wade *et al.* 2006, 2008, 2010; Colford *et al.* 2007, 2012), but due to multiple comparisons these results must be interpreted with caution. The lack of association between indicator levels and swimmer illness despite increased illness incidence among swimmers in the days following the beach visit has several possible interpretations: either the excess risk is not associated with swimming (e.g., outcome reporting bias, as discussed above); the excess risk results from other exposures in the water (e.g., swallowing salt water); random errors in assigning exposure biased the associations toward the null (Fleisher 1990, Hutcheon *et al.* 2010); or the indicator bacteria used in this analysis are an inadequate characterization of water quality risk at this beach. Bacteriophages were positively associated with swimmer illness at a beach with nonpoint sources, despite no association between *Enterococcus* and illness (Colford *et al.* 2007). There may be better-performing indicators of health risk for beaches with nonpoint source pollution than the fecal indicator bacteria considered in this study.

Our finding of no association between indicator levels and swimmer illness is inconsistent with the report by Colford *et al.* (2012), who found strong indicator-health associations at Doheny Beach, California when a stream flowed freely into marine waters; our findings are also inconsistent with beach studies of point-source treated wastewater discharge (Wade *et al.* 2006, 2008, 2010). Our study was located at the same beach as a study conducted in 1995 (Haile *et al.* 1990, 1996), but water quality conditions differed. In 1995, there was an upstream filtered and disinfected sewage discharge, poorer beach water quality, and 2,998 participants who swam within 100 meters of the creek. In 2009, swimmers were less exposed to contaminated water, with no upstream sewage discharges and a restriction that prohibited swimming near the creek (Figure 1). Most swimmers in this study swam ≥400 meters from the creek, and so the sand berm and stream flow likely had less impact on swimmer exposure than in the 1995 study (Haile *et al.* 1990, 1996) and the Doheny beach study (Colford *et al.* 2012). For this reason, conditions may have been more similar to a nonpoint-source beach with more diffuse sources, where indicator bacteria levels have not been associated with gastrointestinal illness (Colford *et al.* 2007, Fleisher *et al.* 2010).

We report a large set of analyses that explore the associations among water exposure, indicator bacteria levels, and swimmer illness. Part of the challenge of interpreting such large sets of results is that the choice of exposure assignment and follow-up period is not standardized in the field. Our stability analysis showed that the distributions of OR estimates can be sensitive to the choice of exposure definition, outcome measurement, and model specification, but that the scenario in the pre-specified, primary analysis was representative of the range of results obtained across all scenarios (Figures SI-6 through SI-9). This observation reinforced our conclusion of no relationship between indicator levels and swimmer illness in this study; it also suggests that similar studies can pre-specify a small set of comparisons to be included in the primary analysis and obtain representative results.

In summary, we found that swimmers with body immersion, head immersion, or who swallowed water were at higher risk of contracting diarrhea, gastrointestinal illness, skin rash, and earache compared to nonswimmers, and that the excess incidence occurred in the three days following the beach visit. Despite more illness among swimmers, we found no consistent association between fecal indicator bacteria measured with both culture and molecular methods and swimmer illness under conditions of relatively good water quality. Our results suggest that the use of longer follow-up periods that are traditionally used in these recreational water studies (10 - 14 days) could in some cases underestimate the swimming associated risk by averaging over a risk period that is partially irrelevant to the exposure of interest. Our findings were stable across a wide range of assumptions typically used in the analysis of similar studies.

Literature Cited

American Public Health Association (APHA). 2009. Standard Methods for the Examination of Water and Wastewater. Washington, DC.

Baqui, A.H., R.E. Black, M. Yunus, A.R. Hoque, H.R. Chowdhury and R.B. Sack. 1991. Methodological issues in diarrhoeal diseases epidemiology: Definition of diarrhoeal episodes. *International Journal of Epidemiol*ogy 20:1057-1063.

California State Water Resources Control Board. 2006. California's 2006 Clean Water Act Section 303(d) List of Water Quality Limited Segments. http://www.waterboards.ca.gov/water_issues/programs/tmdl/docs/303dlists2006/epa/r4_06_303d_reqtmdls.pdf.

City of Los Angeles. 2012. Ocean Water Public Monitoring Data. City of Los Angeles, Department of Public Works, Bureau of Sanitation, Environmental Monitoring Division. Los Angeles, CA.

Colford, J.M., K.C. Schiff, J.F. Griffith, V. Yau, B.F. Arnold, C.C. Wright, J.S. Gruber, T.J. Wade, S. Burns, J. Hayes, C. McGee, M. Gold, Y. Cao, R.T. Noble, R. Haugland and S.B. Weisberg. 2012. Using rapid indicators for Enterococcus to assess the risk of illness after exposure to urban runoff contaminated marine water. *Water Research* 46:2176-2186.

Colford, J.M., T.J. Wade, K.C. Schiff, C.C. Wright, J.F. Griffith, S.K. Sandhu, S. Burns, M. Sobsey, G. Lovelace and S.B. Weisberg. 20007. Water quality indicators and the risk of illness at beaches with nonpoint sources of fecal contamination. *Epidemiology* 18:27-35.

Dorevitch, S., P. Pratap, M. Wroblewski, D.O. Hryhorczuk, H. Li, L.C. Liu and P.A. Scheff. 2012. Health risks of limited-contact water recreation. *Environmental Health Perspectives* 120:192-197.

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 Epidemiol*ogy 19:1100-1106.

Fleisher, J.M., L.E. Fleming, H.M. Solo-Gabriele, J.K. Kish, C.D. Sinigalliano, L. Plano, S.M. Elmir, J.D. Wang, K. Withum, T. Shibata, M.L. Gidley, A. Abdelzaher, G. He, C. Ortega, X. Zhu, M. Wright, J. Hollenbeck and L.C. Backer. 2010. The BEACHES Study: Health effects and exposures from non-point source microbial contaminants in subtropical recreational marine waters. *International Journal of Epidemiology* 39:1291-1298.

Griffith, J.F., Y. Cao, C.D. McGee and S.B. Weisberg. 2009. Evaluation of rapid methods and novel indicators for assessing microbiological beach water quality. *Water Research* 43:4900-4907.

Haile, R.W., J. Alamillo, K. Barrett, R. Cressey, J. Dermond, C. Ervin, A. Glasser, N. Harawa, P. Harmon, J. Harper, C. McGee, R.C. Millikan, M. Nides and J.S. Witte. 1996. An Epidemiological Study of Possible Adverse Health Effects of Swimming in Santa Monica Bay: Final Report. The Santa Monica Bay Restoration Project (The Project). Monterey Park, CA.

Haile, R.W., J.S. Witte, M. Gold, R. Cressey, C. McGee, R.C. Millikan, A. Glasser, N. Harawa, C. Ervin, P. Harmon, J. Harper, J. Dermand, J. Alamilo, K. Barrett, M. Nides and G. Wang. 1999. The health effects of swimming in ocean water contaminated by storm drain runoff. *Epidemiology* 10:355-363.

Haugland, R.A., S.C. Siefring, L.J. Wymer, K.P. Brenner and A.P. Dufour. 2005. Comparison of *Enterococcus* measurements in freshwater at two recreational beaches by quantitative polymerase chain reaction and membrane filter culture analysis. *Water Research* 39:559-68.

Heaney, C.D., E. Sams, A.P. Dufour, K.P. Brenner, R.A. Haugland, E. Chern, S. Wing, S. Marshall, D.C. Love, M. Serre, R. Noble and T.J. Wade. 2012. Fecal indicators in sand, sand contact, and risk of enteric illness among beachgoers. *Epidemiology* 23:95-106.

Heaney, C.D., E. Sams, S. Wing, S. Marshall, K. Brenner, A.P. Dufour and T.J. Wade. 2009. Contact with beach sand among beachgoers and risk of illness. *American Journal of Epidemiology* 170:164-172.

Hutcheon, J.A., A. Chiolero and J.A. Hanley. 2010. Random measurement error and regression dilution bias. *BMJ* 340:c2289.

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 randomised exposure. *Lancet* 344:905-909.

Layton, B.A., S.P. Walters, L.H. Lam and A.B. Boehm. 2010. *Enterococcus* species distribution among human and animal hosts using multiplex PCR. *Journal of Applied Microbiology* 109:539-547.

Lipsitch, M., E.T. Tchetgen and T. Cohen. 2010. Negative controls: A tool for detecting confounding and bias in observational studies. *Epidemiology* 21:383-388.

Los Angeles County Department of Public Health. 2012. Los Angeles County Department of Public Health, Environmental Health Ocean Water Monitoring Data. Los Angeles County Department of Public Health. http://lapublichealth.org/eh/ehftp/.

Noble, R.T., A.D. Blackwood, J.F. Griffith, C.D. McGee and S.B. Weisberg. 2010. Comparison of rapid quantitative PCR-based and conventional culture-based methods for enumeration of *Enterococcus* spp. and *Escherichia coli* in recreational waters. *Applied and Environmental Microbiology* 76:7437-7443.

Rosenbaum, P. 1999. Choice as an alternative to control in observational studies. *Statistical Science* 14:259-278.

Soller, J.A., T. Bartrand, N.J. Ashbolt, J. Ravenscroft and T.J. Wade. 2010. Estimating the primary etiologic agents in recreational freshwaters impacted by human sources of faecal contamination. *Water Research* 44:4736-4747.

US Environmental Protection Agency (USEPA). 2012. Recreational Water Quality Criteria. USEPA Office of Water. http://water.epa.gov/scitech/swguidance/standards/criteria/health/recreation/upload/RWQC2012.pdf.

US Environmental Protection Agency (USEPA). 2006. Method 1600: Enterococci in Water by Membrane Filtration Using membrane-Enterococcus Indoxyl-beta-D-Glucoside Agar (mEI). EPA/821/R-06/009. USEPA Office of Water. Washington, DC.

Wade, T.J., R.L. Calderon, K.P. Brenner, E. Sams, M. Beach, R. Haugland, L. Wymer and A.P. Dufour. 2008. High sensitivity of children to swimming-associated gastrointestinal illness: results using a rapid assay of recreational water quality. *Epidemiology* 19:375-383.

Wade, T.J., R.L. Calderon, E. Sams, M. Beach, K.P. Brenner, A.H. Williams and A.P. Dufour. 2006. Rapidly measured indicators of recreational water quality are predictive of swimming-associated gastrointestinal illness. *Environmental Health Perspectives* 114:24-28.

Wade, T.J., E. Sams, K.P. Brenner, R. Haugland, E. Chern, M. Beach, L. Wymer, C.C. Rankin, D. Love, Q. Li, R. Noble and A. P. Dufour. 2010. Rapidly measured indicators of recreational water quality and swimming-associated illness at marine beaches: A prospective cohort study. *Environmental Health* 9:66.

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Supplemental Information

Supplemental Information is available at ftp://ftp.sccwrp.org/pub/download/DOCUMENTS/AnnualReports/2013AnnualReport/ar13_611_624SI.pdf.