

Acute gastroenteritis and illness burden attributable to recreational water exposure in the United States: higher sensitivity and burden among children

Supplemental Appendices

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1 Beach, Water Quality, and Participant Details

All of the primary research studies that contributed to the present pooled analysis have been previously published with extensive details about recruitment, locations, sampling, and laboratory methods. Readers seeking additional details beyond those presented here can refer to those previously published articles and technical reports.¹⁻⁹ Figure S1 maps the 13 study beaches and summarizes some of their key characteristics. All 4 freshwater beaches in the study were in the great lakes region.

Field teams collected composite water samples from between 3 and 5 locations per beach. Water samples were collected in the morning (typically 8:00), at mid-day (typically 12:00), and in the afternoon (typically 15:00) at shin depth (0.3 to 0.5 m). There were a few exceptions to this. At Malibu, samples were only collected twice per day: in the morning and at mid-day. At Mission Bay, samples were collected hourly between 12:00 and 14:00. At Doheny and Malibu there were no samples collected at waist depth (1 m). The present analyses used all of the samples collected at each beach (across sampling times, locations, depths) when calculating averages.

Water samples were placed on ice and were analyzed within 6 hours for *Enterococcus* using the culture-based EPA Method 1600¹⁰ on mEI agar. We quantified *Enterococcus* using colony forming units (CFU) per 100 ml. The 2003 Mission Bay study analyzed a single sample per day using the EPA 1600 method, but 11 samples per day using the *Enterococcus* Enterolert™ chromogenic substrate and the Quantitray system (IDEXX, Westbrook, ME). Since there was significantly more information collected using the Enterolert assay in Mission Bay, at a level comparable to the other beaches, the study team decided it was most appropriate to use the Enterolert results from Mission Bay.

In addition to the analysis of samples using culture methods, frozen water samples were filtered, DNA extracted, and then those samples were tested at the EPA lab (all beaches) for *Enterococcus* using the TaqMan® quantitative polymerase chain reaction (qPCR) EPA Method 1611.¹¹ The lab used the TaqMan PCR product detection system; PCR amplification was conducted in a thermal cycling instrument (Smart-Cycler System, Cepheid, Sunnyvale, CA) to automate the detection and quantitative measurement of the fluorescent signals produced by the TaqMan probe. We quantified *Enterococcus* using the qPCR assay in calibrator cell equivalents (CCE) using the delta-delta method – refer to Wade et al.⁵ and Siefring et al.¹² for technical details regarding this calculation.

Table S1 summarizes the number of water samples analyzed using the different assays at each beach. At some beaches there were fewer samples analyzed using the qPCR assay than the culture assay mainly because of cost. Below, we have also summarized the sample characteristics for *Enterococcus* culture methods (Table S2) and the *Enterococcus* EPA 1611 qPCR assay (Table S3). Figure S3 compares daily average values for the two types of *Enterococcus* assays used in the analysis.

At most beaches, we calculated a daily average of \log_{10} *Enterococcus* concentrations and then matched those concentrations to swimmers who were exposed on that day. Before calculating the average, we imputed samples that were below the limit of detection at 0.1. However, at the California beaches (Avalon, Doheny, Malibu, Mission Bay) the water sampling locations included a diverse set of conditions. We summarized water quality data separately for some sub-locations and matched water quality measures to swimmers with

higher spatial resolution to reflect the heterogeneous conditions. Figure S2 summarizes the sampling locations for the California beaches. At Avalon beach, there was a single site D that was outside the main cove, had much cleaner water, and was retained as a separate location in the analysis. At Doheny beach, site E was nearly 1 mile south of the other water sampling points, further from freshwater inputs, and was retained as a separate location in the analysis. At both Doheny and Malibu beaches, sampling site C was in the lagoon, with few individuals exposed but very high concentrations of fecal indicator bacteria – for this reason, we kept the lagoon data separate from the other pooled averages. Finally, at Mission Bay in San Diego, CA, we kept the 6 beach locations separate for water quality averaging and for matching the water quality data to swimmer exposure.

● 1 Malibu		
2009	Beachgoers	Swimmers
All Ages	5,674	2,559
0 - 4 Years	547	244
5 - 10 Years	652	495

▲ 2 Avalon		
2007, 2008	Beachgoers	Swimmers
All Ages	6,165	3,891
0 - 4 Years	401	264
5 - 10 Years	925	851

● 3 Doheny		
2007, 2008	Beachgoers	Swimmers
All Ages	9,525	4,335
0 - 4 Years	916	382
5 - 10 Years	1,629	1,241

● 4 Mission Bay		
2003	Beachgoers	Swimmers
All Ages	8,797	3,238
0 - 4 Years	955	437
5 - 10 Years	1,608	1,026

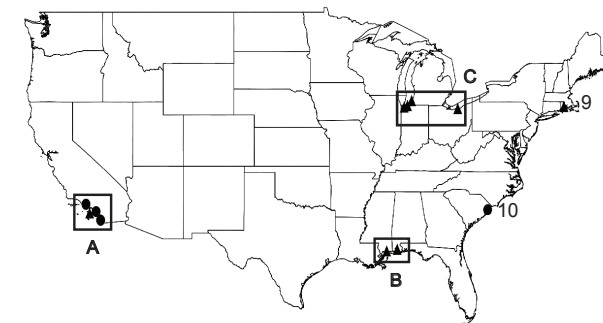
▲ 5 West		
2003	Beachgoers	Swimmers
All Ages	2,877	1,668
0 - 4 Years	214	132
5 - 10 Years	315	258

▲ 6 Washington Park		
2004	Beachgoers	Swimmers
All Ages	4,377	2,360
0 - 4 Years	344	214
5 - 10 Years	575	506

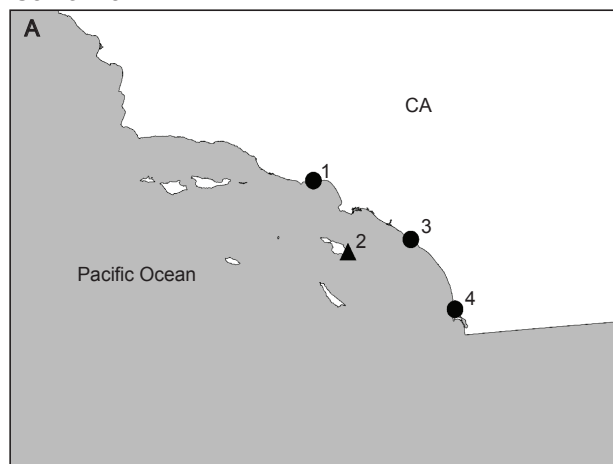
▲ 7 Silver		
2004	Beachgoers	Swimmers
All Ages	10,921	5,651
0 - 4 Years	909	542
5 - 10 Years	1,427	1,197

▲ 8 Huntington		
2003	Beachgoers	Swimmers
All Ages	2,840	757
0 - 4 Years	185	87
5 - 10 Years	327	195

▲ 9 Goddard		
2007	Beachgoers	Swimmers
All Ages	2,977	1,080
0 - 4 Years	238	130
5 - 10 Years	312	236



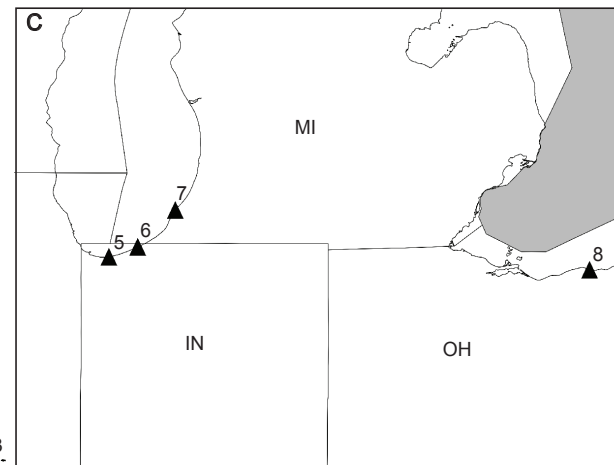
California



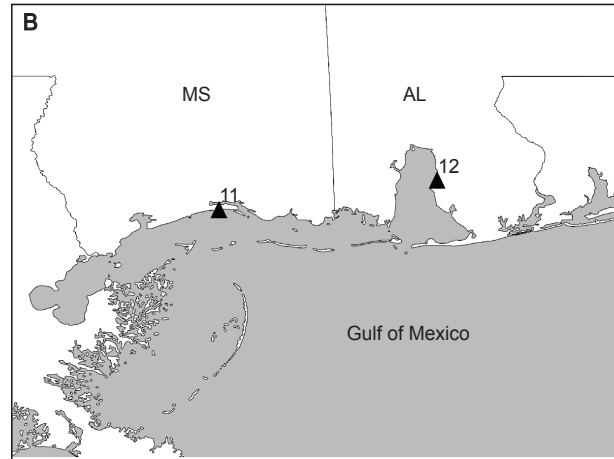
● 10 Surfside		
2009	Beachgoers	Swimmers
All Ages	11,159	8,073
0 - 4 Years	647	483
5 - 10 Years	1,105	1,033

▲ 11 Fairhope		
2007	Beachgoers	Swimmers
All Ages	2,022	823
0 - 4 Years	241	134
5 - 10 Years	333	236

Great Lakes



Gulf Coast



▲ 12 Edgewater		
2005	Beachgoers	Swimmers
All Ages	1,351	741
0 - 4 Years	75	62
5 - 10 Years	141	112

▲ 13 Boqueron		
2009	Beachgoers	Swimmers
All Ages	15,726	12,111
0 - 4 Years	908	764
5 - 10 Years	1,473	1,381

Figure S1: Summary of beach locations, study years, and enrollment totals for all beachgoers and body immersion swimmers. Shape differentiates pollution type: known point source of human sewage discharge (triangles) versus non-point source (circles).

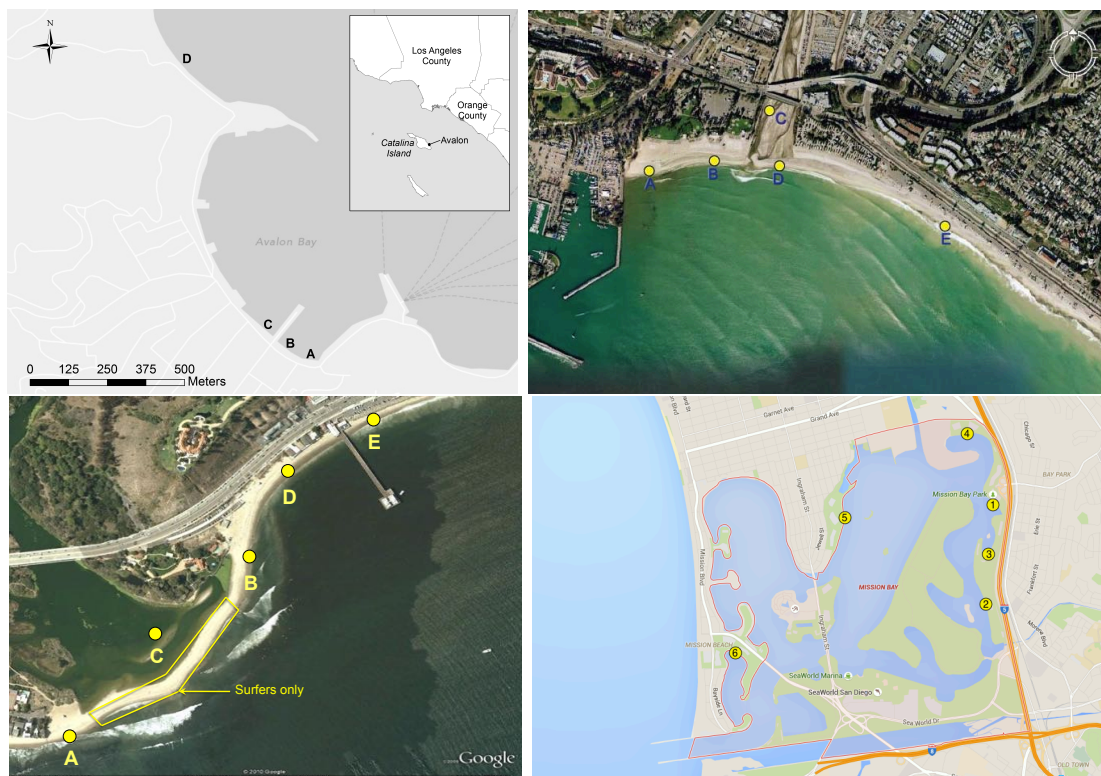


Figure S2: Summary of water quality and participant recruitment sampling locations for the California beaches, where sublocations were retained in the present analysis to match water quality measurements to swimmers. Clockwise from top left: Avalon, Doheny, Mission Bay, and Malibu.

Table S1: Summary of the number of samples analyzed and the number of samples below detection for *Enterococcus* measured using EPA 1600 or EPA qPCR 1611 assays. For Avalon, Dohney, Malibu, and Mission Bay beaches, results are summarized separately for sub-locations within the study beach consistent with how they were assigned to swimmers in the analysis.

Beach -Locations	<i>Enterococcus</i> EPA 1600*		<i>Enterococcus</i> qPCR EPA 1611	
	N Samples	N Non-Detects	N Samples	N Non-Detects
Avalon-ABC	675	30	530	63
Avalon-D	30	18	30	9
Boqueron	600	63	600	333
Doheny-ABD	306	116	234	65
Doheny-C	33	2	23	0
Doheny-E	102	52	77	26
Edgewater	395	48	396	1
Fairhope	431	29	438	97
Goddard	426	78	426	28
Huntington	420	17	420	12
Malibu-ABDE	306	67	305	37
Malibu-C	39	0	32	0
Mission Bay 1	216	63	86	8
Mission Bay 2	324	115	129	4
Mission Bay 3	540	145	215	13
Mission Bay 4	216	64	86	5
Mission Bay 5	324	164	129	14
Mission Bay 6	324	76	128	2
Silver	423	5	423	46
Surfside	530	61	532	173
Washington Park	421	0	421	48
West	336	39	336	12
Total	7417	1252	5996	996

* For all beaches, *Enterococcus* was measured using EPA method 1600 except for Mission Bay, where it was measured using EnterolertTM chromogenic substrate on the Quantitray system (IDEXX).

Table S2: *Enterococcus* measured using culture methods water sample summary by beach. Min, Max, and Geometric Mean values are in colony forming units (CFU) per 100 ml after values below detection were imputed at 0.1. For Avalon, Dohney, Malibu, and Mission Bay beaches, results are summarized separately for sub-locations within the study beach consistent with how they were assigned to swimmers in the analysis. For all beaches, *Enterococcus* was measured using EPA method 1600 except for Mission Bay, where it was measured using Enterolert™ chromogenic substrate on the Quantitray system (IDEXX).

Beach -Locations	N Samples	N Non-Detects	Min	Max	Geometric Mean
Avalon-ABC	675	30	0.1	10000	35
Avalon-D	30	18	0.1	20	1
Boqueron	600	63	0.1	580	6
Doheny-ABD	306	116	0.1	2000	4
Doheny-C	33	2	0.1	41000	1527
Doheny-E	102	52	0.1	1900	1
Edgewater	395	48	0.1	920	8
Fairhope	431	29	0.1	3000	21
Goddard	426	78	0.1	960	4
Huntington	420	17	0.1	48100	25
Malibu-ABDE	306	67	0.1	1740	2
Malibu-C	39	0	18.0	6710	511
Mission Bay 1	216	63	0.1	644	6
Mission Bay 2	324	115	0.1	7030	5
Mission Bay 3	540	145	0.1	57940	9
Mission Bay 4	216	64	0.1	1043	7
Mission Bay 5	324	164	0.1	487	1
Mission Bay 6	324	76	0.1	1723	12
Silver	423	5	0.1	2800	31
Surfside	530	61	0.1	640	3
Washington Park	421	0	1.0	750	25
West	336	39	0.1	3700	7
Total	7417	1252	0.1	57940	9

Table S3: *Enterococcus* EPA qPCR 1611 water sample summary by beach. Min, Max, and Geometric Mean values are in calibrator cell equivalents (CCE) per 100 ml (delta-delta method) after values below detection were imputed at 0.1. For Avalon, Dohney, Malibu, and Mission Bay beaches, results are summarized separately for sub-locations within the study beach consistent with how they were assigned to swimmers in the analysis.

Beach -Locations	N Samples	N Non-Detects	Min	Max	Geometric Mean
Avalon-ABC	530	63	0.1	14696	64
Avalon-D	30	9	0.1	152	5
Boqueron	600	333	0.1	983722	2
Doheny-ABD	234	65	0.1	3198	6
Doheny-C	23	0	25.7	16531	1301
Doheny-E	77	26	0.1	113	3
Edgewater	396	1	0.1	10188	361
Fairhope	438	97	0.1	98995	56
Goddard	426	28	0.1	25622	111
Huntington	420	12	0.1	114286	133
Malibu-ABDE	305	37	0.1	2829	12
Malibu-C	32	0	156.1	3656	727
Mission Bay 1	86	8	0.1	141093	53
Mission Bay 2	129	4	0.1	9004	87
Mission Bay 3	215	13	0.1	46867	84
Mission Bay 4	86	5	0.1	13837	46
Mission Bay 5	129	14	0.1	4502	23
Mission Bay 6	128	2	0.1	4309	58
Silver	423	46	0.1	123483	35
Surfside	532	173	0.1	109297	17
Washington Park	421	48	0.1	1986	32
West	336	12	0.1	15778	119
Total	5996	996	0.1	983722	38

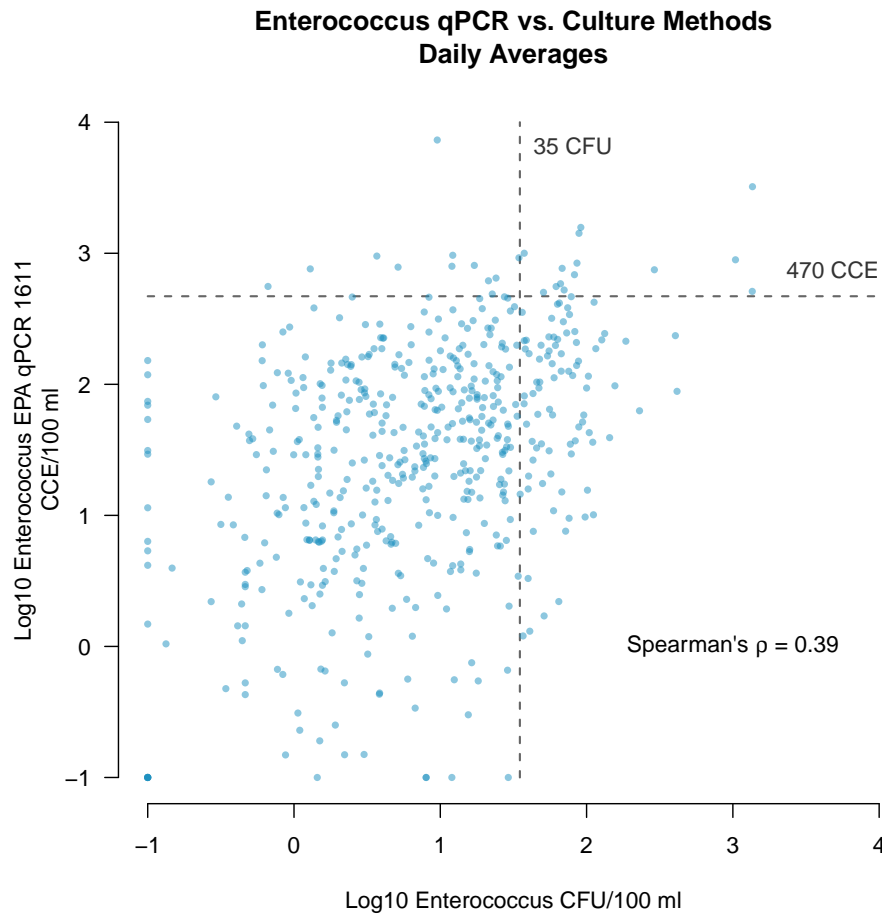


Figure S3: Scatter plot of *Enterococcus* EPA qPCR 1611 values versus *Enterococcus* culture method (EPA 1600 or Enterolert) values in water sample daily averages. The figure includes EPA regulatory guidelines for each type of indicator.

The study included 84,411 individuals with outcome measurements across the 13 cohorts. The studies tended to enroll families with children – for this reason the age distribution of the study population was approximately bi-modal (Figure S4). Teenagers were under-represented in the cohorts due to the inclusion criteria that required an adult present from the household.

Figure S5 summarizes the distribution of *Enterococcus* sample concentrations and swimmer exposure concentrations in the study cohort for the EPA 1600 assay and the EPA 1611 qPCR assay. We excluded from the distribution plots 73 water samples from freshwater lagoons at Dohney and Malibu beaches because although our team collected the water samples, very few individuals were actually exposed to that water – only 54 (Doheny) and 68 (Malibu) participants were exposed to lagoon water.

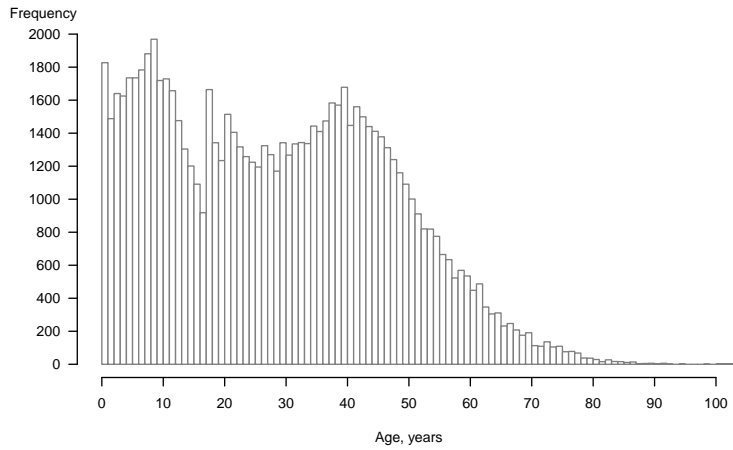
Table S4: Participant Characteristics by Age Category

	All Ages ^a			Age 0 to 4 Years			Age 5 to 10 Years			Age >10 Years		
	N	%	Median (IQR)	N	%	Median (IQR)	N	%	Median (IQR)	N	%	Median (IQR)
Number of Participants	84,411			6,580			10,822			65,854		
Gastrointestinal illness at enrollment	1,948	2.3		186	2.8		189	1.7		1,559	2.4	
Individuals at risk for gastrointestinal illness	82,463			6,394			10,633			64,295		
Incident diarrhea within 10 days	3,409	4.1		398	6.2		393	3.7		2,585	4.0	
Age in years			29 (13,43)			2 (1,3)			8 (6,9)			35 (22,46)
Female	45,562	54.0		3,207	48.7		5,357	49.5		36,454	55.4	
Race/ethnicity												
White/caucasian	48,829	57.8		3,429	52.1		5,843	54.0		39,026	59.3	
Hispanic	27,276	32.3		2,279	34.6		3,578	33.1		20,992	31.9	
African American	2,600	3.1		204	3.1		386	3.6		1,960	3.0	
Asian	2,018	2.4		173	2.6		257	2.4		1,564	2.4	
American Indian	240	0.3		13	0.2		23	0.2		202	0.3	
Multiple Races	1,753	2.1		315	4.8		492	4.5		924	1.4	
Other	1,008	1.2		115	1.7		149	1.4		717	1.1	
Missing	687	0.8		52	0.8		94	0.9		469	0.7	
No water contact	25,762	30.5		1,557	23.7		963	8.9		22,940	34.8	
Any water contact	58,649	69.5		5,023	76.3		9,859	91.1		42,914	65.2	
Body immersion	47,287	56.0		3,875	58.9		8,767	81.0		33,951	51.6	
Head immersion	36,832	43.6		2,753	41.8		7,646	70.7		25,869	39.3	
Swallowed water	10,860	12.9		1,626	24.7		3,055	28.2		6,031	9.2	
Hours spent in the water ^b			1.0 (0.5,2.0)			1.0 (0.5,2.0)			2.0 (1.0,3.0)			1.0 (0.5,2.0)
Hours spent in the water (cat) ^b												
0 – 1	26,287	54.9		2,235	56.2		3,424	38.4		20,264	59.1	
1.1 – 2	11,281	23.6		958	24.1		2,604	29.2		7,528	21.9	
2.1 – 3	5,565	11.6		415	10.4		1,503	16.9		3,584	10.4	
3.1 – 4	3,005	6.3		234	5.9		826	9.3		1,909	5.6	
4.1 – 5	856	1.8		60	1.5		277	3.1		499	1.5	
>5	651	1.4		39	1.0		209	2.3		385	1.1	
Missing	249	0.5		37	0.9		66	0.7		137	0.4	

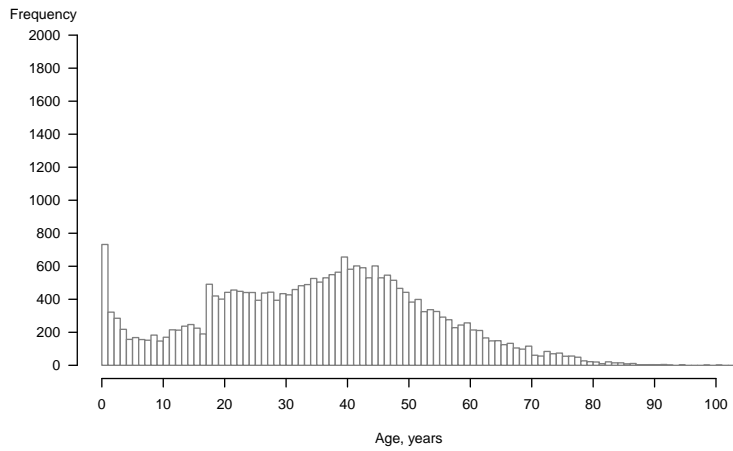
^a All ages category includes 1,155 individuals with no age information.

^b Time spent in the water limited to beachgoers with body immersion, head immersion, or swallowed water exposure.

A) All Beachgoers



B) Non-Swimmers



C) Body Immersion Swimmers

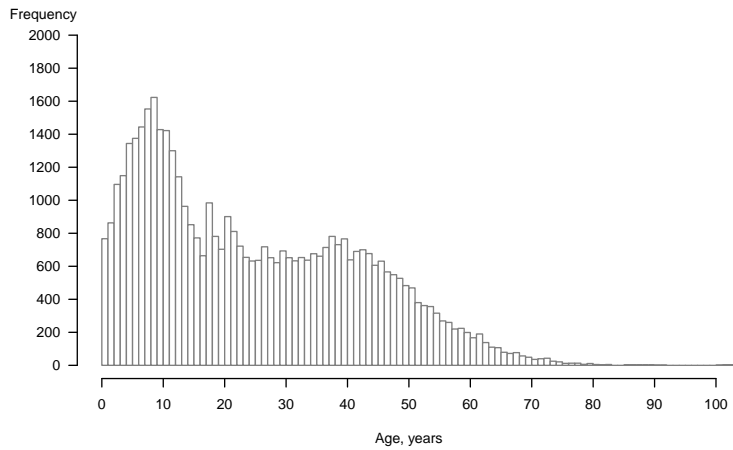
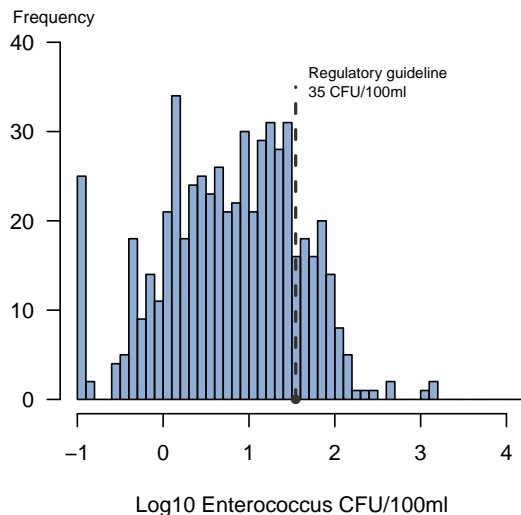
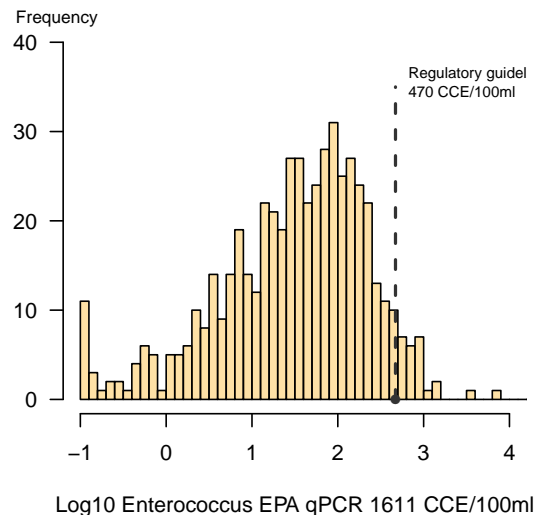


Figure S4: Age distribution of the study population; bin width is 1 year. **A)** All participants; **B)** Non-swimmers (no water contact); **C)** Body immersion swimmers (entered the water to waist depth or more).

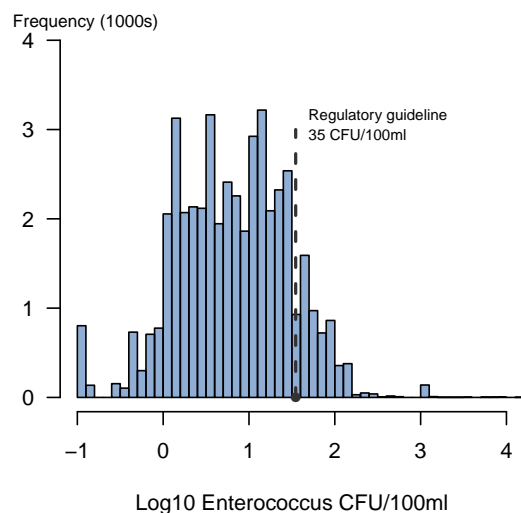
**A) Enterococcus EPA 1600 and Enterolert
Distribution of Daily Averages**



**B) Enterococcus EPA qPCR 1611
Distribution of Daily Averages**



**C) Enterococcus EPA 1600 and Enterolert
Distribution of Body Immersion Swimmer Exposure**



**D) Enterococcus EPA qPCR 1611
Distribution of Body Immersion Swimmer Exposure**

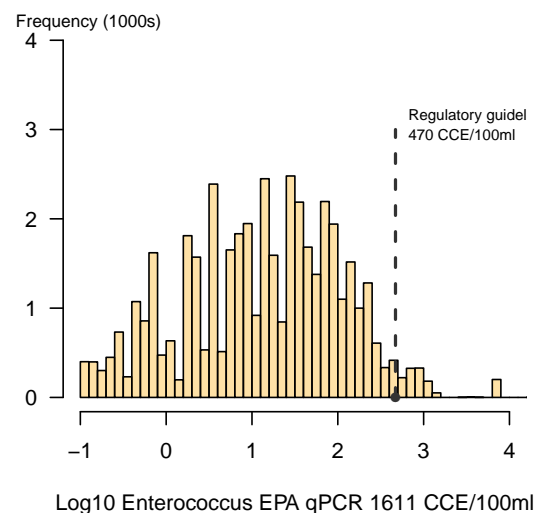


Figure S5: Distribution of Enterococcus Water Samples and Enterococcus Exposure Among Body Immersion Swimmers Matched to Water Samples. Bin width is 0.1. **A)** Enterococcus EPA 1600 or Enterolert water sample distribution. **B)** Enterococcus EPA qPCR 1611 water sample distribution. **C)** Enterococcus EPA 1600 or Enterolert body immersion swimmer distribution. **D)** Enterococcus EPA qPCR 1611 body immersion swimmer distribution. All figures exclude 73 water samples from Malibu and Doheny beaches freshwater lagoons.

2 Beach-Specific Results

This section reports beach-specific estimates of the association between: i) water exposure and diarrhea risk, and ii) *Enterococcus* exposure and diarrhea risk, corresponding to the first two study objectives.

Figure S6 summarizes the adjusted cumulative incidence ratio (CIR) of diarrhea associated with different levels of water exposure at each beach. The results are sorted by fresh versus marine water and then by the CIR for body immersion swimmers. There was evidence for effect modification by fresh versus marine water conditions, with stronger associations between water exposure and incident diarrhea at freshwater beaches: P -value for effect modification = 0.05 (body immersion), 0.08 (head immersion), 0.05 (swallowed water). Summary statistics of heterogeneity across beaches^{13,14} were consistent with low to moderate levels of effect heterogeneity in the estimates: body immersion (Cohen's $Q = 18.52$, $\text{Pr}(Q, \text{df}=12)=0.10$, $I^2 = 35\%$), head immersion (Cohen's $Q = 15.73$, $\text{Pr}(Q, \text{df}=12)=0.20$, $I^2 = 24\%$), and swallowed water (Cohen's $Q = 21.60$, $\text{Pr}(Q, \text{df}=12)=0.04$, $I^2 = 44\%$).

Figure S7 summarizes the adjusted CIR of diarrhea associated with exposure to water above EPA regulatory guidelines for body immersion swimmers at each beach. We observed more heterogeneity in the effects compared to the swim exposure analysis, particularly for the EPA 1611 qPCR indicator analyses. Part of this is due to relatively small numbers of individuals exposed above the qPCR regulatory guideline of 470 CCE/100ml (Figure S5). Indeed, we have not presented the beach-specific CIRs associated with quartiles of *Enterococcus* concentrations due to small sample sizes at each beach for that type of granular analysis. There was some evidence for effect modification by point versus non-point source beaches for the culture method (EPA 1600 or Enterolert) exposure (P -value for effect modification = 0.07). The indicator was associated with increased diarrhea incidence only at beaches with known point sources of treated human sewage (Figure S7). In contrast, we observed no effect modification by beach type of the relationship between the EPA 1611 qPCR indicator and diarrhea incidence (P -value for effect modification = 0.36). Summary statistics of heterogeneity across beaches^{13,14} were consistent with high levels of effect heterogeneity in the estimates: EPA 1600 (Cohen's $Q = 35.26$, $\text{Pr}(Q, \text{df}=12)=0.00$, $I^2 = 72\%$), EPA qPCR 1611 (Cohen's $Q = 70.63$, $\text{Pr}(Q, \text{df}=12)=0.00$, $I^2 = 86\%$).

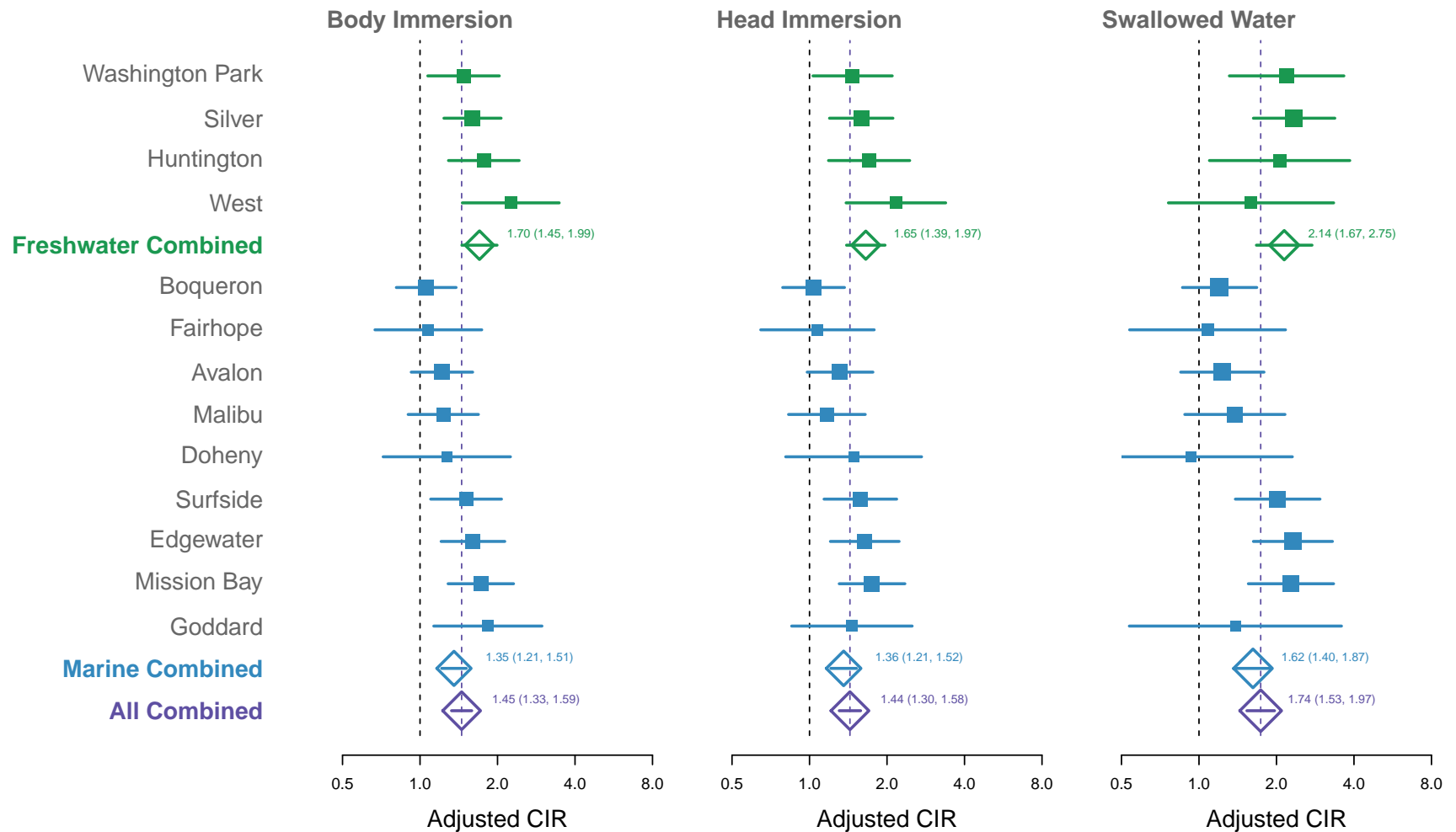


Figure S6: Forest Plot of Adjusted Cumulative Incidence Ratios (CIR) of Diarrhea Comparing Swimmers with Nonswimmers by Beach, Water Type, and Water Exposure Level. Beach-specific estimates (boxes) are scaled by the inverse of the standard error of the CIR, pooled estimates are represented by diamonds, and horizontal lines mark 95% confidence intervals. Beaches are sorted by water type (fresh, marine) and by body immersion CIR.

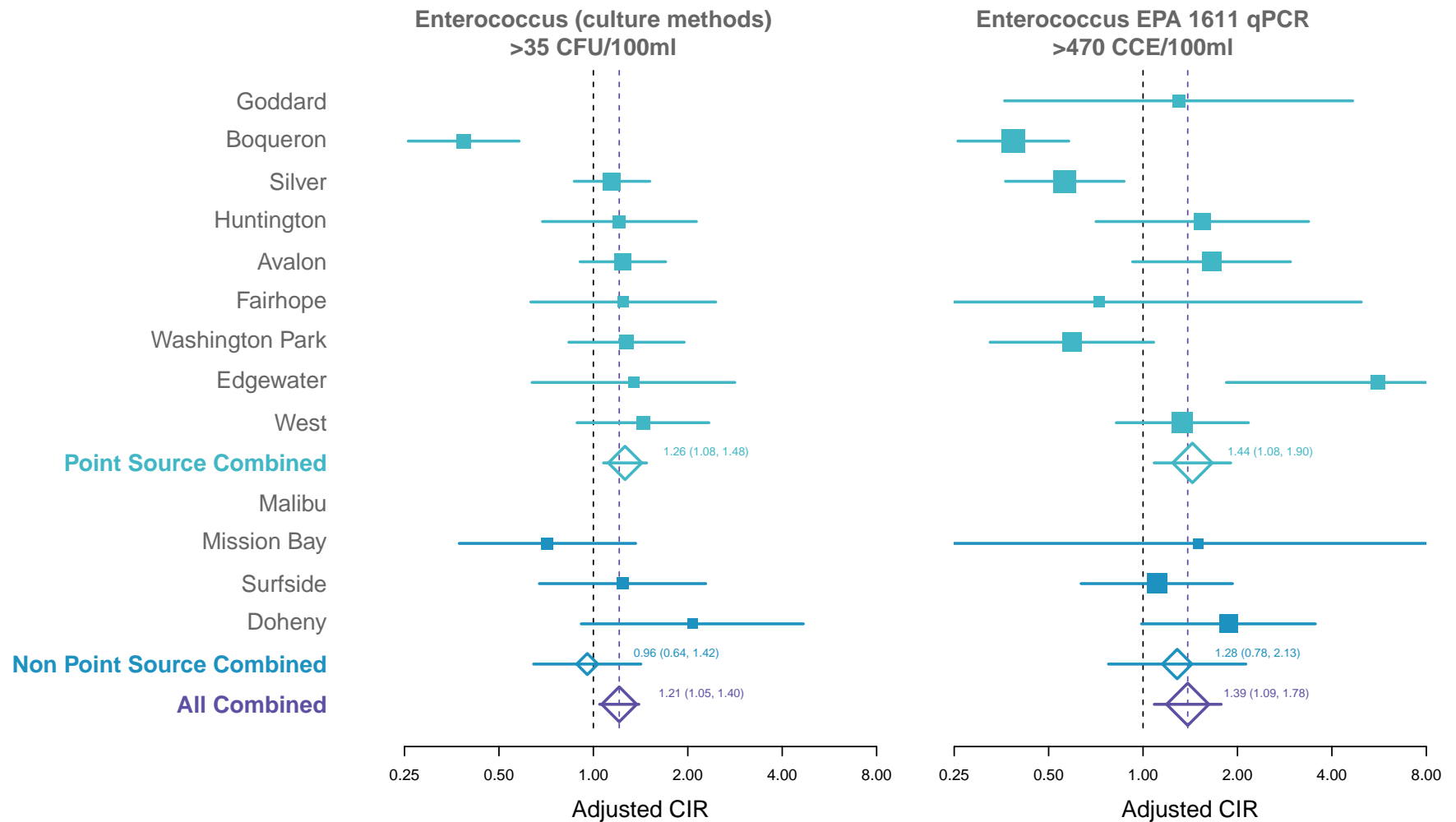


Figure S7: Forest Plot of Adjusted Cumulative Incidence Ratios (CIR) of Diarrhea Among Body Immersion Swimmers Associated with Levels of *Enterococcus* Above EPA Regulatory Guidelines for culture-based methods (EPA 1600 or Enterolert) and EPA qPCR method 1611. The reference group is swimmers below the regulatory guidelines. There are no estimates for Malibu (either method) or Goddard (culture method) because too few swimmers were exposed above the regulatory level, but those beaches still contributed to combined estimates. Beach-specific estimates (boxes) are scaled by the inverse of the standard error of the CIR, pooled estimates are represented by diamonds, and horizontal lines mark 95% confidence intervals. Beaches are sorted by pollution type and by *Enterococcus* culture method CIR.

3 Associations Between Water Exposure, *Enterococcus* and Diarrhea Not Presented in the Main Text

Analysis of water exposure:

Due to space limitations in the journal, the main text Figure 1 only included water exposure related risk estimates for Ages 0-4 and All Ages. Figure S8 includes the full results of the age-stratified analyses of water exposure.

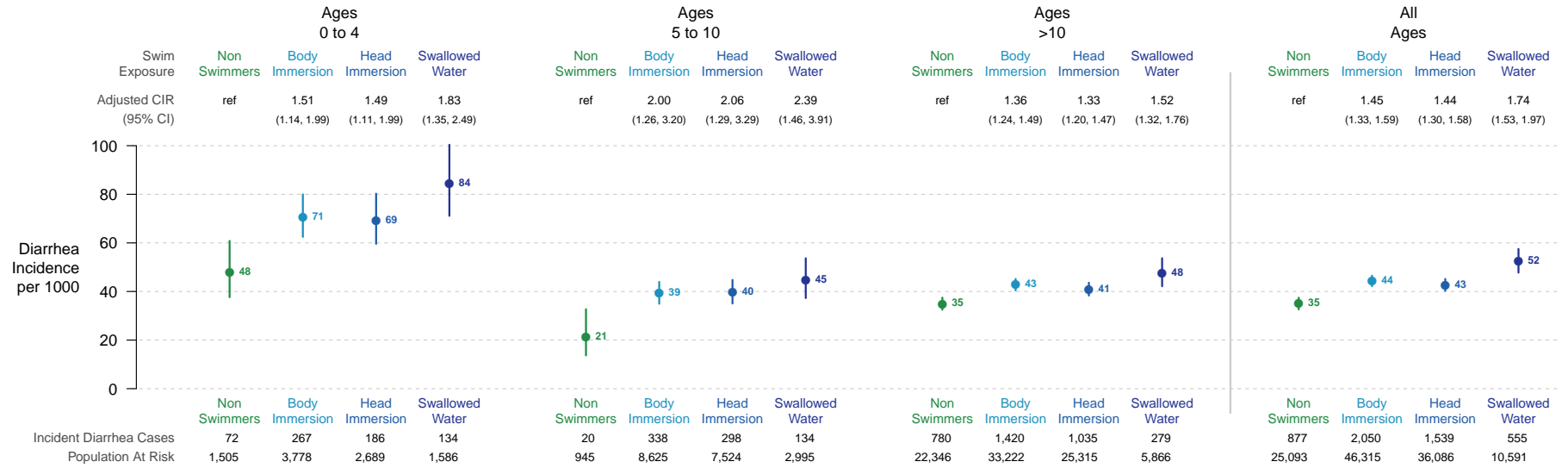


Figure S8: Incident Diarrhea Associated with Water Exposure Stratified by Age. The estimates for ages 0-4 years and all ages are presented in Fig 1 of the main text. Adjusted cumulative incidence ratios (CIRs) were estimated with non-swimmers as the reference group. Estimates from a pooled analysis of 13 prospective cohorts in the United States, 2003-2009.

Analysis by quartiles of *Enterococcus*:

In this section we summarize the association between *Enterococcus* levels measured using culture methods (EPA 1600 or Enterolert) and using EPA 1611 qPCR and diarrhea incidence among body immersion swimmers, using quartiles of the indicators. We present results stratified by age and stratified by type of pollution source – those with a known point-source of human fecal pollution, and those without (“non-point source” beaches).

There was effect modification by age of the association between *Enterococcus* measured using culture methods (effect modification $P = 0.004$), but not for *Enterococcus* measured using qPCR (effect modification $P = 0.67$). Figure S9 summarizes diarrhea incidence among swimmers by quartile of *Enterococcus* exposure, stratified by age.

There was also effect modification by pollution source: higher *Enterococcus* concentrations were associated with higher diarrhea incidence at beaches with known point-sources of fecal pollution but not at non-point source beaches with diffuse pollution (Figure S10).

Analysis by levels *Enterococcus* above and below regulatory guidelines:

Figure 2 of the main text presented diarrhea incidence for *Enterococcus* levels above and below regulatory guidelines for the culture based methods, stratified by age. We also summarized the association between *Enterococcus* levels above and below regulatory guidelines and diarrhea incidence for the qPCR 1611 assay, and for both assays stratified by different sources of pollution. Similar to the quartile analysis, there was some evidence for effect modification by age of the association between *Enterococcus* measured by qPCR and diarrhea incidence (Figure ??).

When stratified by point-source versus non-point source pollution type (Figure S12), there was some suggestion of effect modification for *Enterococcus* measured using culture methods but not for *Enterococcus* measured using qPCR. We note that the qPCR indicator consistently measured increases in risk at every quartile increase at point-source beaches, while the monotonic increase in risk for the culture method obtained only in quartiles 2-4 (Figure S12).

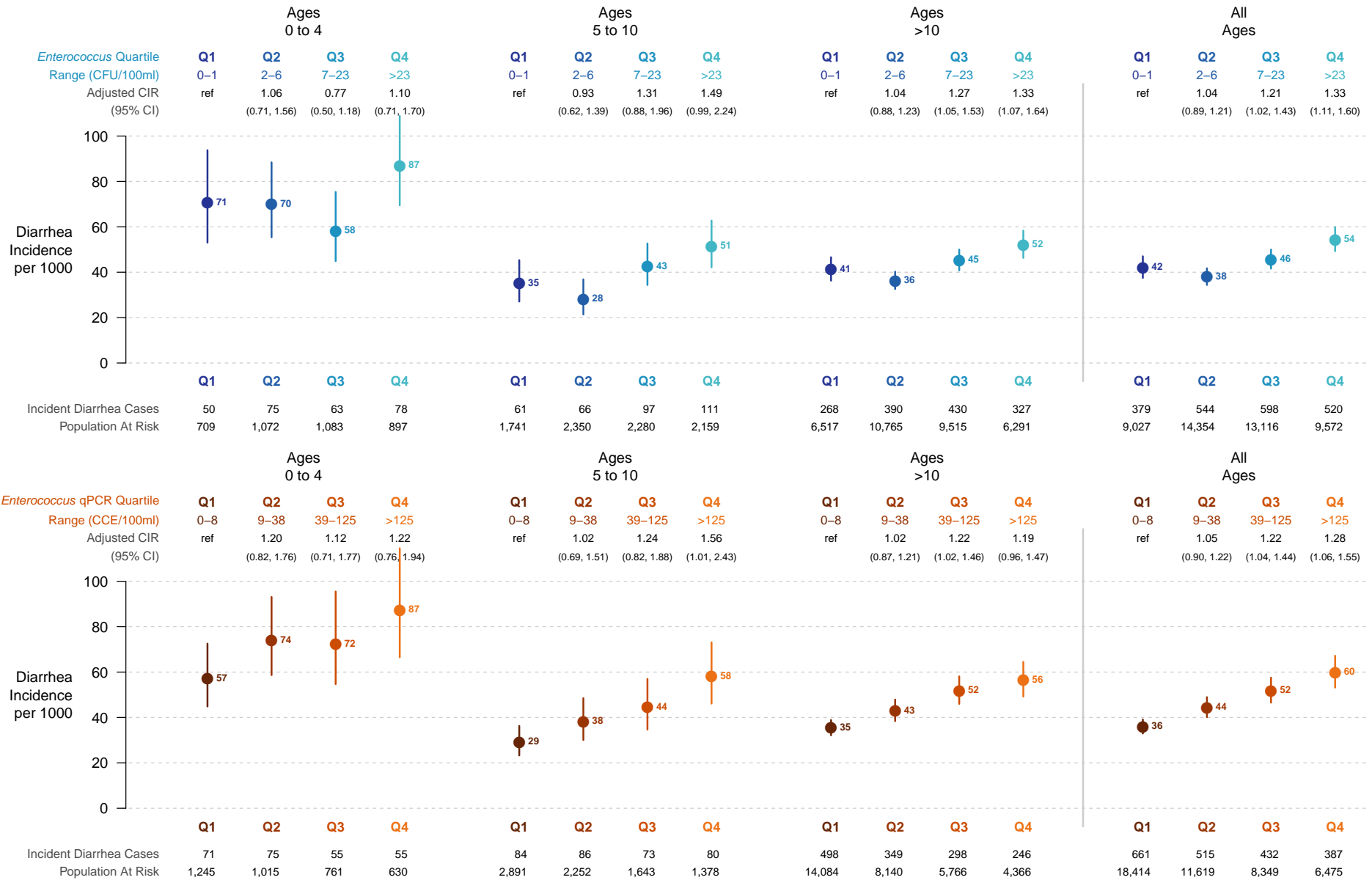


Figure S9: Incident Diarrhea Among Body Immersion Swimmers Associated with Quartiles of Enterococcus, Stratified by Age. The top row summarizes estimates using *Enterococcus* measured using culture methods (EPA 1600 or Enterolert; effect modification $P = 0.004$), and the bottom row summarizes estimates using EPA qPCR 1611 (effect modification $P = 0.67$). Cumulative incidence ratios (CIRs) were adjusted for a range of potential confounders and beach-level fixed effects.

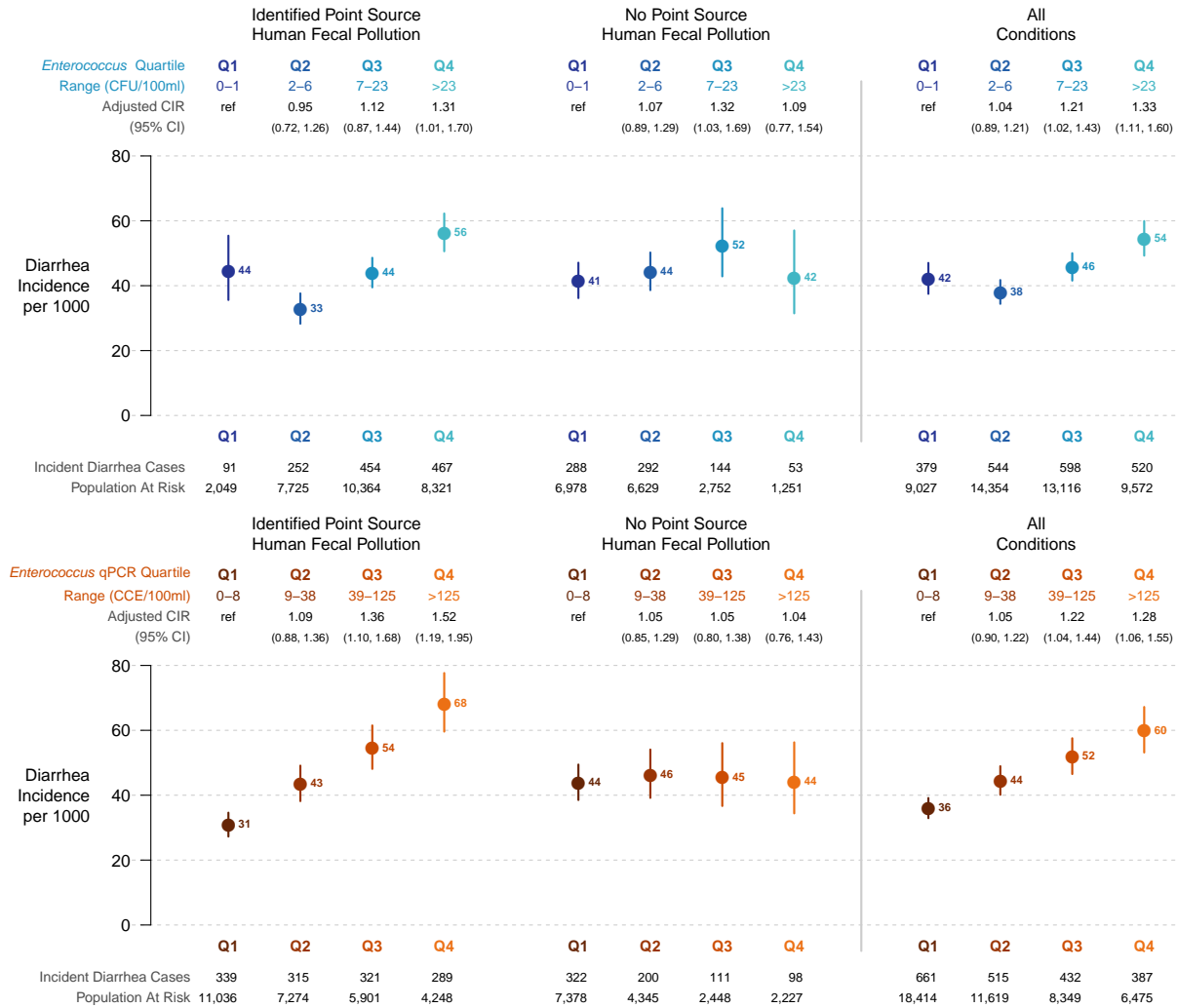


Figure S10: Incident Diarrhea Among Body Immersion Swimmers Associated with Quartiles of *Enterococcus* Concentration, Stratified by Type of Pollution. The top panel plots incidence data by quartiles of *Enterococcus* measured using culture methods (effect modification $P = 0.21$) and the bottom panel plots incidence data by quartiles of *Enterococcus* measured using qPCR (effect modification $P = 0.11$). Cumulative incidence ratios (CIRs) are adjusted for a range of potential confounders and beach level fixed-effects.

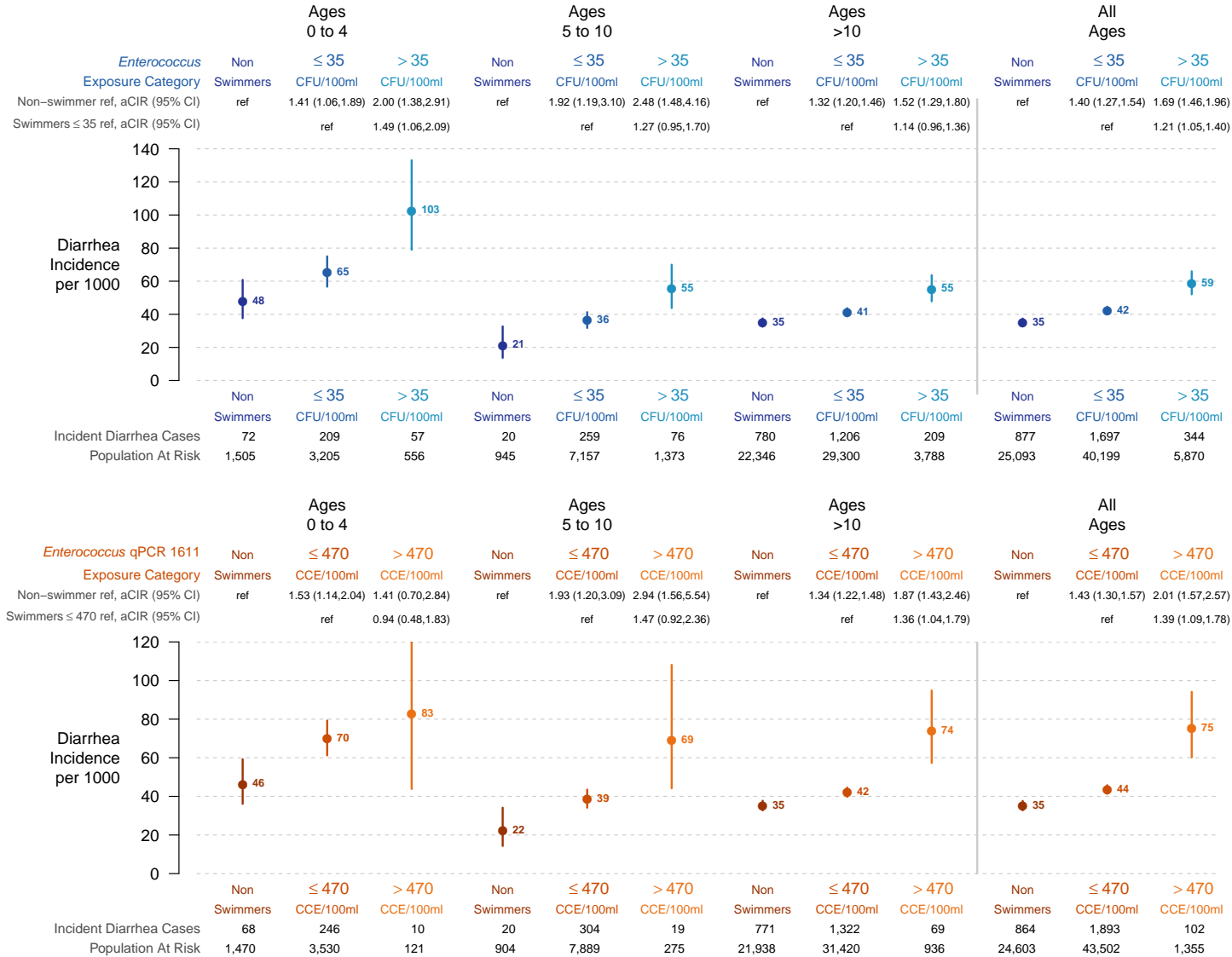


Figure S11: Incident Diarrhea Among Body Immersion Swimmers Associated with *Enterococcus* Levels Above and Below Regulatory Guidelines, Stratified by Age. The top panel plots incidence data by categories of *Enterococcus* measured using culture methods (effect modification $P = 0.01$ [additive] $P = 0.08$ [multiplicative]) and the bottom panel plots incidence data by categories of *Enterococcus* measured using qPCR (effect modification $P = 0.02$ [additive] $P = 0.12$ [multiplicative]). Cumulative incidence ratios (CIRs) are adjusted for a range of potential confounders and beach level fixed-effects. Figure 2 of the main text presents results for *Enterococcus* measured using culture methods for ages 0-4 and all ages.

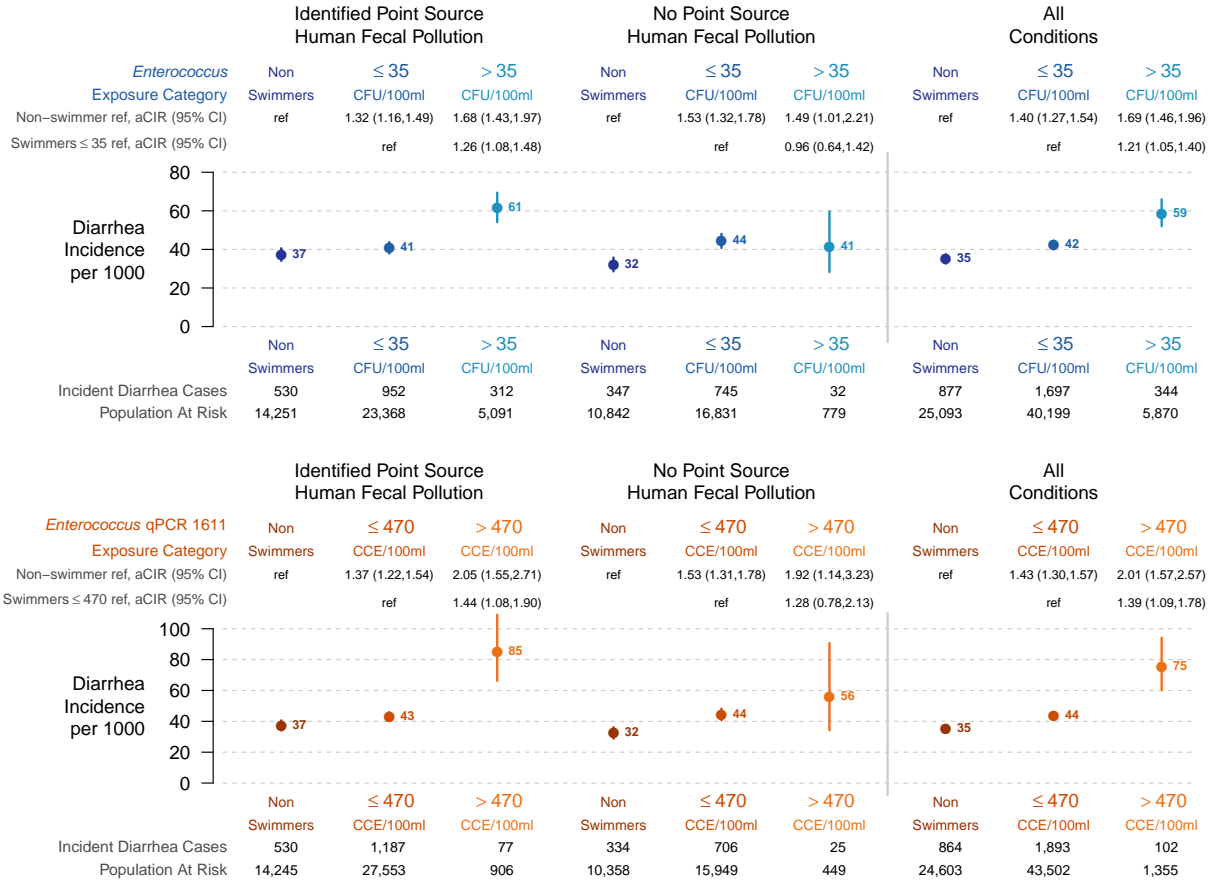


Figure S12: Incident Diarrhea Among Body Immersion Swimmers Associated with *Enterococcus* Levels Above and Below Regulatory Guidelines, Stratified by Type of Pollution. The top panel plots incidence data by quartiles of *Enterococcus* measured using culture methods (effect modification $P = 0.16$), and the bottom panel plots incidence data by quartiles of *Enterococcus* measured using qPCR (effect modification $P = 0.45$). Cumulative incidence ratios (CIRs) are adjusted for a range of potential confounders and beach level fixed-effects.

4 Gastrointestinal Illness Associated With Swim Exposure and *Enterococcus* Exposure

In addition to our primary outcome of incident diarrhea, we also estimated the association between swim exposure and *Enterococcus* level exposure and gastrointestinal illness. As described in the main text, gastrointestinal illness was defined in the same way as prior studies,⁵⁻⁹ using the composite definition: i) diarrhea or ii) vomiting or iii) nausea and stomachache, or iv) nausea or stomachache and missed regular activities as a result of illness. For the gastrointestinal illness outcome, we limited the analyses to our primary exposures delineated in our prespecified analysis plan: levels of swim exposure and *Enterococcus* measured using culture methods.

Overall, the results were highly similar both qualitatively and quantitatively to the diarrhea results. Since diarrhea is an important component of the composite gastrointestinal illness outcome, this is internally consistent. As with the diarrhea analysis, we observed higher incidence among children ages 0-4 compared with the older age groups, as well as a larger increase in incidence on the absolute and relative scale associated with water exposure (effect modification $P = 0.07$; Figure S13). When examining the association between *Enterococcus* levels and illness, we observed a similar pattern for gastrointestinal illness as for diarrhea: stronger associations among younger children (Figure S14). When stratifying beaches by type of pollution source in the gastrointestinal illness analysis, there was no statistical evidence for effect modification ($P = 0.56$), though qualitatively incidence patterns were similar for gastrointestinal illness (Figure S15) and diarrhea (Figure S12).

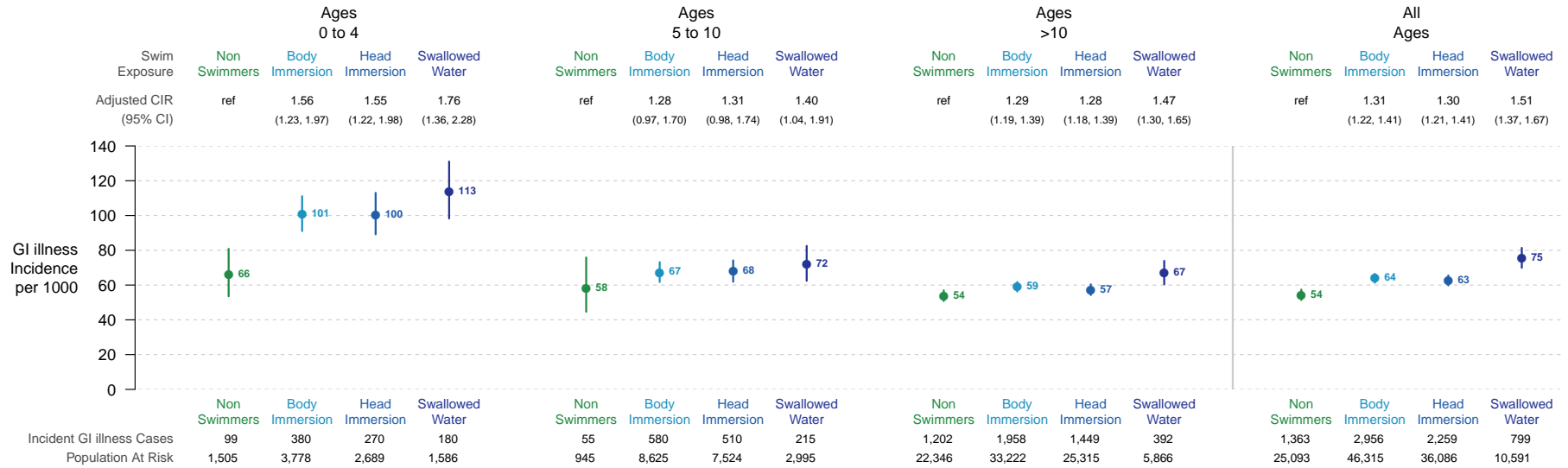


Figure S13: Incident GI Illness Associated with Water Exposure Stratified by Age. Cumulative Incidence Ratios (CIRs) are adjusted for a range of potential confounders and beach level fixed-effects. Tests of effect modification by age: body immersion ($P = 0.06$), head immersion ($P = 0.16$), swallowed water ($P = 0.31$).

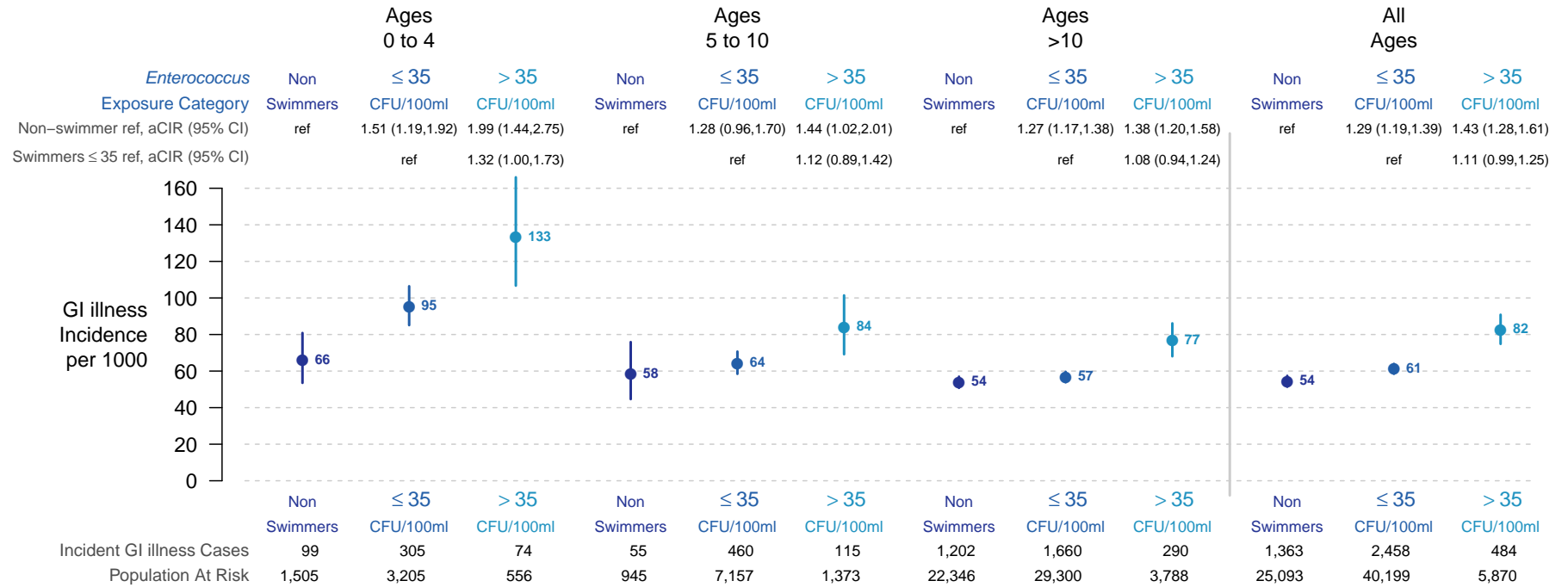


Figure S14: Incident GI illness Among Beachgoers Associated with *Enterococcus* Concentrations Above and Below Regulatory Guidelines, Stratified by Age. Adjusted cumulative incidence ratios (aCIRs) are adjusted for a range of potential confounders and beach level fixed-effects, and are computed using two different reference groups: non-swimmers and swimmers exposed below EPA regulatory guidelines for *Enterococcus*: ≤35 colony forming units (CFU) per 100ml. Test of effect modification by age: $P = 0.07$.

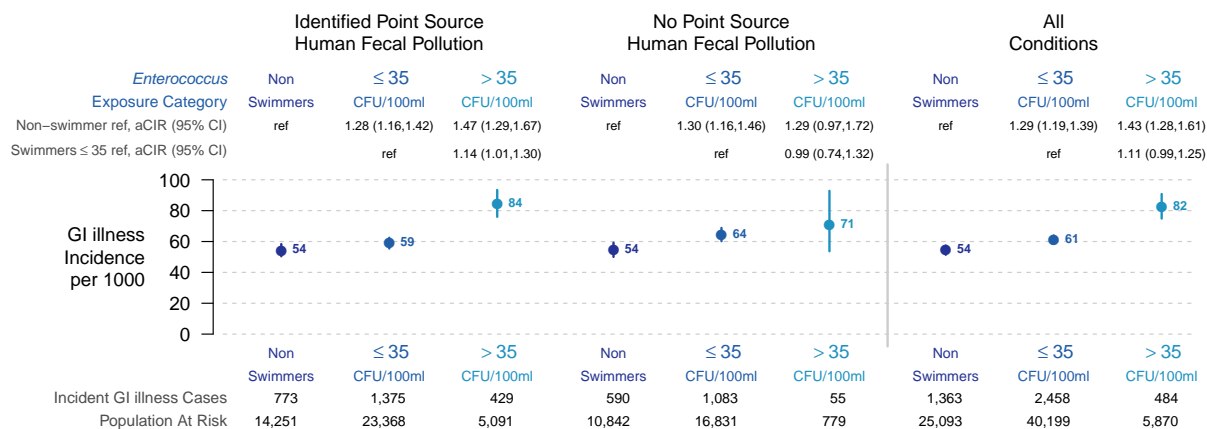


Figure S15: Incident GI illness Among Beachgoers Associated with *Enterococcus* Concentrations Above and Below Regulatory Guidelines, Stratified by Pollution Type. Adjusted cumulative incidence ratios (aCIRs) are adjusted for a range of potential confounders and beach level fixed-effects, and are computed using two different reference groups: non-swimmers and swimmers exposed below EPA regulatory guidelines for *Enterococcus*: ≤ 35 colony forming units (CFU) per 100ml. Test of effect modification by pollution type: $P = 0.61$.

5 Negative Control and Sensitivity Analyses

This section includes a summary of pre-specified negative control analyses to test the robustness of the findings to bias from unmeasured confounding or measurement error, as well as pre-specified sensitivity analyses to ensure that our results were not an artifact of decisions made about swim exposure level, choice of modeling strategy (quartiles versus continuous exposure) or length of follow-up period (ranging from 1 to 10 days).

Negative Control Analyses: We matched *Enterococcus* levels measured in the water to people who were at the beach that day but did not have any water contact as a negative control exposure analysis^{15,16} – without water exposure, the association between *Enterococcus* levels and diarrhea incidence should be null, and indeed that is what we observed (Table S5). This lends additional credibility to the results because it suggests that the effects observed are not an artifact of unobserved confounding or measurement error.^{15,16}

Table S5: Negative Control Analyses. The relationship between *Enterococcus* and diarrhea incidence among non-swimmers (no water contact). *Enterococcus* exposures were considered as quartiles and as a categorical exposure above and below EPA regulatory guidelines.

Exposure	N at Risk	N Cases	Incidence per 1,000 (95% CI)	Adjusted CIR (95% CI)
Enterococcous				
Quartiles				
Q1	4,247	139	33 (28, 39)	ref
Q2	6,992	222	32 (28, 36)	0.99 (0.79, 1.25)
Q3	6,809	253	37 (33, 42)	1.06 (0.83, 1.37)
Q4	6,555	250	38 (34, 43)	1.05 (0.80, 1.38)
Enterococcus				
≤ 35 CFU/100ml	19,804	677	34 (32, 37)	ref
>35 CFU/100ml	4,799	187	39 (34, 45)	1.02 (0.85, 1.23)
Enterococcous qPCR				
Quartiles				
Q1	6,276	203	32 (28, 37)	ref
Q2	6,433	242	38 (33, 43)	1.03 (0.82, 1.29)
Q3	5,466	191	35 (30, 41)	1.01 (0.78, 1.32)
Q4	5,138	188	37 (32, 42)	0.92 (0.70, 1.20)
Enterococcus qPCR				
≤ 470 CCE/100ml	22,253	779	35 (33, 38)	ref
>470 CCE/100ml	1,060	45	42 (31, 57)	0.97 (0.70, 1.34)

Sensitivity Analysis - Water Exposure Level: As described in our statistical analysis plan (available in the supporting information), we chose body immersion swimming as our primary swim exposure level for the *Enterococcus* analyses and population attributable risk analyses to ensure that we included the largest number of participants in the analysis and because we had observed in the individual studies that contributed to this pooled analysis that the risk was similar for body immersion swimming and head immersion swimming. When we estimated the association between *Enterococcus* levels and illness among swimmers with higher levels of exposure, we found similar results to those observed for body immersion swimmers in terms of relative risk measured by the cumulative incidence ratio, albeit with lower precision due to smaller sample sizes (Figure S16). Consistent with the swim exposure analyses, the overall incidence of diarrhea was higher among individuals who swallowed water, but the relative increase in risk with increasing concentration of *Enterococcus* was very similar to body immersion swimmers.

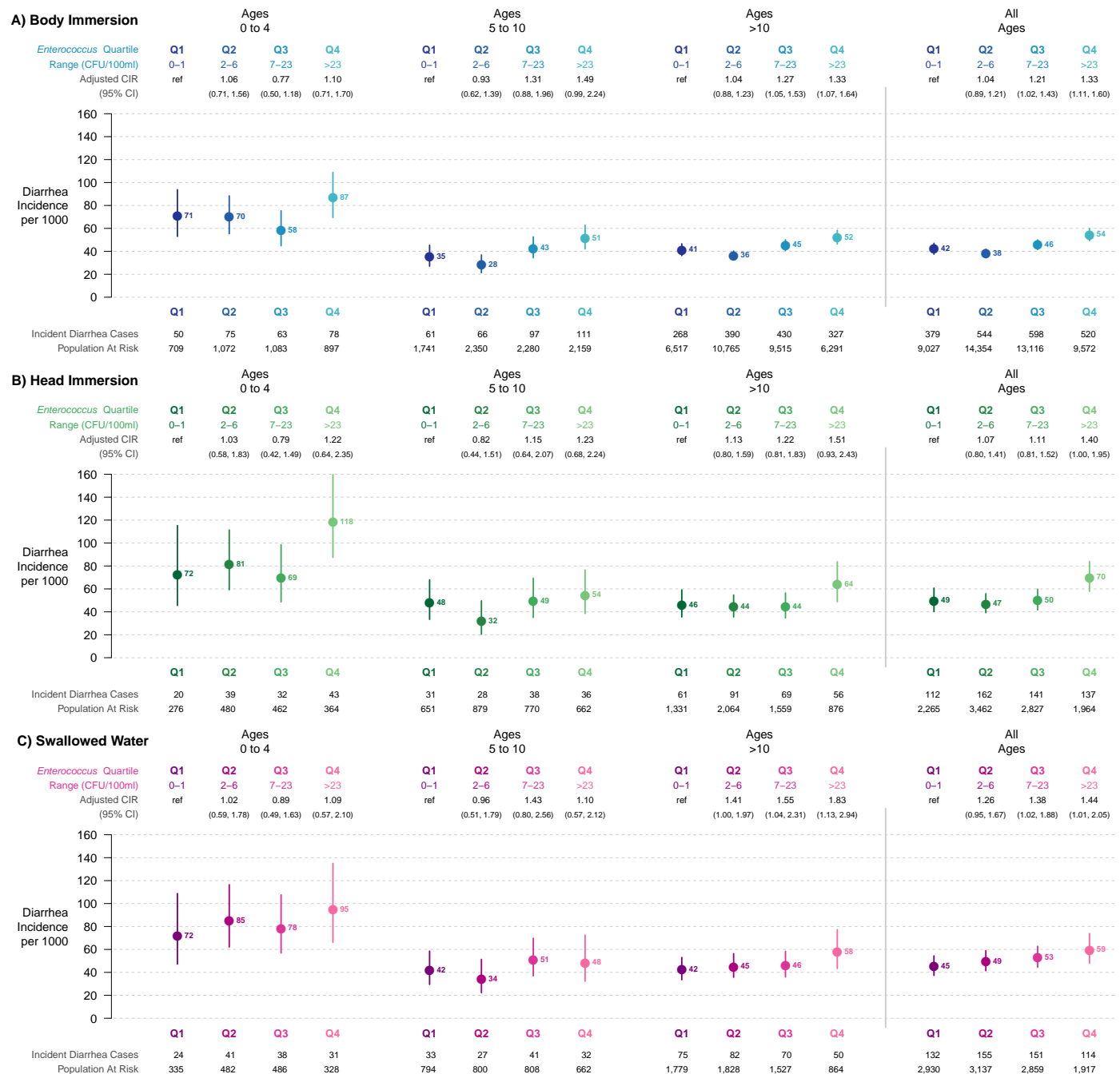


Figure S16: Association between *Enterococcus* quartiles and diarrhea incidence by age. **A)** Among body immersion swimmers (identical to Figure 2 in the main text). **B)** Among head immersion swimmers. **C)** Among beachgoers who swallowed water. *Enterococcus* was measured using EPA method 1600 except at the Mission Bay beach, where it was measured using the Enterolert assay.

Sensitivity Analysis - Continuous Exposure: To provide summary results that were consistent with the modeling approach used in many previously published studies, we estimated the relationship between \log_{10} *Enterococcus* concentrations on a continuous scale and the risk of diarrhea during follow-up. This analysis imposes stronger modeling assumptions on the relationship between *Enterococcus* concentrations and diarrhea risk, but is a complementary approach to the quartile analysis used in the main text. Consistent with our primary analysis, we modeled the probability of diarrhea during the 10 days of follow-up among body immersion swimmers using a log-linear, modified Poisson model with robust standard errors clustered at the household level. This model estimates the cumulative incidence ratio (CIR) associated with a \log_{10} increase in *Enterococcus* concentration. We estimated adjusted CIRs using the same set of potential confounding variables used in the primary analysis (see the Statistical Analysis Plan in the Supporting Materials for details).

We calculated marginally adjusted exposure-response curves based on the model fit using marginal standardization over the empirical distribution of covariates in the study population.^{17,18} We calculated point-wise standard errors and 95% confidence intervals for the curves using a bootstrap that re-sampled households with replacement, stratified by beach and 1,000 iterations.¹⁷

We estimated that a \log_{10} increase in *Enterococcus* measured using culture methods was associated with a 15% relative increase in the probability of diarrhea in the 10 days following the beach visit (CIR=1.15, 95%CI: 1.05, 1.26; Figure S17). Consistent with the primary analysis, we observed effect modification by age (effect modification $P=0.07$), with no clear relationship among children ages 0-4 years, and the strongest relationship among children ages 5-10 years (Figure S18). Contrary to the primary analysis, we did not observe effect modification by pollution type in this analysis (effect modification $P=0.97$) (Figure S19).

The increase in diarrhea incidence associated with a \log_{10} increase in *Enterococcus* EPA 1611 qPCR was similar to the EPA 1600 assay (CIR=1.13, 95%CI: 1.05, 1.22; Figure S20). We observed no effect modification by age (effect modification $P = 0.34$) (Figure S21), but consistent with the primary analysis, there was some evidence for effect modification by pollution type (effect modification $P = 0.08$) (Figure S22).

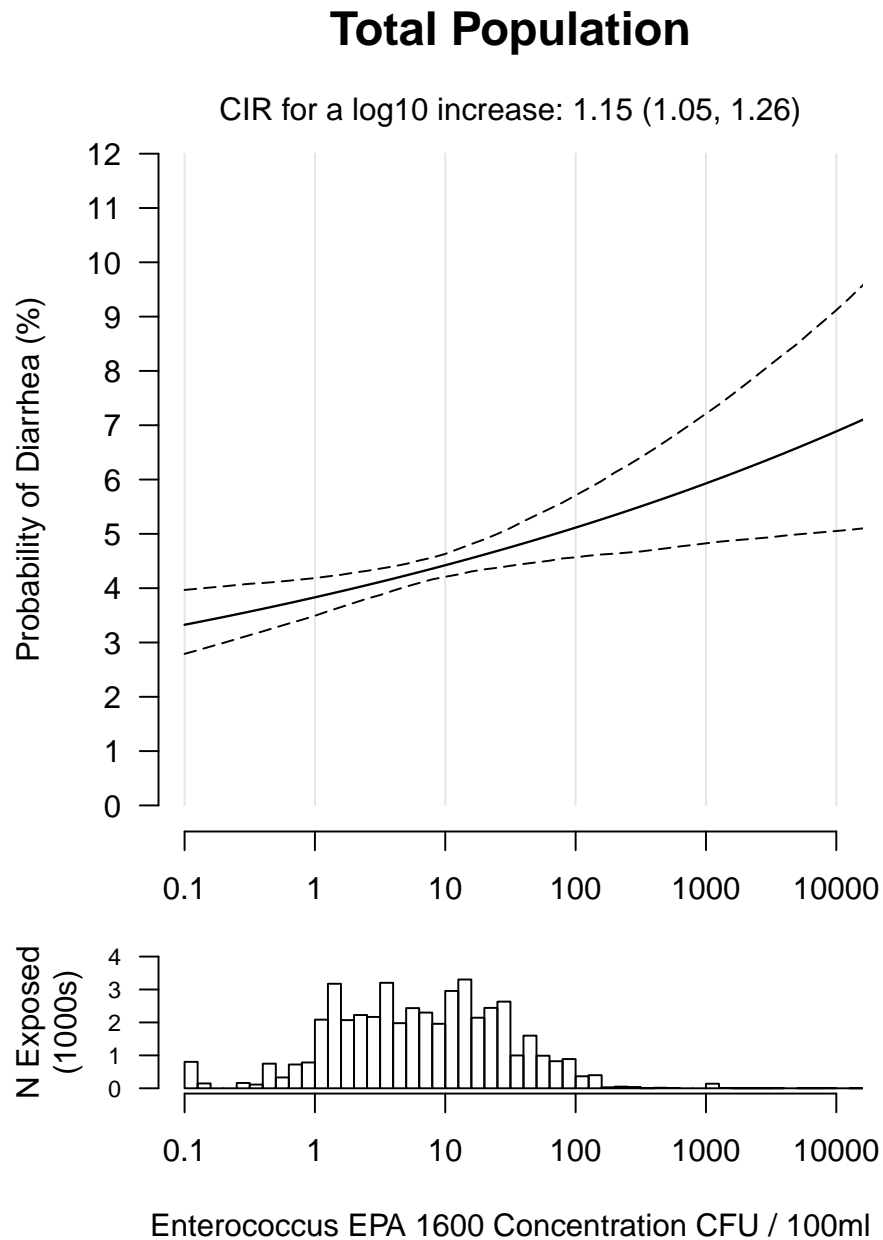


Figure S17: Association between *Enterococcus* concentration (EPA 1600 or Enterolert) and incident diarrhea among body immersion swimmers. The cumulative incidence ratio (CIR) and exposure-response curve were estimated using an adjusted log-linear regression model. Dashed lines are bootstrapped 95% confidence intervals.

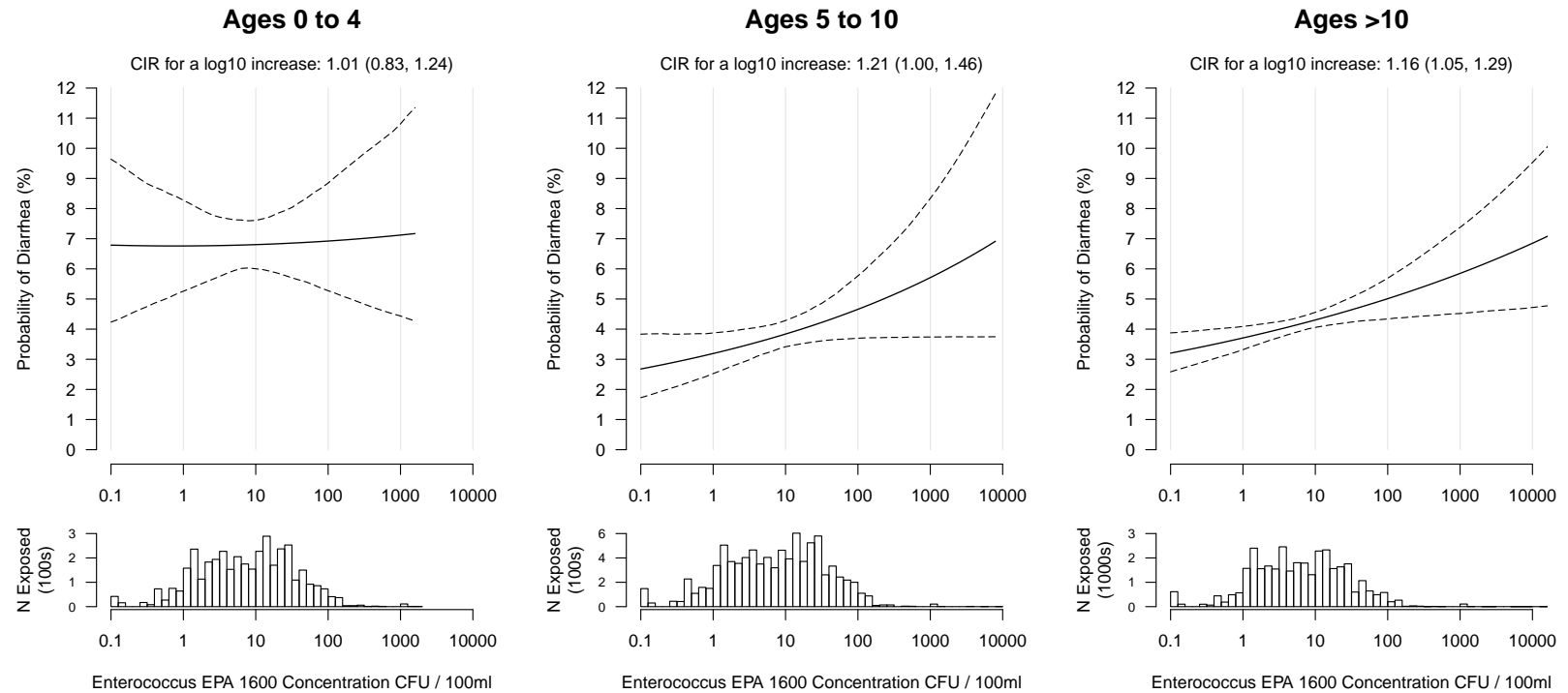


Figure S18: Association between *Enterococcus* concentration (EPA 1600 or Enterolert) and incident diarrhea among body immersion swimmers, stratified by age group. Cumulative incidence ratios (CIRs) and curves were estimated using a log-linear regression model. Note that the Y-scale differs on the number exposed across plots to display the distributions more clearly given differences in sample size across age groups. Dashed lines are bootstrapped 95% confidence intervals.

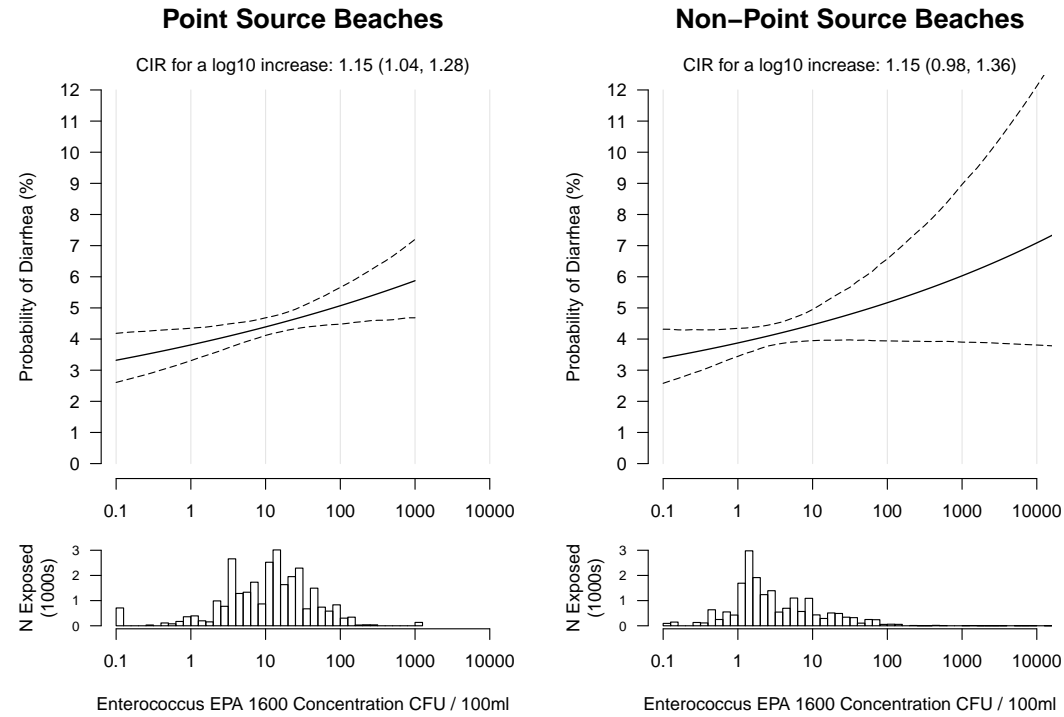


Figure S19: Association between *Enterococcus* concentration (EPA 1600 or Enterolert) and incident diarrhea among body immersion swimmers, stratified by type of pollution. Cumulative incidence ratios (CIRs) and curves were estimated using a log-linear regression model. Dashed lines are bootstrapped 95% confidence intervals.

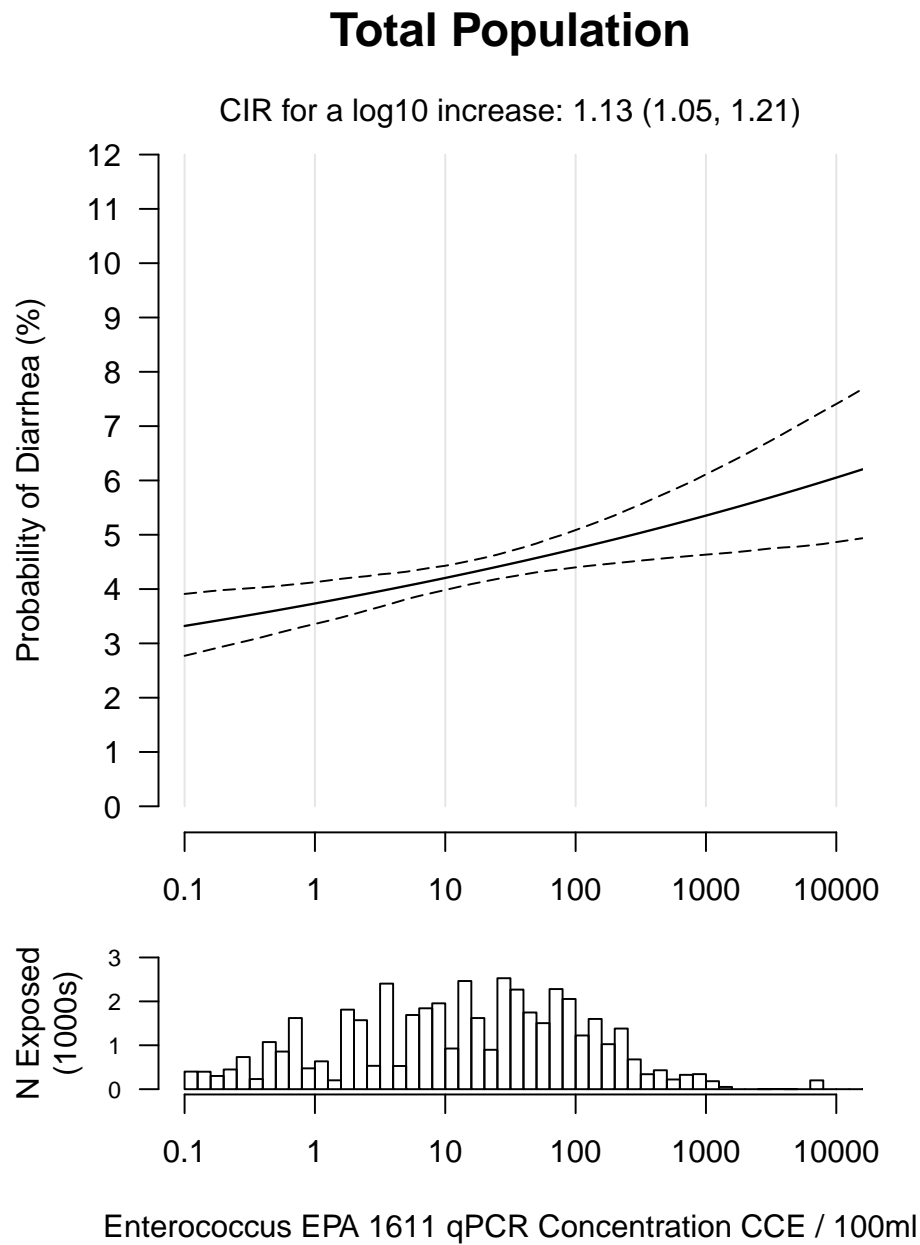


Figure S20: Association between *Enterococcus* qPCR EPA 1611 qPCR concentration and incident diarrhea among body immersion swimmers. The cumulative incidence ratio (CIR) and exposure-response curves were estimated using a log-linear regression model. Dashed lines are bootstrapped 95% confidence intervals.

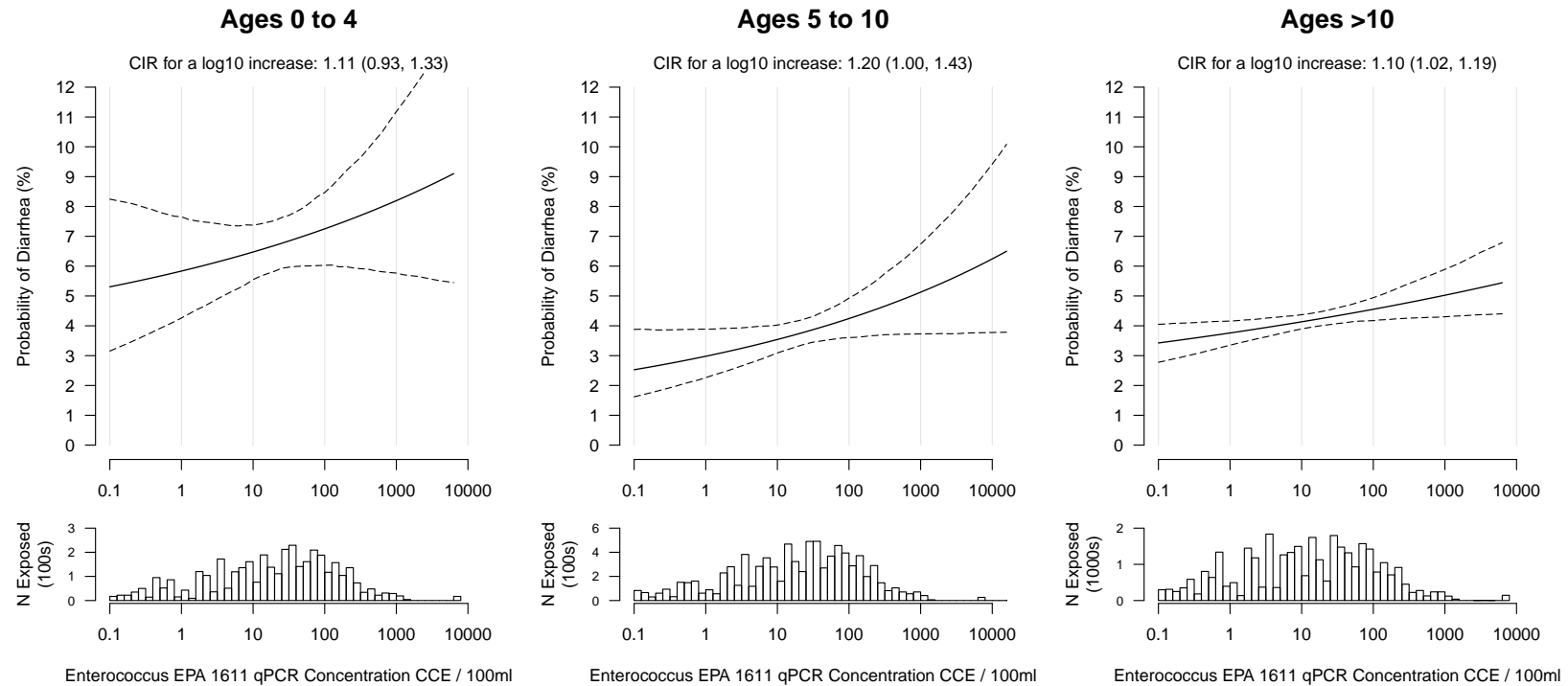


Figure S21: Association between *Enterococcus* qPCR EPA 1611 concentration and incident diarrhea among body immersion swimmers, stratified by age group. Cumulative incidence ratios (CIRs) and curves were estimated using an adjusted log-linear regression model. Note that the Y-scale differs on the number exposed across plots to display the distributions more clearly given differences in sample size across age groups. Dashed lines are bootstrapped 95% confidence intervals.

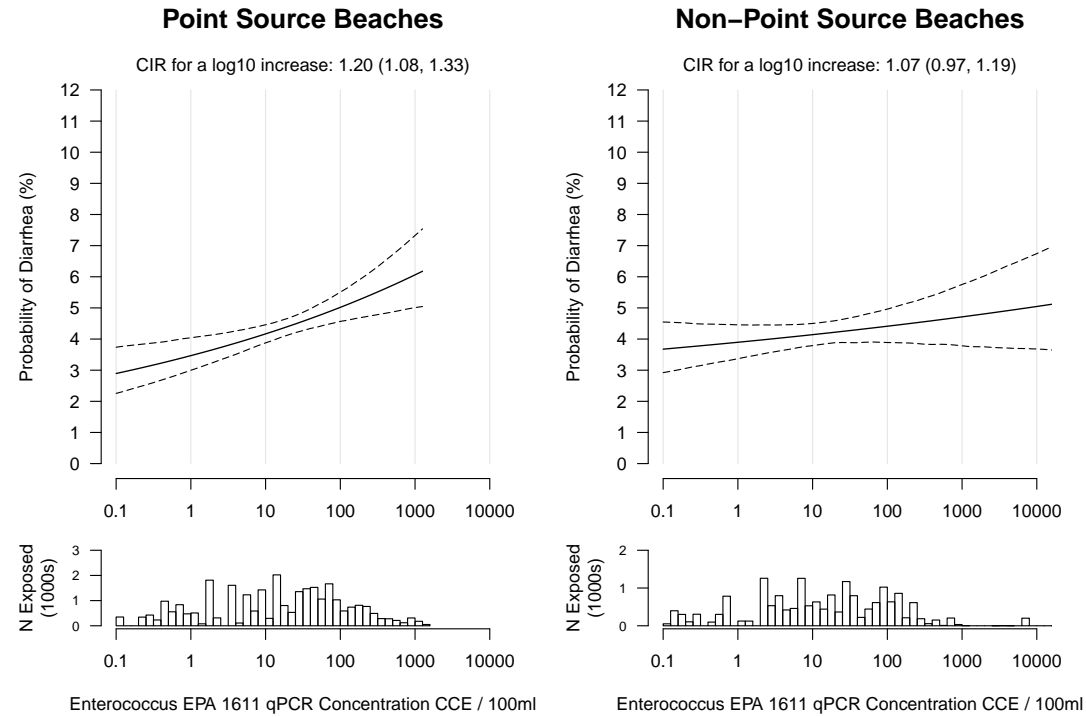


Figure S22: Association between *Enterococcus* qPCR EPA 1611 concentration and incident diarrhea among body immersion swimmers, stratified by type of pollution. Cumulative incidence ratios (CIRs) and curves were estimated using a log-linear regression model. Dashed lines are bootstrapped 95% confidence intervals.

Sensitivity Analysis - Length of Follow-up: An earlier analysis from the Malibu cohort demonstrated that the greatest increase daily diarrhea incidence among swimmers compared to non-swimmers was in the first 3 days following the beach visit.⁸ When we examined daily incidence patterns across all cohorts, we observed a similar pattern (Figure S23). We additionally re-estimated the association between body immersion swim exposure and incident diarrhea, as well as *Enterococcus* exposure and incident diarrhea among swimmers (Figure S24). Consistent with the analysis of the Malibu cohort,⁸ there was an attenuation of the cumulative incidence ratio (CIR) associated with body immersion swim exposure with follow-up longer than 3 days (Figure S24, A). However, length of follow-up period did not strongly influence the magnitude of association between *Enterococcus* levels and diarrhea incidence – longer follow-up periods had similar CIR estimates to shorter periods, but with narrower confidence intervals due to substantially larger numbers of cumulative incident cases (Figure S24, B-C).

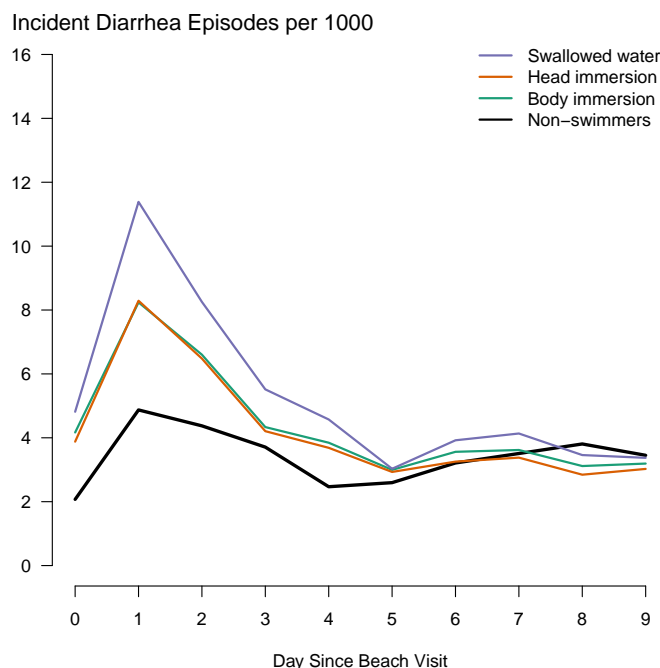
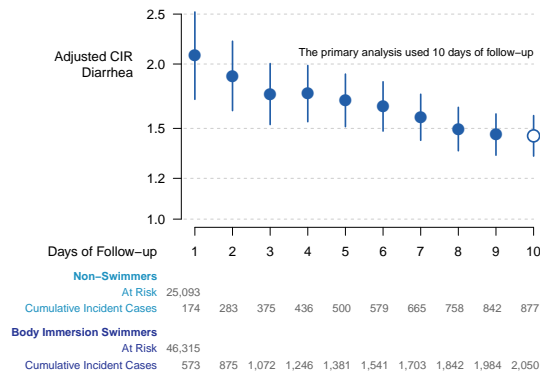
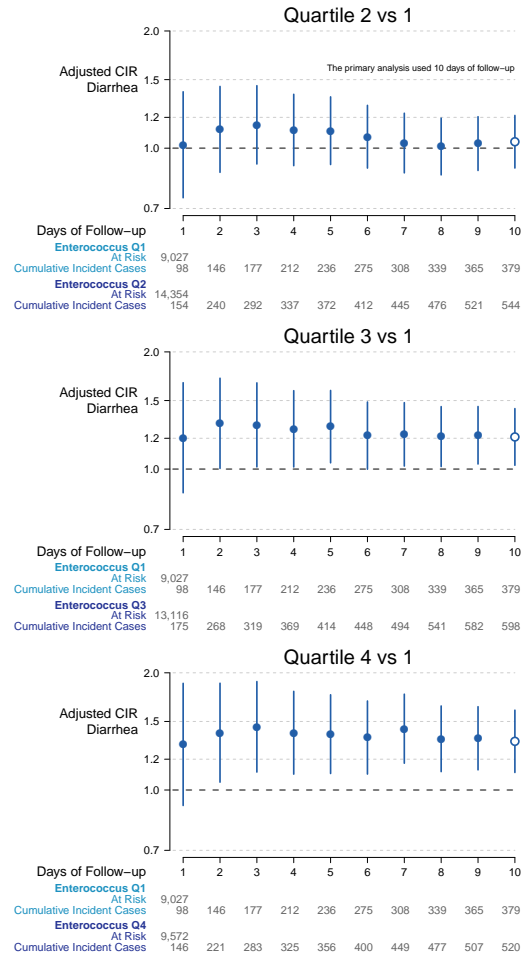


Figure S23: Daily Incidence of Diarrhea by Level of Water Exposure.

A)



B)



C)

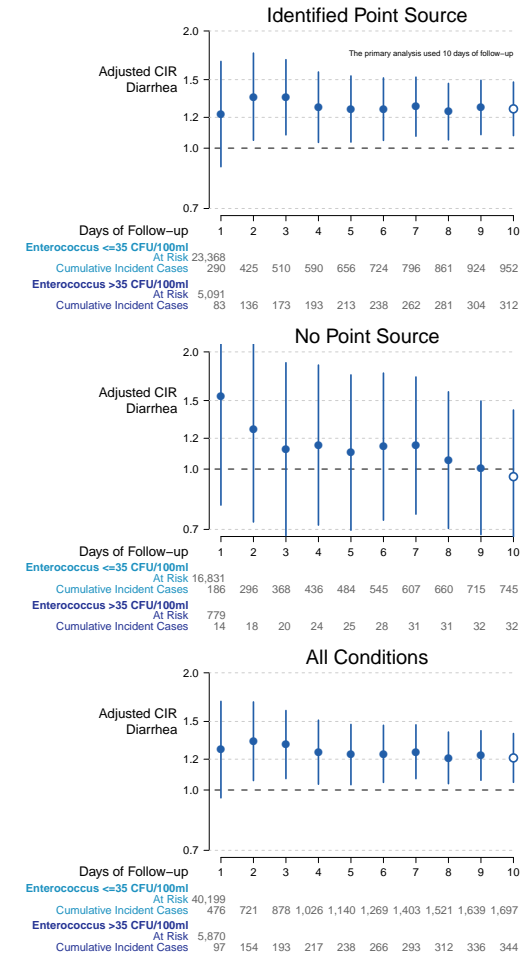


Figure S24: Sensitivity analysis of the length of follow-up period on cumulative incidence ratio (CIR) estimates. **A)** Body immersion swim exposure analysis. **B)** *Enterococcus* by culture methods (EPA 1600 or Enterolert) quartile analysis. **C)** *Enterococcus* >35 CFU/100ml analysis, stratified by beach type.

6 Population Attributable Risk Results for *Enterococcus* Exposure

This section includes detailed estimates of population attributable risk (PAR) and population attributable fraction (PAF) among swimmers for exposure to *Enterococcus* levels above 35 colony forming units (CFU) per 100ml (the current EPA regulatory guideline). There are two separate calculations in this section.

First, we estimated the population attributable risk for exposure to water >35 CFU/100 ml among beachgoers, using non-swimmers as the reference group. The implied intervention is closing beaches when *Enterococcus* levels exceed 35 CFU / 100 ml. These results are summarized in Figure 3 of the main text and in Table S6 for all outcomes. For acute diarrhea, gastroenteritis, and missed daily activities (work, school, vacation) associated with gastroenteritis, we found that the risk attributable to this exposure was far larger among young children. However, we estimated no attributable of days missed paid work or medical visits/consultations associated with this exposure.

Second, we estimated the population attributable risk for exposure to water >35 CFU/100 ml among *swimmers*, using swimmers exposed below 35 CFU/100 ml as the reference group. The implied intervention in this counterfactual is that beaches would always have water quality that met regulatory guidelines. Since this is a less extreme change in exposure compared with preventing people from swimming on poor water quality days, the attributable risk and fraction values were smaller (Table S7).

Table S6: Population Attributable Risk Among Beachgoers Due to Swimming in Water That Exceeds the EPA Guideline of *Enterococcus* >35 CFU/100ml.

	N Events	N At Risk	Predicted Incidence ¹ per 1000		Population Attributable Risk ² (95% CI)		Population Attributable Fraction ³ (95% CI)	
			Observed Exposure	No Swimming >35 CFU/100ml				
Diarrhea, episodes								
All Ages	3,389	82,021	41	39	2.0	(1.4, 2.6)	5%	(3%, 6%)
Age Stratified								
Ages 0 to 4	396	6,344	62	57	5.5	(2.1, 8.7)	9%	(3%, 14%)
Ages 5 to 10	389	10,496	37	33	4.5	(1.9, 6.8)	12%	(5%, 18%)
Ages >10	2,571	64,041	40	39	1.4	(0.8, 2.0)	3%	(2%, 5%)
Gastrointestinal illness⁴, episodes								
All Ages	4,992	82,021	61	59	2.0	(1.3, 2.8)	3%	(2%, 5%)
Age Stratified								
Ages 0 to 4	560	6,344	88	81	7.3	(3.5, 11.1)	8%	(4%, 12%)
Ages 5 to 10	689	10,496	66	62	3.3	(-0.1, 6.4)	5%	(-0%, 10%)
Ages >10	3,694	64,041	58	56	1.5	(0.8, 2.2)	3%	(1%, 4%)
Missed Daily Activities⁵, days								
All Ages	4,509	82,021	55	54	1.3	(0.2, 2.4)	2%	(0%, 4%)
Age Stratified								
Ages 0 to 4	438	6,344	70	62	7.8	(2.5, 13.4)	11%	(4%, 19%)
Ages 5 to 10	676	10,496	64	63	1.5	(-3.0, 6.3)	2%	(-5%, 10%)
Ages >10	3,357	64,041	52	51	1.0	(-0.0, 2.1)	2%	(-0%, 4%)
Missed Paid Work⁶, days								
All Ages ⁸	1,043	82,021	13	13	-0.0	(-0.4, 0.4)	na	
Medical Visits⁷, events								
All Ages ⁸	915	82,021	11	11	0.1	(-0.2, 0.4)	1%	(-2%, 4%)

1. Predicted incidence per 1000 among body immersion swimmers under the empirical distribution of *Enterococcus* exposure (observed) and under a counterfactual scenario where nobody entered the water in conditions >35 CFU/100ml. Estimates are from a multivariable regression model adjusted for a range of potential confounders and beach level fixed-effects (see statistical analysis plan for details).
2. Population Attributable Risk is the number of events per 1000 swimmers that would be prevented if the exposure of swimming in water with *Enterococcus* ≥ 35 CFU/100ml were removed from the population. The proportion of beachgoers who swam in water with *Enterococcus* >35 CFU/100ml was: all ages (10%), ages 0-4 (12%), ages 5-10 (15%), ages >10 (8%).
3. Population Attributable Fraction is the percentage of events among beachgoers attributable to swimming in water with *Enterococcus* ≥ 35 CFU/100ml.
4. Gastrointestinal illness was defined as (i) diarrhea or (ii) vomiting or (iii) stomach cramps and missed daily activities or (iv) nausea and missed daily activities.
5. Includes days of school, work, or vacation missed because of gastrointestinal illness.
6. Includes work days missed because of gastrointestinal illness.
7. Includes phone consultations, outpatient visits, and emergency room visits due to gastrointestinal illness.
8. Outcome incidence was too rare to calculate age-stratified estimates.

Table S7: Population Attributable Risk Among Body Immersion Swimmers Due to Swimming in Water That Exceeds the EPA Guideline of *Enterococcus* >35 CFU/100ml.

			Predicted Incidence ¹ per 1000		Population Attributable Risk ² (95% CI)		Population Attributable Fraction ³ (95% CI)	
	N Events	N At Risk	Observed Exposure	All ≤35 CFU/100ml				
Diarrhea, episodes								
All Ages	2,041	46,069	44	43	1.3	(0.4, 2.3)	3%	(1%, 5%)
Age Stratified								
Ages 0 to 4	266	3,761	71	66	5.0	(0.7, 9.5)	7%	(1%, 14%)
Ages 5 to 10	335	8,530	39	37	1.9	(-0.4, 4.5)	5%	(-1%, 11%)
Ages >10	1,415	33,088	43	42	0.8	(-0.3, 1.9)	2%	(-1%, 4%)
Gastrointestinal illness⁴, episodes								
All Ages	2,942	46,069	64	63	1.0	(-0.2, 2.2)	2%	(-0%, 3%)
Age Stratified								
Ages 0 to 4	379	3,761	101	95	5.6	(0.6, 10.8)	6%	(1%, 11%)
Ages 5 to 10	575	8,530	67	66	1.4	(-1.5, 4.3)	2%	(-2%, 6%)
Ages >10	1,950	33,088	59	58	0.5	(-0.6, 1.8)	1%	(-1%, 3%)
Missed Daily Activities⁵, days								
All Ages	2,677	46,069	58	57	1.1	(-0.7, 3.0)	2%	(-1%, 5%)
Age Stratified								
Ages 0 to 4	328	3,761	87	78	8.6	(0.7, 17.3)	10%	(1%, 19%)
Ages 5 to 10	557	8,530	65	65	-0.0	(-4.1, 4.0)	na	
Ages >10	1,770	33,088	54	53	0.7	(-1.2, 2.9)	1%	(-2%, 5%)
Missed Paid Work⁶, days								
All Ages ⁸	596	46,069	13	13	0.1	(-0.6, 0.8)	1%	(-5%, 6%)
Medical Visits⁷, events								
All Ages ⁸	583	46,069	13	13	0.1	(-0.4, 0.7)	1%	(-3%, 5%)

1. Predicted incidence per 1000 among body immersion swimmers under the empirical distribution of *Enterococcus* exposure (observed) and under a counterfactual scenario where water conditions never exceeded 35 CFU/100ml. Estimates are from a multivariable regression model adjusted for a range of potential confounders and beach level fixed-effects (see statistical analysis plan for details).
2. Population Attributable Risk is the number of events per 1000 swimmers that would be prevented if the exposure of swimming in water with *Enterococcus* ≥35 CFU/100ml were removed from the population. The proportion of swimmers exposed to water with *Enterococcus* >35 CFU/100ml was: all ages (13%), ages 0-4 (15%), ages 5-10 (16%), ages >10 (11%).
3. Population Attributable Fraction is the percentage of events among swimmers attributable to swimming in water with *Enterococcus* ≥35 CFU/100ml.
4. Gastrointestinal illness was defined as (i) diarrhea or (ii) vomiting or (iii) stomach cramps and missed daily activities or (iv) nausea and missed daily activities.
5. Includes days of school, work, or vacation missed because of gastrointestinal illness.
6. Includes work days missed because of gastrointestinal illness.
7. Includes phone consultations, outpatient visits, and emergency room visits due to gastrointestinal illness.
8. Outcome incidence was too rare to calculate age-stratified estimates.

References

1. Colford Jr. JM, Wade TJ, Schiff KC, et al. Recreational Water Contact and Illness in Mission Bay, California. Tech. rep. 449. SCCWRP, 2005.
2. Colford JM, Wade TJ, Schiff KC, et al. Water quality indicators and the risk of illness at beaches with nonpoint sources of fecal contamination. *Epidemiology* 2007;18:27–35.
3. Wade TJ, Calderon RL, Sams E, et al. Rapidly measured indicators of recreational water quality are predictive of swimming-associated gastrointestinal illness. *Environ. Health Perspect.* 2006;114:24–28.
4. Wade TJ, Calderon RL, Brenner KP, et al. High sensitivity of children to swimming-associated gastrointestinal illness: results using a rapid assay of recreational water quality. *Epidemiology* 2008;19:375–383.
5. Wade TJ, Sams E, Brenner KP, et al. Rapidly measured indicators of recreational water quality and swimming-associated illness at marine beaches: a prospective cohort study. *Environ. Health* 2010;9:66.
6. Wade TJ, Sams EA, Haugland R, et al. Report on 2009 National Epidemiologic and Environmental Assessment of Recreational Water Epidemiology Studies. Tech. rep. EPA/600/R-10/168. United States Environmental Protection Agency, 2010.
7. Colford JM, Schiff KC, Griffith JF, et al. Using rapid indicators for *Enterococcus* to assess the risk of illness after exposure to urban runoff contaminated marine water. *Water Res.* 2012;46:2176–2186.
8. Arnold BF, Schiff KC, Griffith JF, et al. Swimmer illness associated with marine water exposure and water quality indicators: impact of widely used assumptions. *Epidemiology* 2013;24:845–853.
9. Yau VM, Schiff KC, Arnold BF, et al. Effect of submarine groundwater discharge on bacterial indicators and swimmer health at Avalon Beach, CA, USA. *Water Res.* 2014;59:23–36.
10. EPA. Method 1600: Enterococci in Water by Membrane Filtration Using membrane-Enterococcus Indoxyl-Beta-D-Glucoside Agar (mEI). Tech. rep. EPA-821-R-09-016. U.S. Environmental Protection Agency, 2009.
11. EPA. Method 1611: Enterococci in Water by TaqMan® Quantitative Polymerase Chain Reaction (qPCR) Assay. Tech. rep. EPA-821-R-12-008. U.S. Environmental Protection Agency, 2012.
12. Siefring S, Varma M, Atikovic E, Wymer L, and Haugland RA. Improved real-time PCR assays for the detection of fecal indicator bacteria in surface waters with different instrument and reagent systems. *J. Water Health* 2008;6:225–237.
13. Higgins J and Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat. Med.* 2002;21:1539–1558.
14. Higgins JPT, Thompson SG, Deeks JJ, and Altman DG. Measuring inconsistency in meta-analyses. *BMJ* 2003;327:557–560.

15. Lipsitch M, Tchetgen ET, and Cohen T. Negative controls: a tool for detecting confounding and bias in observational studies. *Epidemiology* 2010;21:383–388.
16. Arnold BF, Ercumen A, Benjamin-Chung J, and Colford JM. Negative controls to detect selection bias and measurement bias in epidemiologic studies. *Epidemiology* 2016;(in press).
17. Ahern J, Hubbard A, and Galea S. Estimating the effects of potential public health interventions on population disease burden: a step-by-step illustration of causal inference methods. *Am. J. Epidemiol.* 2009;169:1140–1147.
18. Muller CJ and MacLehose RF. Estimating predicted probabilities from logistic regression: different methods correspond to different target populations. *Int. J. Epidemiol.* 2014;43:962–970.