Human viruses and viral indicators in marine water at two recreational beaches in Southern California, USA

David C. Love, Roberto A. Rodriguez, Christopher D. Gibbons, John F. Griffith, Qilu Yu, Jill R. Stewart and Mark D. Sobsey

ABSTRACT

Waterborne enteric viruses may pose disease risks to bather health but occurrence of these viruses has been difficult to characterize at recreational beaches. The aim of this study was to evaluate water for human virus occurrence at two Southern California recreational beaches with a history of beach closures. Human enteric viruses (adenovirus and norovirus) and viral indicators (F+ and somatic coliphages) were measured in water samples over a 4-month period from Avalon Beach, Catalina Island ($n = 324$) and Doheny Beach, Orange County ($n = 112$). Human viruses were concentrated from 40 L samples and detected by nested reverse transcriptase polymerase chain reaction (PCR). Detection frequencies at Doheny Beach were 25.5% (adenovirus) and 22.3% (norovirus), and at Avalon Beach were 9.3% (adenovirus) and 0.7% (norovirus). Positive associations between adenoviruses and fecal coliforms were observed at Doheny ($p = 0.02$) and Avalon ($p = 0.01$) Beaches. Human viruses were present at both beaches at higher frequencies than previously detected in the region, suggesting that the virus detection methods presented here may better measure potential health risks to bathers. These virus recovery, concentration, and molecular detection methods are advancing practices so that analysis of enteric viruses can become more effective and routine for recreational water quality monitoring.

Key words | adenovirus, bathing water, coliphage, detection methods, norovirus

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INTRODUCTION

Enteric viruses can enter recreational waters from runoff or sewage and pose risks to human health (Okoh *et al.* 2010). Some of the viruses with a history of causing human waterborne illnesses like gastroenteritis are in the *Adenoviridae*, *Astroviridae*, *Caliciviridae*, *Picornaviridae*, and *Reoviridae* families (Bosch 1998; Okoh *et al.* 2010). Exposure to even low doses of these viruses in water can cause infections. For example, the probability of human infection from a single virus is as high as 30% for rotavirus (Gerba *et al.* 1996) and 50% for norovirus (Teunis *et al.* 2008).

Los Angeles and Orange Counties, California, USA, are in a heavily urbanized region with a favorable climate and 160 km of coastal shoreline that attracts recreational bathers in large numbers. Self-limiting diseases, like many of the gastrointestinal, skin, and respiratory illnesses acquired by beachgoers, may not require medical attention and therefore go unreported to public health agencies. However, it is estimated that beachgoers in Southern California experience between 627,800 and 1,479,200 gastrointestinal illnesses each year (Given *et al.* 2006). Epidemiologic studies in Southern California have confirmed infectious disease risks and health effects associated with water contact for beachgoers (Colford *et al.* 2007; 2012).

To protect bathers, the United States Environmental Protection Agency (USEPA) has developed water quality criteria and guidelines based on the levels of fecal indicator bacteria (FIB), e.g. *Escherichia coli* and enterococci, which correlate with risks of disease symptoms (Cabelli 1983;

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Dufour 1984; USEPA 2012). Molecular tools to detect these bacteria have been developed (Haugland *et al.* 2005; Noble *et al.* 2010) and benchmarked to health outcomes (Wade *et al.* 2006, 2010). However, the actual agents that cause disease among bathers have not been well characterized. Research suggests that human viruses may be a major contributor (World Health Organization 2003), and a quantitative microbial risk assessment has recently identified enteric viruses as potentially responsible for observed illnesses in swimmers (Soller *et al.* 2010).

The aim of this study was to quantify the occurrence of human adenoviruses and human noroviruses at two Southern California recreational beaches. This work also applied the newly developed Nanoceram© (Argonite Corp.) positively-charged cartridge filter for viral monitoring of beach water. This filter has been demonstrated to be highly efficient for the concentration and recovery of noroviruses from large volumes of marine waters using a modified adsorption-elution method described previously (Gibbons *et al.* 2010). To our knowledge this is the first report of using a Nanoceram© cartridge filter for the detection of human adenovirus and human norovirus during a beach monitoring study. This study aimed to compare the occurrence of viruses with other microbial water parameters at beaches impacted by non-point source contamination.

METHODS

Site description and sample collection

Doheny and Avalon Beaches, CA, were selected because of their high summer use, history of beach postings or closures, and potential impact from non-point source pollution. Sampling events were scheduled on weekends and during summer months because these dates are most popular among beach visitors (Dwight *et al.* 2007). An intensive sampling approach allowed for the capture of transient conditions of viral contamination in beaches used for recreation. At Doheny Beach in Dana Point, water sampling was conducted one weekend day per week from May 28 to July 1 2007, and July 4 2007, for a total of eight sampling days. A total of 112 water samples were collected from Doheny Beach at a depth 0.5 m below the water surface along a transect (Figure 1(a), sites: A, B, D, E) three times per day (07:00 hours, 13:00 hours, and 15:00 hours), and at 07:00 hours from a channelized urban stream (San Juan Creek) (Figure 1(a), site C) that forms a lagoon behind the beach between sites B and D. The lagoon was reported as a source of fecal pollution impacting the beach (Colford *et al.* 2012), and during this study, the lagoon did not breach the berm separating it from the ocean.

At Avalon Beach on Catalina Island, water sampling was conducted daily Thursday through Sunday from July 26 to September 2 2007 and September 8 and 9 2007, for a total of 26 sampling days. Water samples at Avalon

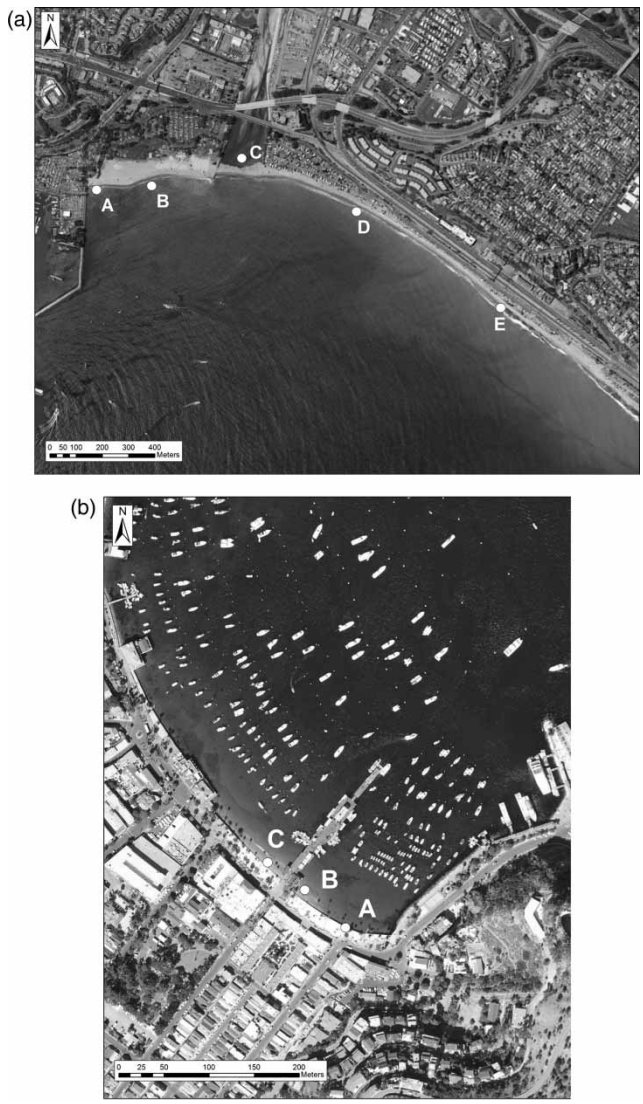

Figure 1 | Site map of (a) Doheny Beach, CA and (b) Avalon Beach, CA.

Beach were collected along a beach transect (Figure 1(b), sites: A, B, C) three times per day (08:00 hours, 12:00 hours, and 15:00 hours). Samples were collected at a depth 0.5 m below the surface of the water at all sampling times, as well as at 1 m depth at the 12:00 hours sampling time. There were 324 water samples in total. Previous research has identified that fecal pollution comes from a variety of sources including a leaky sewage system (Boehm *et al.* 2003), which contaminates groundwater and discharges into Avalon Bay during outgoing tides (Boehm *et al.* 2009).

Adenovirus and norovirus concentration

Adenoviruses and noroviruses were concentrated from 40 L water samples by positively-charged Nanoceram© alumina cartridge filters (Argonide Corporation, Sanford, FL, USA), using a previously described method with known adenovirus and norovirus recovery efficiencies (Gibbons *et al.* 2010). Filtrations were performed at a field laboratory near the beach by Southern California Coastal Water Research Project (SCCWRP) staff, and at a filtration rate of approximately 2.5 L per min (Emerson self-priming pump, ITT Jabsco, St Louis, MO). To preserve the samples during shipment, 350 mL of transport medium consisting of sterile 3% (w/v) beef extract (Becton, Dickinson and Company, Sparks, MD) – 0.1 M glycine (BE, pH 7.3) solution was poured into each filter cartridge housing. The housing was sealed, and shipped overnight at 4 °C to the University of North Carolina (UNC) laboratory in Chapel Hill, NC, for analysis.

To elute viruses at UNC, an additional 150 mL of BE was added to the existing 350 mL BE transport medium solution, and the liquid was recirculated through the cartridge filters using a peristaltic pump. The sample pH was adjusted to pH 9.5 using 1 M NaOH during recirculation, and maintained at pH 9.5 for 15 min (changing the flow direction every 5 min). Sample eluents were then transferred to a 1 L bottle, and the pH was adjusted to pH 7.3 using a 1 M HCl solution. Between samples, the pH meter probe was disinfected with 0.1 M HCl for 2 min. Each sample eluent was then split into two Corning polypropylene 250 mL conical bottles (Corning Inc., Corning, NY) and 9% (w/v) polyethylene glycol (PEG 8000, Fisher Scientific, Fair Lawn, NJ) and 0.3 M sodium chloride (NaCl) were added for PEG precipitation. Samples were shaken at 200 rpm (Lab-Line platform shaker, Barnstead International, Dubuque, IA, USA) at 4 °C overnight. The eluents were then centrifuged for 1 h at 5,100 × *g* and 4 °C, after which point the supernatant was discarded. The pellet was resuspended in 2 mL of phosphate-buffered saline (PBS, pH 7.2; 0.027 M KCl, 0.88 M KH_2HPO_4, 0.0064 M Na_2HPO_4, 0.14 M NaCl) and stored at –80 °C.

In seawater, the efficiency of virus adsorption-elution recovery by the Nanoceram© filter was published previously and was 99% for norovirus and 3.5% for human adenovirus (Gibbons *et al.* 2010). The efficiency of virus recovery by the secondary concentration step with PEG precipitation was 59% for norovirus and 39% for adenovirus. The total recovery efficiency of the method was 58% for norovirus and 1.4% for adenovirus.

Viral nucleic acid extraction

A guanidinium thiocyanate (GuSCN) extraction method was utilized for viral nucleic acid extraction of Doheny Beach and Avalon Beach sample concentrates (Boom *et al.* 1990; Jothikumar *et al.* 2010). Sample concentrates (1.2 mL for each Doheny Beach sample and 0.2 mL for each Avalon Beach sample) were mixed with equal volumes of GuSCN extraction buffer containing 120 g of GuSCN, 100 mL of TE Buffer (10 mM of Tris (tris(hydroxymethyl) aminomethane) and 1 mM of EDTA (ethylenediaminetetraacetic acid)) pH 8.0 (Ambion, Austin, TX), 55 mM sodium chloride, 33 mM sodium acetate, and 4.4 mg of polyadenylic acid (5′) potassium salt for a total volume of 240 mL. The solution was vortex mixed for 1 min then incubated for 10 min at room temperature. Then, two volumes of 100% ethanol were added to the extract and the mixture was vortex mixed for 15 sec. Six hundred microliters of solution was transferred to a HiBind RNA mini-column (OMEGA Bio-Tek, Doraville, GA) and centrifuged at 16,000 × *g* for 1 min; this step was repeated until the entire sample was passed through the column. Three separate HiBind RNA mini-columns were used to extract the 1.2 mL Doheny Beach concentrate sample (one mini-column per 0.4 mL volume). Only one column was used for each 0.2 mL Avalon Beach concentrate sample. The columns containing viral nucleic acids were washed twice with 0.5 mL of 75%

ethanol, and then spun again in a clean collection tube to remove the ethanol. Nucleic acids were eluted from the column with 50 μL of nuclease-free water and stored at −80 °C until further analysis. One blank control (PBS) was run for every three samples.

At Avalon Beach, it was initially determined that decreasing the volume of sample from 1.2 to 0.2 mL for nucleic acid extraction improved virus detection (data not shown). These modifications reduced the amount of the 40-L water sample analyzed per nested polymerase chain reaction (PCR) for adenovirus from 6% at Doheny Beach to 1% at Avalon Beach, and similarly reduced the amount of sample analyzed per nested reverse transcriptase PCR (RT-PCR) reaction for norovirus from 2% at Doheny Beach to 1% at Avalon Beach. Some of the authors conducted additional analyses of the inhibitory effect of beach water, and found that Avalon Beach samples were more inhibitory than Doheny Beach samples for norovirus detection by quantitative reverse transcriptase PCR (qRT-PCR) (Rodríguez *et al.* 2012b).

Adenovirus detection by nested PCR

Adenovirus (species A–F) were detected using previously described methods (Ko *et al.* 2003). The first round PCR reaction mixture consisted of a 5 μL of 10× PCR buffer, 2.0 mM $MgCl_2$, 10 μL 5× Q solution (Qiagen, Valencia, CA, USA), 200 μM each deoxyribonucleotide triphosphate (dNTP), 1 μM each primer (Hex 1 and Hex 2), 1.5 units of Hot Star Taq (Qiagen), 15 μL of extracted viral nucleic acid and water for a reaction volume of 50 μL. For Avalon Beach samples, the volume of viral nucleic acid was decreased from 15 to 5 μL to reduce environmental inhibitors to PCR, based upon preliminary studies characterizing the degree of sample-volume-related PCR inhibition. The nested PCR mixture was the same as described above, except the primer Hex 3 replaced Hex 2 (Ko *et al.* 2003) and 2 μL of the first PCR product was added. Conditions for both PCR reactions was 94 °C for 10 min, 40 cycles of 94 °C for 30 sec, 50 °C for 30 sec, and 30 sec at 72 °C, and a final extension step of 72 °C for 7 min (Peltier Thermal Cycler, MJ Research, Waltham, MA, USA). A negative control PBS blank and a positive control of adenovirus 41 genomic DNA were included in each run. PCR products were then visualized by gel electrophoresis through 2% agarose in 0.1 Tris/borate/EDTA and 5 μg/mL of ethidium bromide.

Norovirus detection by nested RT-PCR

Norovirus region A was detected using previously described methods (Vinjé *et al.* 2004). A Qiagen One-Step RT-PCR Kit (Qiagen, Valencia, Ca) was used as described by the manufacturer. The One-step RT-PCR consisted of 5 μL of 5× Qiagen One-Step RT-PCR buffer, 5 μL of Qsolution, 1 μL of Qiagen enzyme mix, 10 units of RNase inhibitor, 400 μM each dNTP, 2 μM MJV12 primer and 2 μM RegA primer, 5 μL of the extracted viral nucleic acid, and water for a reaction volume of 25 μL. The RT-PCR conditions were 50 °C for 30 min, 94 °C for 15 min, 40 cycles of 94 °C for 15 sec, 50 °C for 15 sec, and 72 °C for 30 sec, then a final extension step of 72 °C for 7 min. A nested PCR developed by Maloney (2006) was then run on the products of the first reaction. The nested PCR reaction consisted of 5 μL of 10× PCR buffer, 2 mM $MgCl_2$, 200 μM each dNTP, 1 μM RegA primer and 1 μM MP290 primer, 1.5 units Hot Star Taq, 2 μL of the first PCR reaction product and water for a reaction volume of 50 μL. The sequence of the MP 290 primer is 5′GAY TAC TCY CS/inosine TGG GAY TC 3′ (degenerate base symbols: Y = C/T; S = C/G). Nested PCR conditions were 94 °C for 10 min, 40 cycles of 94 °C for 20 sec, 49 °C for 30 sec, and 72 °C for 1 min, and a final extension step of 72 °C for 7 min. Negative control PBS blanks and positive control norovirus G2.4 genomic RNA were included in each PCR run, and products were analyzed by gel electrophoresis as for adenoviruses.

Coliphage detection

For coliphage analysis, water samples were collected in 2 L sterile wide-mouth bottles and shipped overnight at 4 °C to the UNC laboratory. A modified version of the two-step enrichment US EPA Method 1601 (USEPA 2001) was used for the quantification and detection of male-specific (F+) and somatic coliphages as described previously (USEPA 2001; Rodríguez *et al.* 2012a). In short, multiple volumes were analyzed up to a total volume of 1 L per coliphage group (F+ and somatic) in order to calculate a most probable number (MPN) of coliphages present in each sample.

Fecal indicator bacteria detection

FIB were assayed in water samples by SCCWRP staff at a field laboratory near the beach. Concentrations of total and fecal coliforms were determined as previously described in Standard Methods 9222 B and D, respectively (Eaton *et al.* 2005). Enterococci were quantified using previously described EPA Method 1600 (USEPA 2006). FIB data were only used for modeling purposes and comparison to enteric virus data, hence no FIB summary statistics are presented in this manuscript, although portions of these FIB data have been published previously (Colford *et al.* 2012).

Environmental parameters

Various parameters were recorded at Avalon Beach to model their association with viruses. Water temperature data were collected by Ali Boehm, Stanford University, using a probe fixed to a pier in Avalon Beach, and matched with sampling times. Rainfall data were collected by the Catalina Airport (National Climate Data Center 2012), and coded as a binary variable as previous rainfall within the past 48 h or not. A beach advisory report for beach closures in 2007 was collected from a State of California website that is no longer active. Rainfall, water temperature, surf height, sea state, water color, wind, current, odor, trash, and kelp presence were collected at Doheny Beach during four of the eight sampling days in 2007, and this incomplete data set was not used in data modeling.

Statistical analysis and modeling

Fisher's exact test was used to assess beach sampling site effects on human virus detection frequency, while Friedman's test and Dunn's multiple comparison post-test determined sampling site effects on coliphage concentrations. Virus inactivation rates due to sunlight were calculated using linear regression in Prism (Graphpad, La Jolla, CA, USA). Chi-squared analysis was performed on virus frequency at beaches with and without recreational water guidelines. A generalized estimating equation (GEE) model was used to test for time-of-day effects on virus detection frequency and concentration for each beach, which accounted for spurious correlations due to repeated measures. The associations among human viruses, environmental parameters, somatic coliphages, F+ coliphages, and FIB were also measured with a GEE model. Bivariate relationships between predictors and probability of human virus presence was explored. Factors with a p value of less than 0.10 were chosen to enter into the multivariate GEE model. Model selection was then performed based on likelihood-ratio test with a backward selection process. Factors with a significance level of 0.05 or less were kept in the model. Modeling was performed in SAS version 9.2 (Cary, NC).

Quality assurance and quality controls

The UNC laboratory was set up as described in the USEPA manual for quality assurance for using PCR methods applied to environmental samples (USEPA 2004). Each step in the processing of the sample was physically separate: separate rooms were used for mixing PCR reagents, field sample processing, positive control manipulation and post PCR processing. The same equipment was used for the duration of the study. Blank samples were included in between every three field samples for nucleic acid extraction and PCR. One positive control for each virus (adenovirus 41 and norovirus G2.4) was run for each master mix. The manipulation of the positive controls was performed in a separate room from field samples.

RESULTS

Virus detection at beaches

Adenoviruses and noroviruses were detected in 25.5 and 22.3%, respectively, of water samples at Doheny Beach, and in 9.3 and 0.7% of water samples at Avalon Beach, respectively, (Table 1). The larger proportion of virus-positive samples at Doheny Beach may be a function of differences in fecal pollution sources at the two beaches and the fact that larger water sample volumes were analyzed by molecular methods at Doheny Beach than Avalon Beach.

F+ and somatic coliphages were detected in 26.7 and 82.8% of samples at Doheny Beach, and 56.9 and 41.5% of samples at Avalon Beach, respectively. Among samples with detectable concentrations, the median concentrations

Table 1 | Detection frequency and concentration of viruses during the summer of 2007 at Doheny and Avalon Beaches

	Doheny Beach, CA (May 28 to July 4 2007)				Avalon Beach, CA (July 26 to September 9 2007)			
Virus type (sample volume)	% positive	*N*	Median[a] (MPN / 100 mL)	Range (MPN / 100 mL)	% positive	*n*	Median[a] (MPN / 100 mL)	Range (MPN / 100 mL)
Adenovirus (40 L)	25.5	94	n/a	n/a	9.3	291	n/a	n/a
Norovirus (40 L)	22.3	94	n/a	n/a	0.7	291	n/a	n/a
F+ coliphages (1 L)	26.7	101	0.3	<0.09 to 140	56.9	318	0.3	<0.1 to 37
Somatic coliphages (0.1 L)	82.2	101	4.9	<1 to 150,000	41.5	318	3.1	<1 to > 370

[a]Median among samples with a detectable concentration.

of F+ coliphages were the same at the two beaches (0.3 MPN/100 mL), and median concentrations of somatic coliphages were similar (4.9 and 3.1 MPN/100 mL at Doheny and Avalon Beaches, respectively) (Table 1).

Effects of sampling location on virus detection

No noroviruses (zero of seven samples) and only one adenovirus positive (one of seven samples) was detected in the lagoon at Doheny Beach, while the viruses were readily detected in beach water at frequencies as high as 32% for noroviruses (Figure 2(a), site D) and 41% for adenoviruses at beach sampling sites (Figure 2(a), site E). Along the beach transect (sites A, B, D, E), no significant sampling site effect was observed for adenoviruses ($p = 0.2$) or noroviruses ($p = 0.8$).

At the Doheny Beach lagoon, frequencies were 100% for somatic coliphage and 88% for F+ coliphage (Figure 2(b)). Along the beach transect, there was a significant sampling site effect for F+ coliphage concentrations ($p = 0.03$) and somatic coliphage concentrations ($p = 0.008$) (Figure 2(c)), but not for somatic coliphage ($p = 0.7$) or F+ coliphage detection frequency ($p = 0.1$) (Figure 2(b)). The only significant pair-wise comparisons between sites were for somatic coliphage, where site E had a significantly lower concentration of somatic coliphages than site A ($p < 0.05$).

At Avalon Beach, sampling was conducted at three sites along the beach and there was no significant sampling site effect for either adenoviruses ($p = 0.6$) or noroviruses ($p = 0.7$) (Figure 2(d)). Adenovirus detection frequency ranged from 7 to 12% at the sampling sites at Avalon Beach. Noroviruses were only detected in two of 291 samples. There was a significant site effect for somatic coliphage concentration ($p = 0.007$) (Figure 2(f)), although this effect was not significant when measured by somatic coliphage detection frequency ($p = 0.7$) (Figure 2(e)). Neither detection frequency ($p = 0.8$) nor concentration ($p = 0.09$) showed a significant site effect for F+ coliphages. These results were consistent with a diffuse source of pollution to the beach.

Effects of time of day

There was a significant time of day effect, in which F+ and somatic coliphage concentrations and detection frequencies decreased throughout the day at both beaches (Figure 3(c) and (f)), presumably due to inactivation by sunlight. F+ coliphage inactivation rate constants and 95% confidence intervals were somewhat similar at Avalon and Doheny Beaches ($k_{\text{obsAvalon F+ coliphage}} = 0.097 \pm 0.30\ \text{h}^{-1}$; $k_{\text{obsDoheny F+ coliphage}} = 0.069 \pm 0.16\ \text{h}^{-1}$), as were somatic coliphage rate constants at both beaches ($k_{\text{obs Avalon somatic}} = 0.248 \pm 1.2\ \text{h}^{-1}$; $k_{\text{obsDoheny somatic}} = 0.239 \pm 0.64\ \text{h}^{-1}$). F+ coliphages were inactivated more slowly than somatic coliphages. Linear regression models could account for most of the variability in the data ($R^2 = 0.873$ to 0.969), although there were only three time points used to develop the models.

Time of day effects were variable for adenovirus and norovirus. At Doheny Beach, time of day was significant for norovirus detection frequency, but in the opposite direction as expected; samples were 61% more likely to be positive for norovirus at 15:00 than 07:00 hours ($p = 0.05$). For adenovirus, detection was 73% more likely at 07:00 than 13:00 hours ($p = 0.002$), and no difference was observed between 07:00 or 13:00 and 15:00 hours ($p = 0.33$; $p = 0.08$,

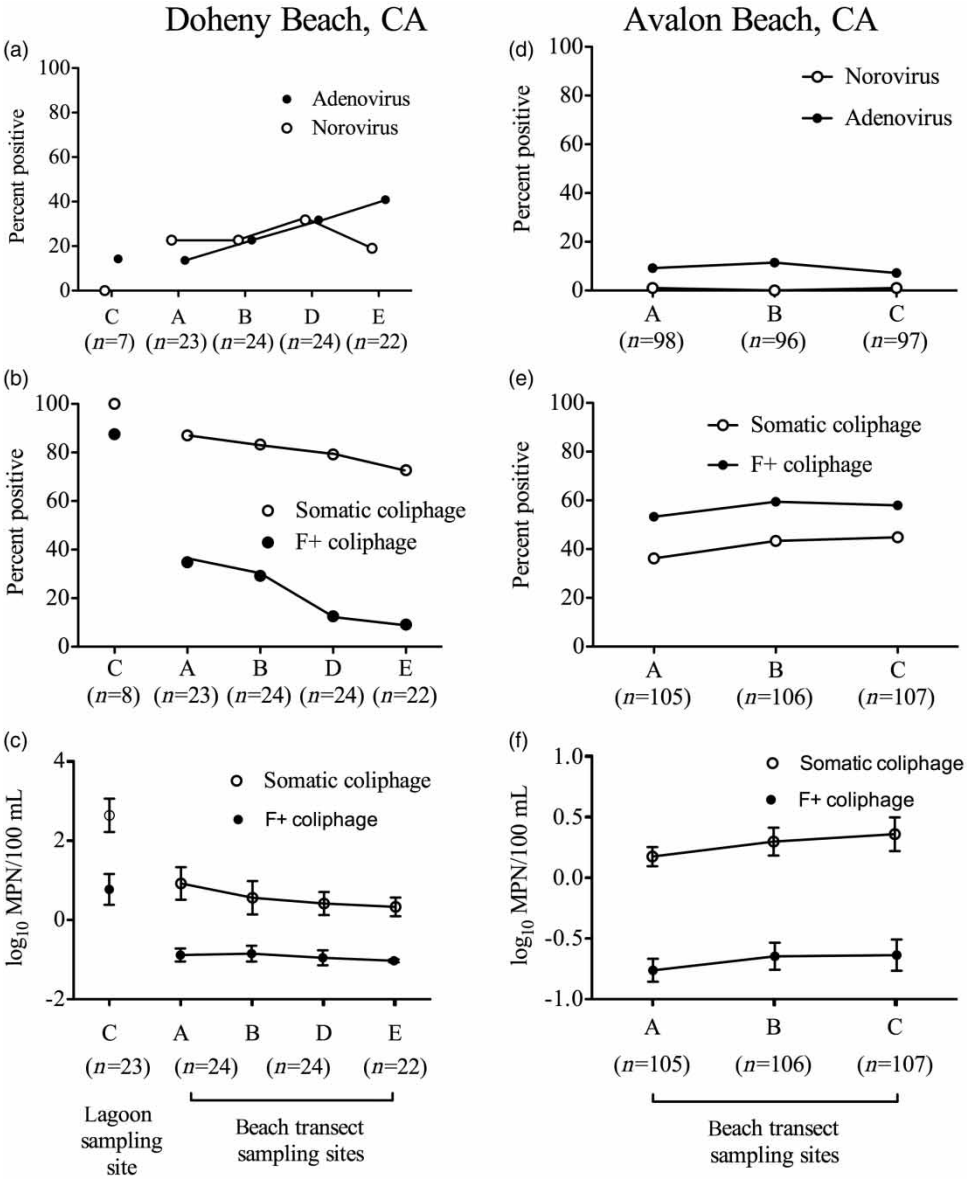

Figure 2 | Virus detection frequency and concentration at sampling sites along beach transects at Doheny (Figure 2(a), (b) and (c)) and Avalon (Figure 2(d), (e) and (f)) beaches, and at the lagoon at Doheny Beach. Adenovirus and norovirus virus detection frequency (Figure 2(a) and (d)) and F+ and somatic coliphage detection frequency (Figure 2(b) and (e)) and concentration (Figure 2(c) and (f)) are reported. Ninety-five percent confidence intervals are provided for F+ and somatic coliphage concentrations.

respectively). At Avalon Beach, no time of day effects were observed for adenoviruses. Noroviruses were detected in one 07:00 hours sample and one 15:00 hours sample, which was not enough to perform statistics.

Effect of sample depth

To explore potential differences in exposure between wading versus swimming, a 'waist-deep' water sample (1 m depth) was collected at noon at the same time as a 'knee-deep' water sample (0.5 m depth) at Avalon Beach. For samples collected at the same time, there was no significant effect of sample depth on coliphage concentration (F+ coliphage, $p = 0.78$; somatic coliphage, $p = 0.28$) (Table 2). Furthermore, agreement between sampling depths was observed when comparing detection frequency using Fisher's exact test (F+ coliphage, $p < 0.0001$; somatic coliphage, $p = 0.002$). Based on the agreement between sample depths for coliphage

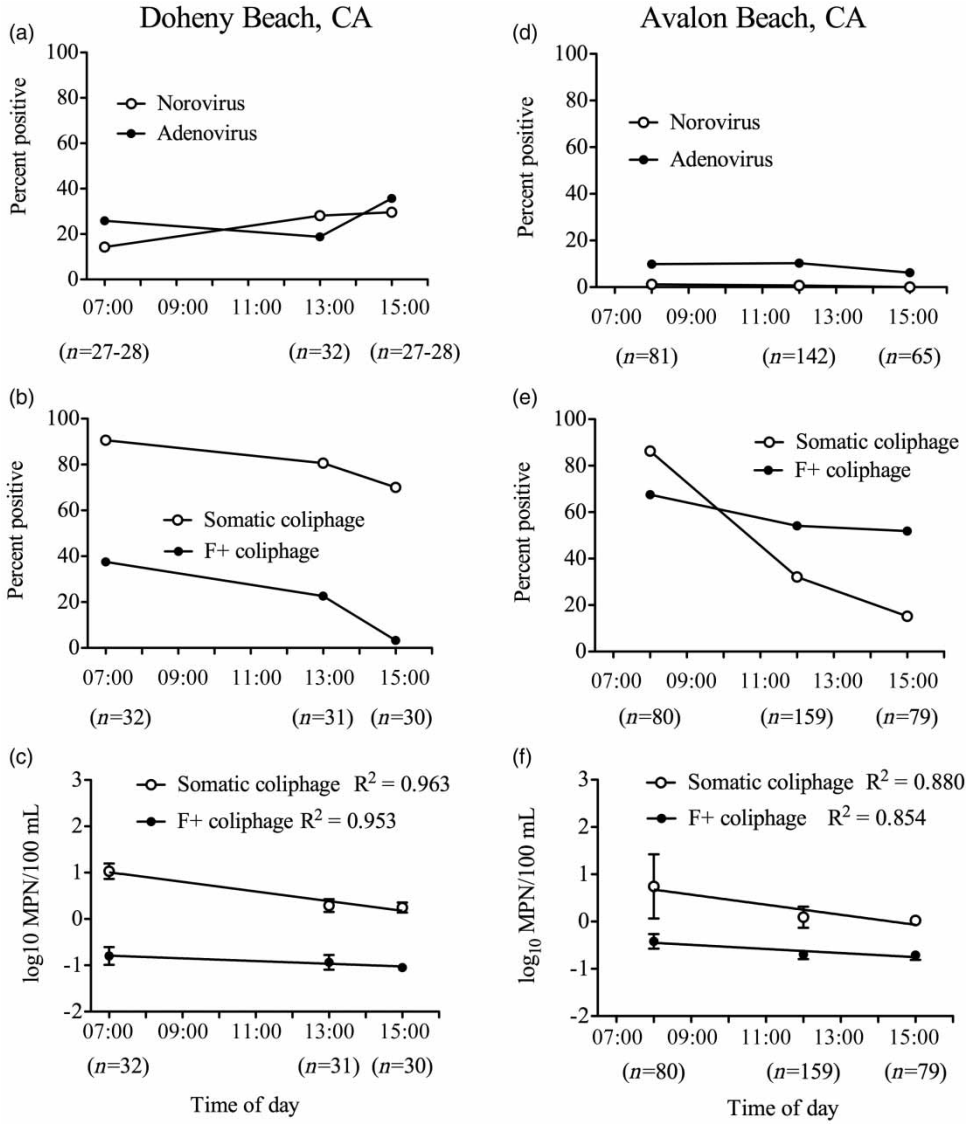

Figure 3 | Time-of-day effects on virus detection frequency and concentration in samples from Doheny Beach, CA (a, b, c) and Avalon Beach, CA (d, e, f). Adenovirus and norovirus detection frequency (Figure 3(a) and (d)), F+ and somatic coliphage detection frequency (Figure 3(b) and (e)), and concentration (Figure 3(c) and (f)) are presented. Linear regression trendlines are listed for F+ and somatic coliphages (Figure 3(c) and (f)).

concentrations and frequencies, results of both sample depths were pooled for analyses. Adenovirus detection frequencies at the two sample depths were not significantly associated ($p = 1$). Similarly, norovirus detection frequencies at sampling depths were not significantly associated ($p = 1$).

Day of study

Sampling results were presented by day of study for both beaches (Figure 4). The values presented by day of study represent the frequency of positive samples (or concentration) detected from all sites on that day. For F+ and somatic coliphages, values were reported only for morning samples, which presents the best-case scenario for their detection, because solar inactivation occurred in afternoon samples. One notable finding at Doheny Beach was very high frequencies of noroviruses (54% positive) and adenoviruses (85% positive) on day 21 of the study (Figure 4(a)), with no increases in F+ or somatic coliphage frequencies. There were no remarkable environmental conditions on

Table 2 | Effects of water sample collection depth on the detection frequency and concentration of viruses at Avalon Beach

	Water sample depth[a]								Statistical tests	
	0.5 m				1.0 m					
Virus type	% positive	*n*	Median [b] (MPN/100 mL)	Range (MPN/100 mL)	% positive	*N*	Median [b] (MPN / 100 mL)	Range (MPN / 100 mL)	Fisher's exact test, *p* value	Wilcoxon matched pairs test, *p* value
Adenovirus	8.8	77	n/a	n/a	12.3	65	n/a	n/a	1.0	n/a
Norovirus	0	77	n/a	n/a	1.5	65	n/a	n/a	1.0	n/a
F+ coliphage	55.1	78	0.38	<1 to 8	53.1	81	0.31	<0.1 to >37	<0.0001	0.78
Somatic coliphage	32.1	78	2.10	<1 to 370	32.1	81	1.20	<1 to 14	0.008	0.28

[a]All samples collected at noon.
[b]Median among samples with a detectable concentration.

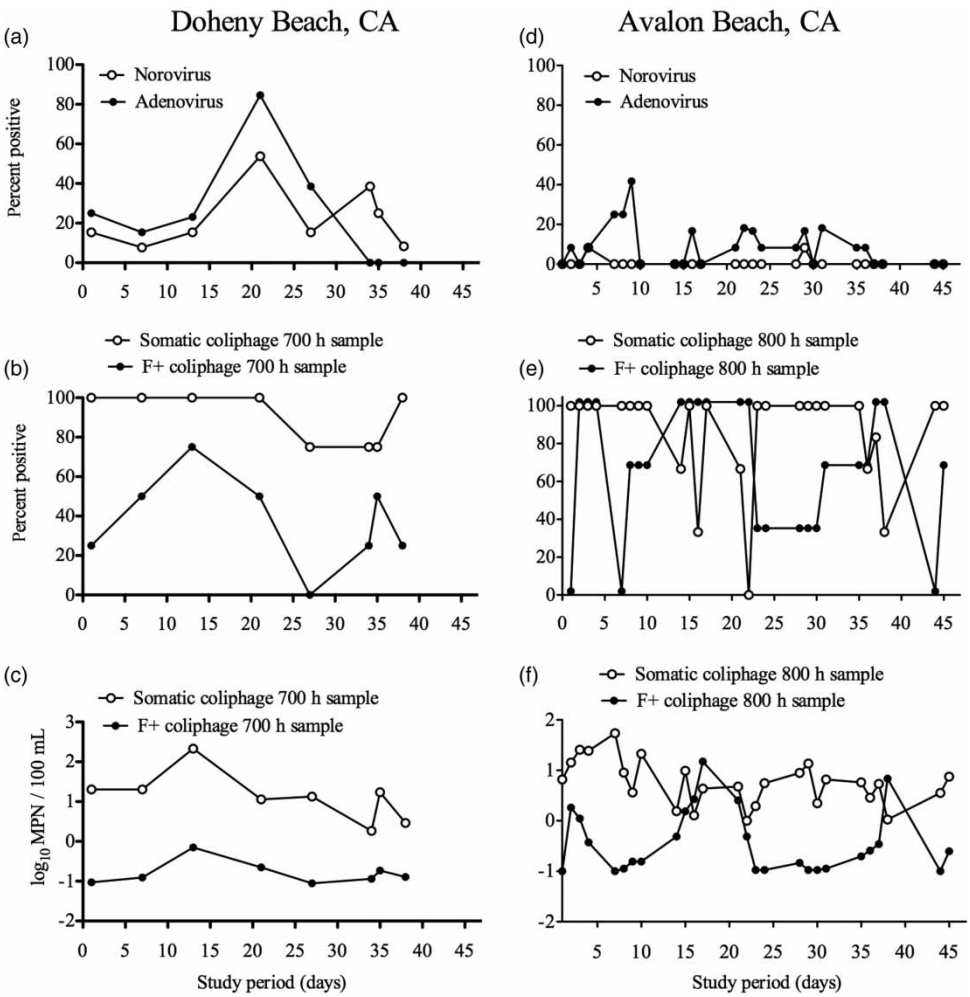

Figure 4 | Daily time-series and detection frequency of viruses in water samples from Doheny Beach, CA (Figure 4(a), (b) and (c)) and Avalon Beach, CA (Figure 4(d), (e) and (f)). F+ and somatic coliphage detection frequencies and concentrations were based solely on the morning water samples to control for time-of-day effects described in Figure 3. Sample sizes: Doheny Beach $n = 4$ to 13 samples/day (norovirus and adenovirus), $n = 4$ samples/day (F+ and somatic coliphages); Avalon Beach: $n = 6$ to 11 samples/day (norovirus and adenovirus), $n = 3$ samples/day (F+ and somatic coliphages).

that day, which was overcast at 07:00 hours with calm seas and a light wind, at 13:00 hours the sky was clear, and at 15:00 hours a moderate breeze and moderate choppy waves were recorded. At some sites and some times of the day kelp cover was observed, the water was green or blue/green, and some litter was seen in the water in the morning.

At Avalon Beach, noroviruses were rarely detected and adenoviruses were detected often, but rarely at frequencies higher than 25% in any sampling day (Figure 4(d)). Comparing coliphage frequencies (Figure 4(b) and (e)) versus continuous coliphage data (Figure 4(c) and (f)) shows the importance of quantification for generating high-resolution water quality data. Correlations among enteric viruses, colphages, and other parameters were then performed.

Correlation among viruses and environmental parameters

A GEE model assessed correlation at Doheny Beach among human viruses, FIB, and coliphages, controlling for time of day. Only those model parameters that produced significant models are reported. The probability of detecting adenovirus was greater in the absence of F+ coliphages ($p = 0.002$; odds ratio (OR) = 0.24) and enterococci ($p = 0.001$; OR = 0.95), in the presence of fecal coliforms ($p = 0.02$; OR = 1.004), and no significant associations were observed between somatic coliphages and adenovirus. Norovirus was not significantly associated with either type of coliphages or FIB. Environmental parameters were not consistently collected at Doheny Beach, but were at Avalon Beach.

At Avalon Beach, significant associations among adenoviruses, FIB, and coliphages were determined using a GEE model. The probability of detecting adenovirus was significantly associated with higher fecal coliform concentrations ($p = 0.01$; OR = 1.99) and total coliform concentrations ($p = 0.002$; OR = 1.44). The presence of F+ coliphage ($p = 0.1$; OR = 1.98) was positively associated with the probability of detecting adenovirus, although this association was only marginally significant. The probability of detecting adenovirus was not associated with water temperature, rainfall in the past 48 h, or the presence of municipal beach advisories. There were too few norovirus positive samples at Avalon Beach for model convergence.

Relationship between human viruses and recreational water guidelines

Relationships between viruses and recreational water guidelines were further studied using 2×2 tables (Tables 3 and 4). At Doheny Beach, 35.5 and 34.4% of samples from sites without a beach advisory were positive for norovirus and adenovirus, respectively (Table 3). There was a significant relationship between the absence of a beach advisory and virus frequency at Doheny Beach for norovirus ($p = 0.03$) but not for adenovirus ($p = 0.3$). At Avalon Beach, 8.7% of samples from sites without a beach advisory were positive for adenovirus, although the relationship between advisory and adenovirus was not significant.

Comparing virus frequency with enterococci guidelines, for Doheny Beach there was no significant relationship between virus detection and enterococci guidelines (Table 4), mainly because there were too few samples collected with enterococci levels greater than 104 colony forming units (CFU)/100 mL. Only one of the 25 and 21

Table 3 | Frequency of human viruses detected in samples collected with and without a beach advisory

Beach	Beach advisory	No. samples[a]	Human virus detection frequency (%) Adenovirus	Norovirus
Avalon Beach	No	115	8.7	0
	Yes	111	8.1	0.9
Doheny Beach	No	31	34.4	35.5
	Yes	63	23.2	15.9

[a]At Avalon Beach, only samples collected at 0.5 m depth were used for comparability to enterococci data in Table 4.

Table 4 | Frequency of human viruses detected in samples with different concentrations of enterococci

Beach	Enterococci[a]	No. samples[b]	Human virus detection frequency (%) Adenovirus	Norovirus
Avalon Beach	<104 CFU/100 mL	162	5.6	0
	>104 CFU/100 mL	64	15.6	1.0
Doheny Beach	<104 CFU/100 mL	79	30.0	25.3
	>104 CFU/100 mL	15	6.7	6.7

[a]California single sample standard.
[b]At Avalon Beach, only samples collected at 0.5 m depth were used because enterococci data was not available for samples collected at 1 m depth.

samples positive for norovirus and adenovirus, respectively, also contained enterococci levels greater than 104 CFU/100 mL. Higher rates of virus detection were observed in samples from Doheny Beach with lower concentrations of enterococci. At Avalon Beach, there was a significant relationship between the frequency of adenovirus and the presence of enterococci ($p = 0.01$); the frequency of adenovirus occurrence was higher in the samples above the established level for enterococci (Table 4).

DISCUSSION

Human enteric viruses, specifically adenoviruses and noroviruses, were detected at two beaches with different types of fecal contamination: a known source at Avalon Beach and unknown, probably diffuse source(s) at Doheny Beach. There have been several studies on the occurrence of human adenovirus in California beaches (Jiang *et al.* 2001, 2007; McQuaig *et al.* 2012). To our knowledge this is the first report on the detection of human norovirus at California beaches, and in general, there is limited information on the occurrence of human noroviruses in recreational use beaches in the USA. At Doheny Beach, a contamination event was detected during the fourth sampling weekend when up to 80% of samples were positive for either human adenovirus and human norovirus without any increase in concentration and detection frequency of traditional fecal indicator microorganisms. The contamination event occurred during a relatively calm, rain-free day, with no obvious cause or source.

A Nanoceram© positively-charged cartridge filter method was used for this study, which is the first report of use of this filter to monitor the virological quality of recreational beach water. This cartridge filter is efficient at adsorbing human adenovirus and adsorbing and recovering human norovirus from seawater, although the recovery efficiency of human adenovirus is low (Gibbons *et al.* 2010). An advantage of using this filter is that it allows for the rapid filtration of larger volumes of water (40 L) than the most commonly used methods using negatively charged membranes or ultrafilters (0.5–1 L water) (Jiang *et al.* 2007; Sassoubre *et al.* 2012), and sample water does not require cumbersome and time-consuming pre-conditioning to an acidic pH as with the negatively charged Filterite filter.

Noroviruses were detected less frequently than adenoviruses during this study, which may be due to the smaller fraction of the sample analyzed for norovirus than adenovirus at Doheny Beach. The theoretical detection limits of the assay were around one PCR unit in 2.5 L for adenovirus and 1.2 L for norovirus. However, the method recovery efficiency of norovirus in seawater samples should be greater than for adenovirus, which can compensate for the difference in extraction volumes assayed between these viruses. Furthermore, the detection frequency of each virus was greater than previous reports from waters along the California coast and other coastal waters (Jiang 2006; Jiang *et al.* 2007; Silva *et al.* 2010; McQuaig *et al.* 2012). In a similar study, adenoviruses were detected in less than 10% of samples from Doheny Beach and were not detected at Avalon Beach (McQuaig *et al.* 2012). An epidemiologic study of Mission Bay resulted in only one detection of enteric virus despite analyses of more than 1800 water samples (Colford *et al.* 2007). A study of Newport Bay reported that adenoviruses and enteroviruses were detected in approximately 5% of samples collected during dry weather (Jiang *et al.* 2007). Other studies have reported an association between rainfall, tides and hydrogeographical factors associated with the detection of adenoviruses and other pathogens (Griffin *et al.* 2003; Jiang & Chu 2004; Ahn *et al.* 2005; Abdelzaher *et al.* 2011). Previous studies have also reported a lack of correlation between enteric viruses and coliphages (Jiang & Chu 2004), although one study of runoff-impacted waters demonstrated a correlation between adenovirus and F-specific coliphage (Jiang *et al.* 2001). Therefore, the extent to which enteric virus occurrence and concentrations in coastal seawaters is associated with or predictable by FIB or coliphages or by other environmental variables, such as temperature, rainfall, and hydrological conditions remains uncertain and may be more site-specific rather than generalizable.

Sampling at multiple time points and sites each day provided an opportunity to test existing theories about Avalon and Doheny Beach pollution sources. At Avalon Beach, adenovirus, and F+ coliphage frequency did not differ along the beach transect, which is similar to findings by McQuaig and colleagues for human polyomavirus at Avalon Beach (McQuaig *et al.* 2012). Currents within the Bay caused by

intermittent propeller wash from a passenger ferry (Ho *et al.* 2011) adds variability to the system that was not accounted for in our models. The city of Avalon is addressing poor water quality by repairs to the leaky sewage system (Barboza 2011). Our results are consistent with an intermittent source of contamination and support these remediation actions to reduce fecal pollution loading in Avalon Bay and reduce swimmer health risks.

At Doheny Beach, an urban stream-fed lagoon has been identified as a source of fecal pollution at the beach, and periodic breaks in a sand berm between the lagoon and the ocean are associated with an increase in diseases to bathers (Colford *et al.* 2012). In our study, the lagoon was a source for coliphages as well. Based on a concentration gradient that increased from sites E to A, it appears that coliphage travel from the lagoon into marine water and collect in the more stagnant waters near the jetty (site A), which is a popular surfing area. Human viruses did not follow the same gradient along the beach transect according to the data of this study. Human viruses were rarely detected in the lagoon (one of seven samples positive) compared to marine water (37 of 94 samples positive) and were not associated with coliphages. There was no rainfall during the study period, so our findings may not be representative of wet weather conditions. One year after our study was conducted, McQuaig and colleagues rarely detected human viruses in the lagoon but did detect human viruses in marine water (McQuaig *et al.* 2012). Considered together our studies suggest that sources of human fecal contamination other than the lagoon exist at Doheny Beach.

Microcosm studies with sunlight or simulated sunlight (Sinton *et al.* 1999; Kohn *et al.* 2007; Kohn & Nelson 2007; Love *et al.* 2010) are expanding our understanding of virus photoinactivation, and these concepts are being applied to recreational water settings. We now recognize that sunlight affects microbe survival in marine waters (Boehm *et al.* 2009), and may indirectly affect bather health risks. Morning water samples of FIB had stronger relationships to health outcomes than afternoon water samples at Doheny Beach (Colford *et al.* 2012). In the present study, the relationship between time of day (i.e., a proxy for solar radiation) and virus survival was observed within sampling days at both beaches; coliphages were more prevalent in morning samples at both beaches and decreased throughout the day, although a comparison to solar radiation is needed to strengthen this finding. Given the presumed effects of sunlight on microorganisms, the timing of water sampling (i.e., sampling in the morning instead of the afternoon) is an approach water managers should consider.

While it may appear from our study that coliphages were more susceptible than human viruses to photoinactivation, this is likely an artifact of the methods used. Coliphages were measured using infectivity methods, while viral RNA or DNA was measured for human viruses using molecular methods. In sunlit seawater microcosms, the qPCR signal from RNA or DNA of viruses and bacteria persisted longer than their infectious counterparts (Walters *et al.* 2009). This is because the short sequences used for qPCR detection do not allow for detecting damage in DNA that could eventually affect the infectivity of the microbe (Rodríguez *et al.* 2009). When infectivity assays were used for measuring Adenovirus 2 and coliphage MS2 in laboratory microcosms, similar photoinactivation rates were observed (Love *et al.* 2010).

Associating enteric viruses with health risks has been hampered by limitations in detection technologies (Griffin *et al.* 2003; Fong & Lipp 2005). Use of cell culture involves logistical challenges and drastically increases the cost to the sampling program, while the use of PCR-based methods does not provide information about the infectivity of the virus detected, unless integrated with a cell culture infectivity assay, called integrated cell culture-polymerase chain reaction (ICC-PCR). With the tools we used, potential etiological agents of disease can be identified in water prior to exposure, before clinical identification of disease in bathers. An important limitation of this study, and many previous ones, is that water body characteristics vary greatly, and not all variations could be captured. Current monitoring practices that involve single grab samples for a single type of FIB may not be capturing the complexity in coastal beach environments or be associated with enteric virus presence and concentrations. Another potential limitation of the study was not accounting for environmental inhibition of molecular analysis in several ways: by not measuring inhibition within individual samples, and by assuming one nucleic acid extraction method would be optimal for removing inhibitors to both adenovirus and norovirus amplification, at both beaches, which some authors later found not to be the case (Rodríguez *et al.* 2012b).

CONCLUSION

Noroviruses and adenoviruses were detected in the waters of two California beaches sampled during this study. The relationship between human viruses and microbial indicators of fecal contamination depended on the sources of contamination at the beaches. More agreement between human virus detection and FIB was observed in Avalon Beach where the fecal contamination source is known (leaking sewage infrastructure) than in Doheny Beach (unknown, non-point source of viral contamination). At Doheny Beach, sampling during the dry weather helped to identify sources of enteric virus contamination other than the previously identified source of FIB during rain events (San Juan Creek). Our results comparing enterococci to enteric virus detection demonstrate that beach advisories based on these FIB may not protect the beach users from exposure to human viruses at some California beaches. The direct detection of enteric virus pathogens and the use of more specific microbial source tracking methods may better assess recreational water quality and provide improved analytical tools for management decisions.

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REFERENCES

Abdelzaher, A. M., Wright, M. E., Ortega, C., Hasan, A. R., Shibata, T., Solo-Gabriele, H. M., Kish, J., Withum, K., He, G., Elmir, S. M., Bonilla, J. A., Bonilla, T. D., Palmer, C. J., Scott, T. M., Lukasik, J., Harwood, V. J., McQuaig, S., Sinigalliano, C. D., Gidley, M. L., Wanless, D., Plano, L. R., Garza, A. C., Zhu, X., Stewart, J. R., Dickerson, J. W., Yampara-Iquise, H., Carson, C., Fleisher, J. M. & Fleming, L. E. 2011 Daily measures of microbes and human health at a non-point source marine beach. *J. Water Health* **9**, 443–457.

Ahn, J. H., Grant, S. B., Surbeck, C. Q., DiGiacomo, P. M., Nezlin, N. P. & Jiang, S. 2005 Coastal water quality impact of stormwater runoff from an urban watershed in southern California. *Environ. Sci. Technol.* **39**, 5940–5953.

Barboza, T. 2011 Avalon's dirty little secret: its beach is health hazard. *Los Angeles Times*, July 10, 2011.

Boehm, A. B., Fuhrman, J. A., Mrse, R. D. & Grant, S. B. 2003 Tiered approach for identification of a human fecal pollution source at a recreational beach: case study at Avalon Bay, Catalina Island, California. *Environ. Sci. Technol.* **37**, 673–680.

Boehm, A. B., Yamahara, K. M., Love, D. C., Peterson, B. M., McNeill, K. & Nelson, K. L. 2009 Covariation and photoinactivation of traditional and novel indicator organisms and human viruses at a sewage-impacted marine beach. *Environ. Sci. Techno.* **43**, 8046–8052.

Boom, R., Sol, C. J., Salimans, M. M., Jansen, C. L., Dillen, P. M. W.-v. & Noordaa, J. v. d. 1990 Rapid and simple method for purification of nucleic acids. *J. Clin. Microbiol.* **28**, 495–503.

Bosch, A. 1998 Human enteric viruses in the water environment: A minireview. *Int. Microbiol.* **1**, 191–196.

Cabelli, V. 1983 *Health Effects Criteria for Marine Recreational Waters*. Research Triangle Park, North Carolina, USEPA-600-1-80-031.

Colford Jr., J. M., Schiff, K. C., Griffith, J. F., Yau, V., Arnold, B. F., Wright, C. C., Gruber, J. S., Wade, T. J., Burns, S., Hayes, J., McGee, C., Gold, M., Cao, Y., Noble, R., Haugland, R. & Weisberg, S. B. 2012 Using rapid indicators for *Enterococcus* to assess the risk of illness after exposure to urban runoff contaminated marine water. *Water Res.* **47**, 2176–2186.

Colford Jr., J. M., Wade, T. J., Schiff, K. C., Wright, C. C., Griffith, J. F., Sandhu, S. K., Burns, S., Sobsey, M., Lovelace, G. & Weisberg, S. B. 2007 Water quality indicators and the risk of illness at beaches with nonpoint sources of fecal contamination. *Epidemiology* **18**, 27–35.

Dufour, A. P. 1984 *Health Effect Criteria for Fresh Recreational Waters*. Cincinnati, Ohio, USEPA-600-1-84-004.

Dwight, R., Brinks, M., SharavanaKumar, G. & Semenz, J. 2007 Beach attendance and bathing rates for Southern California beaches. *Ocean Coast. Manage.* **50**, 847–858.

Eaton, A. D., Clesceri, L. S., Rice, E. W., Greenberg, A. E. & Franson, M. A. H. 2005 *Standard Methods for the Examination of Water and Wastewater: Centennial Edition*. American Public Health Association, Washington, D.C.

Fong, T. T. & Lipp, E. K. 2005 Enteric viruses of humans and animals in aquatic environments: health risks, detection, and potential water quality assessment tools. *Microbiol. Mol. Biol. Rev.* **69**, 357–371.

Gerba, C. P., Rose, J. B., Haas, C. N. & Crabtree, K. D. 1996 Waterborne rotavirus: a risk assessment. *Water Res.* **30**, 2929–2940.

Gibbons, C. D., Rodríguez, R. A., Tallon, L. & Sobsey, M. D. 2010 Evaluation of positively charged alumina nanofibre cartridge filters for the primary concentration of noroviruses, adenoviruses and male-specific coliphages from seawater. *J. Appl. Microbiol.* **109**, 635–641.

Given, S., Pendleton, L. H. & Boehm, A. B. 2006 Regional public health cost estimates of contaminated coastal waters: a case study of gastroenteritis at southern California beaches. *Environ. Sci. Technol.* **40**, 4851–4858.

Griffin, D. W., Donaldson, K. A., Paul, J. H. & Rose, J. B. 2003 Pathogenic human viruses in coastal waters. *Clin. Microbiol. Rev.* **16**, 129–143.

Haugland, R. A., Siefring, S. C., Wymer, L. J., Brenner, K. P. & Dufour, A. P. 2005 Comparison of *Enterococcus* measurements in freshwater at two recreational beaches by quantitative polymerase chain reaction and membrane filter culture analysis. *Water Res.* **39**, 559–568.

Ho, L. C., Litton, R. M. & Grant, S. B. 2011 Anthropogenic currents and shoreline water quality in Avalon Bay, California. *Environ. Sci. Technol.* **45**, 2079–2085.

Jiang, S. C. 2006 Human adenoviruses in water: occurrence and health implications: a critical review. *Environ. Sci. Technol.* **40**, 7132–7140.

Jiang, S. C. & Chu, W. 2004 PCR detection of pathogenic viruses in southern California urban rivers. *J. Appl. Microbiol.* **97**, 17–28.

Jiang, S., Noble, R. & Chu, W. 2001 Human adenoviruses and coliphages in urban runoff-impacted coastal waters of Southern California. *Appl. Environ. Microbiol.* **67**, 179–184.

Jiang, S. C., Chu, W. & He, J. W. 2007 Seasonal detection of human viruses and coliphage in Newport Bay, California. *Appl. Environ. Microbiol.* **73**, 6468–6474.

Jothikumar, N., Sobsey, M. D. & Cromeans, T. L. 2010 Development of an RNA extraction protocol for detection of waterborne viruses by reverse transcriptase quantitative PCR (RT-qPCR). *J. Virolog. Method.* **169**, 8–12.

Ko, G., Cromeans, T. L. & Sobsey, M. D. 2003 Detection of infectious adenovirus in cell culture by mRNA reverse transcription-PCR. *Appl. Environ. Microbiol.* **69**, 7377–7384.

Kohn, T., Grandbois, M., McNeill, K. & Nelson, K. L. 2007 Association with natural organic matter enhances the sunlight-mediated inactivation of MS2 coliphage by singlet oxygen. *Environ. Sci. Technol.* **41**, 4626–4632.

Kohn, T. & Nelson, K. L. 2007 Sunlight-mediated inactivation of MS2 coliphage via exogenous singlet oxygen produced by sensitizers in natural waters. *Environ. Sci. Technol.* **41**, 192–197.

Love, D. C., Silverman, A. & Nelson, K. L. 2010 Human virus and bacteriophage inactivation in clear water by simulated sunlight compared to bacteriophage inactivation at a southern California beach. *Environ. Sci. Technol.* **44**, 6965–6970.

Maloney, A. M. 2006 Comparison of conventional and TaqMan® realtime RT-PCR assays for the detection of human noroviruses in environmental water samples. Masters Thesis. University of North Carolina at Chapel Hill.

McQuaig, S., Griffith, J. & Harwood, V. J. 2012 Association of fecal indicator bacteria with human viruses and microbial source tracking markers at coastal beaches impacted by nonpoint source pollution. *Appl. Environ. Microbiol.* **78**, 6423–6432.

National Climate Data Center 2012 Station 23191.

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

Okoh, A. I., Sibanda, T. & Gusha, S. S. 2010 Inadequately treated wastewater as a source of human enteric viruses in the environment. *Int. J. Environ. Res. Public Health* **7**, 2620–2637.

Rodríguez, R. A., Pepper, I. L. & Gerba, C. P. 2009 Application of PCR-based methods to assess the infectivity of enteric viruses in environmental samples. *Appl. Environ. Microbiol.* **75**, 297–307.

Rodríguez, R. A., Love, D. C., Stewart, J. R., Tajuba, J., Knee, J., Dickerson, J. W., Webster, L. F. & Sobsey, M. D. 2012a Comparison of methods for the detection of coliphages in recreational water at two California beaches. *J. Virol. Methods* **181**, 73–79.

Rodríguez, R. A., Thie, L., Gibbons, C. D. & Sobsey, M. D. 2012b Reducing the effects of environmental inhibition in quantitative real-time PCR detection of adenovirus and norovirus in recreational seawaters. *J. Virol. Methods* **181**, 43–50.

Sassoubre, L. M., Love, D. C., Silverman, A., Nelson, K. L. & Boehm, A. B. 2012 Comparison of enterovirus and adenovirus concentration and enumeration methods in seawater from Southern California, USA and Baja Malibu, Mexico. *J. Water Health* **10**, 419–430.

Silva, A. M., Vierira, H., Martins, N., Granja, A. T., Vale, M. J. & Vale, F. F. 2010 Viral and bacterial contamination in recreational waters: a case study in the Lisbon bay area. *J. Appl. Microbiol.* **108**, 1023–1031.

Sinton, L. W., Finlay, R. K. & Lynch, P. A. 1999 Sunlight inactivation of fecal bacteriophages and bacteria in sewage-polluted seawater. *Appl. Environ. Microbiol.* **65**, 3605–3613.

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

Teunis, P. F., Moe, C. L., Liu, P., Miller, S. E., Lindesmith, L., Baric, R. S., Le Pundu, J. & Calderon, R. L. 2008 Norwalk virus: how infectious is it? *J. Med. Virol.* **80**, 1468–1476.

USEPA 2001 *Male-specific (F+) and Somatic Coliphage in Water by Two-step Enrichment Procedure*. United States Environmental Protection Agency, Washington, DC.

USEPA 2004 *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples*. EPA 815-B-04-00. Cincinnati, Ohio.

USEPA 2006 *Enterococci in Water by Membrane Filtration Using Membrane-Enterococcus Indoxyl-B-D-Glucoside Agar (mEI)*. United States Environmental Protection Agency, Washington, DC.

USEPA 2012 *Recreational Water Quality Criteria. Office of Water 820-F-12-058*. United States Environmental Protection Agency, Washington, DC.

Vinjé, J., Hamidjaja, R. A. & Sobsey, M. D. 2004 Development and application of a capsid VP1 (region D) based reverse transcription PCR assay for genotyping of genogroup I and II noroviruses. *J. Virol. Methods* **116**, 109–117.

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

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

Walters, S. P., Yamahara, K. M. & Boehm, A. B. 2009 Persistence of nucleic acid markers of health-relevant organisms in seawater microcosms: implications for their use in assessing risk in recreational waters. *Water Res.* **43**, 4929–4939.

World Health Organization 2003 *Guidelines for Safe Recreational Waters*. WHO, Geneva.

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