Daily measures of microbes and human health at a non-point source marine beach

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ABSTRACT

Studies evaluating the relationship between microbes and human health at non-point source beaches are necessary for establishing criteria which would protect public health while minimizing economic burdens. The objective of this study was to evaluate water quality and daily cumulative health effects (gastrointestinal, skin, and respiratory illnesses) for bathers at a non-point source subtropical marine recreational beach in order to better understand the inter-relationships between these factors and hence improve monitoring and pollution prevention techniques. Daily composite samples were collected, during the Oceans and Human Health Beach Exposure Assessment and Characterization Health Epidemiologic Study conducted in Miami (Florida, USA) at a non-point source beach, and analyzed for several pathogens, microbial source tracking markers, indicator microbes, and environmental parameters. Analysis demonstrated that rainfall and tide were more influential, when compared to other environmental factors and source tracking markers, in determining the presence of both indicator microbes and pathogens. Antecedent rainfall and F+ coliphage detection in water should be further assessed to confirm their possible association with skin and gastrointestinal (GI) illness outcomes, respectively. The results of this research illustrate the potential complexity of beach systems characterized by non-point sources, and how more novel and comprehensive approaches are needed to assess beach water quality for the purpose of protecting bather health.

Key words | beach, epidemiology, health, marine, microbes, non-point

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INTRODUCTION

Illness associated with microbial pathogens in recreational marine beaches can have significant health and economic impacts. Globally, each year, there are in excess of 120 million cases of gastrointestinal disease and in excess of 50 million cases of respiratory diseases caused by swimming and bathing in wastewater-polluted coastal waters (Shuval 2003). A study in Orange County, California, USA demonstrated an estimated 3.3 million US dollars per year in excess illness costs for Newport and Huntington beaches (Dwight *et al*. 2005). Since the 1950s, epidemiologic studies have been performed to evaluate relationships between bathing in point source impacted beaches and health risk (gastrointestinal disease, respiratory, skin, eye, and ear illnesses); and they concluded that symptoms for all these illnesses were increased in bathers compared to non-bathers (Prüss 1998).

Indicator microbes are used routinely to evaluate risk of illness from recreational bathing at beaches. These non-pathogenic microbes serve as surrogates for microbial pollution containing pathogenic microbes and are utilized for several reasons. These reasons include: their high concentrations in raw untreated human sewage, ease of detection, and in some cases, associations with human illness rates. Studies show that the Environmental Protection Agency (EPA)-recommended indicator microbe for marine beaches, enterococci, has demonstrated a significant association with illness in point source impacted marine beaches, at least in temperate climates (Prüss 1998; Wade *et al*. 2003).

As was shown with point source impacted beaches, swimming in non-point source impacted beaches may also be a cause of increased illness when compared to non-bathers (Colford *et al*. 2007; Bonilla *et al*. 2007; Fleisher *et al*. 2010; Sinigalliano *et al*. 2010). However a relationship between enterococci and bather health has not been consistently established at non-point source impacted or subtropical marine beaches (Prüss 1998; Haile *et al*. 1999; Wade *et al*. 2003; Colford *et al*. 2007). Recent studies however have shown preliminary relationships between health outcomes and indicators. A study conducted in Miami, Florida demonstrated a relationship between enterococci measured by membrane filtration and skin illness, between 24 hour prior rainfall and skin illness, and between water temperature and acute febrile respiratory illness (Sinigalliano *et al*. 2010). Another study conducted on the California west coast demonstrated a relationship between male-specific coliphage and several symptoms reported by bathers (Colford *et al*. 2007).

In addition to the lack of a consistent association between enterococci and bather health at non-point source impacted and subtropical marine beaches, there are many limitations to using indicator microbes to monitor for fecal contamination and significant human health risk. Enterococci can originate from sources other than humans, and therefore the ratios of pathogens to enterococci may not be consistent with that of human fecal pollution. Enterococci are also heavily influenced by environmental factors, and they do not necessarily address the risks for non-gastrointestinal illnesses, since their association was originally established specifically with gastrointestinal illness (Boehm *et al*. 2009). Additionally, research has shown that the presence and levels of indicators are not always associated with pathogens in areas impacted by non-point sources of indicator organisms (e.g. rainfall runoff, animals, human shedding, and sand re-suspension) (Deetz *et al*. 1984; Fujioka *et al*. 1981; Gerba & Rose 1990; Jiang *et al*. 2001; Noble & Fuhrman 2001; Jiang & Chu 2004). Furthermore, at both subtropical and temperate climates, the indicator bacteria can multiply in the environment, giving a false impression of an increase of fecal pollution, which is in turn interpreted through current US federal guidelines as an increase in human health risk (Fujioka *et al*. 1981; Solo-Gabriele *et al*. 2000; Desmarais *et al*. 2002; Whitman *et al*. 2003; Shibata *et al*. 2004; Whitman *et al*. 2004; Alm *et al*. 2006; Beversdorf *et al*. 2007).

Monitoring directly for the specific disease-causing pathogens themselves would be ideal. Studies have shown the presence of pathogenic viruses (such as enteroviruses, reoviruses, adenoviruses, hepatitis A virus, and norovirus) in marine waters in many areas (including Greece, Italy, California, Florida, and Hawaii) (Griffin *et al*. 2003; Ortega *et al*. 2009). Pathogenic protozoa and bacteria have also been detected in recreational beaches (Elmir *et al*. 2007; Sunderland *et al*. 2007; Abdelzaher *et al*. 2010; Graczyk *et al*. 2010). However, in both point and non-point source impacted marine beaches in temperate and sub/tropical climates, epidemiologic studies have not established associations between health risk and specific pathogens. To the author's knowledge, Haile *et al*. (1999) was the only study which measured pathogens (enterovirus), other then *S. aureus* (Fujioka *et al*. 1994), in conjunction with human effects. Several epidemiologic studies have been initiated at several California beaches (Avalon, Doheny, and Malibu), which have measured pathogens in conjunction with health effects and publications from these studies are pending. The US EPA has also initiated studies in Mississippi, Rhode Island, Alabama, and Puerto Rico, but these study results are also pending and focus on point source impacted beaches. To date, no published studies have evaluated pathogens and health effects concurrently within a subtropical beach environment.

The objectives of this study were to evaluate the water quality (indicator microbes, pathogens, and environmental factors) and daily cumulative health effects (gastrointestinal, skin, and respiratory illnesses) for bathers at a non-point source subtropical marine recreational beach. This study

evaluated the relationships between microbes and environmental conditions; it also evaluated the inter-relationship between health effects and water quality, and identified possible implications for beach regulation practices. Given cost limitations when screening multiple targets including pathogens and concurrently assessing human health, this study was not intended to establish a conclusive relationship between the various factors and targets, but rather to give further insight on how the non-point source beach system functions. The results of this study should be utilized with results from other epidemiologic and water quality studies to establish conclusive statements about the relationship between water quality and human health at non-point source beaches. This study differs from previously published work as part of the BEACHES study (Fleisher *et al.* 2010; Sinigalliano *et al.* 2010; Shibata *et al.* 2010) by reporting the results from daily composite samples during the BEACHES study therefore allowing many more microbiological measures to be compared to environmental parameters and health outcomes and providing an alternate perspective to beach monitoring through composite as opposed to grab samples.

METHODS

Site description

Samples were collected from a study beach within Miami-Dade County, Florida, USA (Figure 1). The climate of Miami is classified as 'subtropical' because of its geographic location, and its average ambient temperature of 24.8 °C. The study beach is an irregularly narrow beach, with an approximate average distance between the mean water line and the outer edge of the sand of 5 m. The beach is 1.6 km long, relatively shallow, and characterized by weak water circulation (Shibata *et al.* 2004). This beach is the only beach in Miami-Dade County where visitors can bring their pets, particularly dogs. Beach admission is free, and many bathers frequent the beach, particularly during the summer months (Wang *et al.* 2010). Extensive prior evaluation of the vicinity of the study beach had not found point sources of pollution to this beach (such as sewage outfalls, failing lift stations) or other direct sewage inputs (such as through septic tanks) (Shibata *et al.* 2004). However

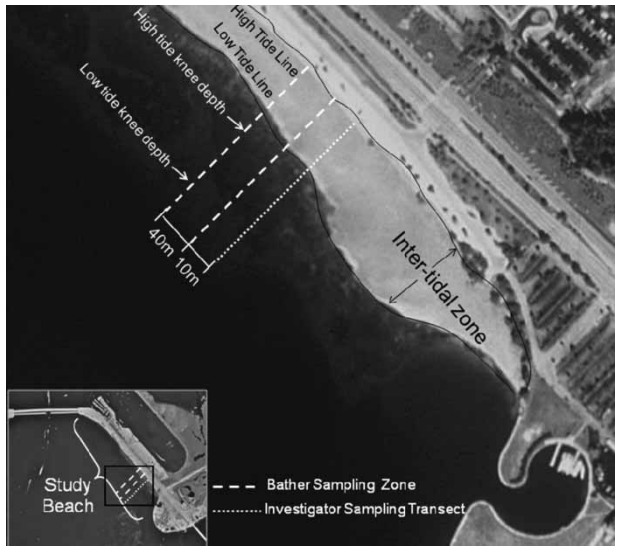


Figure 1 | Study beach aerial photograph and sampling scheme including bather sampling area where bather-collected composite samples were collected and the investigator sampling transect where the investigator-collected samples were collected (aerial photograph courtesy of USGS).

several potential non-point sources of pollution such as sand efflux, trash bins, bather shedding, rainfall runoff, offshore boat dumping, dogs and other animals may impact the study beach (Wright *et al.* 2009; Wright *et al.* 2011). Sand efflux is believed to be significant (Zhu *et al.* accepted) due to elevated levels of indicators observed in the sand along with intermittent detection of pathogens including *Giardia* spp., *Cryptosporidium* spp., enterovirus, and *Vibrio vulnificus* (Abdelzaher *et al.* 2010; Shah *et al.* accepted). This beach is usually in compliance with regulatory monitoring criteria, but periodically (i.e. 2.1 times per year averaged from 2002 to 2010) has been placed under an advisory due to microbial water quality violations (Polk D., personal communication).

Epidemiologic study

Sampling occurred as part of the Oceans and Human Health Beach Exposure Assessment and Characterization Health Epidemiologic Study, 'BEACHES' (Fleisher *et al.* 2010). In brief, this study was the first randomized control exposure epidemiological study to be conducted in the United States. The study design involved randomly assigning adults who report regularly bathing in South Florida to either a 'bather' or 'non-bather' category. Bathers were asked to spend 15 minutes in

the water, while non-bathers spent 15 minutes on the beach. Approval was obtained by the Florida Department of Health Internal Review Board (IRB 1491; DOH IRB Number, H07164) and the University of Miami Internal Review Board (IRB 20070306) to include bathers in this study (Fleisher *et al*. 2010). As part of the BEACHES study two distinct types of water samples were collected, 'individual' samples collected by individual bathers and 'daily composite samples' which were combined water samples collected throughout each sampling day. The earlier published work focused on describing the epidemiologic data (Fleisher *et al*. 2010) and also evaluated, for the 'individual' samples, dose-response relationships between microbes and environmental measures and health effects (Sinigalliano *et al*. 2010). The current manuscript complements these earlier publications by reporting the results from the daily composite samples, comparing microbiological and environmental measurements, as well as evaluating combined human illness rates corresponding to the daily composite samples. The results from the daily composite samples have not been reported elsewhere. Due to the fewer number and larger sample sizes of the daily composite samples, many more microbiological measures were included in the analyses allowing for the comparisons between a much broader range of microbes. In this manuscript, illness among individual participants was combined on a daily basis (number of participants who became ill relative to the total number of participants per day), and then excess illness was computed (average daily difference in percentages for gastrointestinal, skin, and acute respiratory febrile illnesses between bathers and non-bathers). This approach yields, however, an underestimate of risk due to the misclassification of exposure (i.e. the assignment of a single daily microbial exposure value to multiple bathers with different exposures albeit on the same day) (Fleisher *et al*. 1993). Nevertheless this approach provides an alternative perspective to monitoring and hence beach regulation through compositing samples over several hours and from different locations as opposed to grab sampling.

Sample collection and measurement of physical–chemical parameters

Fifteen sampling events were conducted on 15 different sampling days ranging from December 2007 to June 2008 as part of the BEACHES study described above. Sampling occurred over the study day span of 3.5 hours starting at approximately 8:00 am and ending at 11:30 am local time. Two sets of composite water samples were collected and analyzed per sampling day: 'bather-collected composite' and 'investigator-collected composite'. Bathers (30–60 per sampling day), assigned to spend 15 minutes in the water, each collected 5 L water samples in pre-sterilized sampling containers. These bathers collected samples from their sampling zone (area of exposure) at knee depth, which varied with tide (Figure 1). Bathers were trained (asked to conduct a thorough pre-rinse of the sample container, avoid touching the inside of the container, and to sample just below the surface of the water, etc.) on how to collect the water sample to minimize variation between bathers.

One liter of each of these samples was added to a 50 L pre-sterilized carboy, and this sample (30 to 50 L) was called the 'bather-collected composite'. When there were greater than 50 bathers, smaller sample aliquots (0.8 L) were added to the 50 L carboy to not exceed the carboy capacity. The remaining 4 L collected by bathers was also analyzed individually on a 'per bather basis' and was thus not composited; the analysis of the remaining 4 L has been discussed elsewhere (see Fleisher *et al*. 2010; Sinigalliano *et al*. 2010).

Another composite sample was also collected by study staff and called the 'investigator-collected composite'. This sample involved collecting two 10 L water samples at knee depth approximately every 10 minutes from the time the first bather entered the water and throughout the 3.5 hour sampling duration. Investigators collected samples using 5 gallon pre-sterilized buckets supported by a float. Sample collection was done in a manner as to minimize sediment disturbance and minimize contamination from the investigators by standing downstream of the sampling location. These 10 L aliquots were pooled into two 250 L sterilized plastic drums by pouring approximately 5 L from each aliquot into both drums. The total volume of this sample ranged from 200 to 225 L/sampling day. The sampling transect for the investigator-collected composite samples was approximately 10 m away from the bather sampling zone where the bather-collected composite samples were collected in order to minimize the impacts from the individual epidemiologic study participants (Figure 1). Both the 50 L bather-collected carboy and 200 to 225 L investigator collected drum were kept in the

shade during the 3.5 hour sampling time to minimize temperature increase of the sample.

Water temperature, pH, salinity and turbidity were measured for the bather-collected composite samples in the field using a YSI probe (600R series sonde, YSI Inc., Yellow Springs, Ohio) and a turbidimeter (Model 66120-200, VWR, Newark, DE, USA). Additionally, other environmental conditions were obtained from National Ocean and Atmospheric Administration (NOAA) (tidal height) and the University of Miami (National Science Foundation (NSF) National Institute of Environmental Health Sciences (NIEHS) OHH Center Remote Sensing Facilities Core) (rainfall, wind speed, and solar radiation). Solar radiation, tidal height, and wind speed were determined by averaging measured values every 2 to 6 minutes from the time of the first bather-collected sample to the last. Some peaks in microbe measurements were noticed when the majority of sampling for the composites occurred right after peak high tide. This was further analyzed quantitatively by computing the percentage of samples on a given sampling day which were collected beyond peak high tide (post-HT sampling %). For sampling days where sampling did not overlap with peak high tide, this value was set to zero.

Concentration and analysis of bather-collected composite samples

Bather-collected composites were analyzed for many different microbes, including the EPA-recommended marine bacterial indicator, enterococci, using three methods: membrane filtration (MF), chromogenic substrate (CS), and quantitative polymerase chain reaction (qPCR). Additional microbial indicators (fecal coliform, *Escherichia coli*, *Clostridium perfringens* by MF and coliphage via single agar layer method) were measured as well as human and dog-associated microbial source tracking (MST) markers including Bacteriodales (*Bacteroides thetaiotaomicron*, BacHum-UCD, HF8, and DogBac), human polyomavirus (HPyVs) and the *esp* gene of *Enterococcus faecium*. Pathogens evaluated included: bacteria (*Staphylococcus aureus* by MF, *Vibrio vulnificus* with enrichment by MF and confirmation by PCR) and a low volume concentration method (bilayer method, Abdelzaher *et al.* 2009) for processing protozoa (*Cryptosporidium* spp. and *Giardia* spp. via qPCR) and viruses (pan enterovirus, norovirus, and hepatitis A via qPCR). The volumes analyzed for each microbe, as well as a description of the methods used in the analysis, are summarized in the electronic supplement to this article (available at http://www.iwaponline.com/jwh/146.pdf).

Concentration and analysis of investigator-collected composite samples

Investigator-collected composite samples, consisting of two large drums of approximately 225 L, were used to collect samples for pathogen analysis using traditional large volume concentration methods. Filters (Envirochek, Pall™ Ann Arbor, MI, USA) obtained from processing the water from one of the drums was used for protozoan analysis (*Cryptosporidium* spp. and *Giardia* spp. via immunomagnetic separation followed by microscopic analysis), while the filter obtained from the second drum (Filterite, Pall™, after acidification of water in the drum) was used for enterovirus analysis via culture. Details of the corresponding analyses methods are included in the electronic supplement to this article (available at http://www.iwaponline.com/jwh/146.pdf).

Statistical analysis

Data were checked for normality using the Shapiro–Wilk test, and normalized using a log transformation when needed. Parameters that did not meet normality requirements (such as rainfall, post-HT sampling %, pathogens, coliphage, and all of the source tracking markers except Bacteriodales BacHum-UCD), mostly due to being frequently below detection limits (i.e. left censured data), were not evaluated statistically. Parameters that met the normality requirements were analyzed using the following parametric tests: a student *t*-test was used to determine statistical significance of different results between the different enterococci analysis methods using Excel™, and a Pearson analysis was conducted using XLSTAT™ to determine correlation coefficients between parameters as well as their statistical significance. The few values below detection limits within these statistically analyzed parameters were analyzed at their respective detection limits.

RESULTS

Health outcomes

The method for assessing illness, as well as other epidemiologic considerations using the individual exposure data, have been previously described (Fleisher *et al.* 2010). The number of total bathers per day ranged from 29 to 55 (avg = 43 ± 10), while the number of total non-bathers per day ranged from 25 to 60 (avg = 43 ± 11). This yielded a total of 652 bathers and 651 non-bathers for the 15 study days. Daily gastrointestinal, skin, and acute febrile respiratory illness reported in follow up interviews ranged from 0 to 3, 0 to 9, and 0 to 4 participants for bathers, and 0 to 2, 0 to 2, and 0 to 2 for non-bathers, respectively. The total number of cases over the 15 study days for gastrointestinal, skin, and acute febrile respiratory illness were 31, 47, and 12 for bathers, and 18, 9, and 4 for non-bathers, respectively.

Illness rates were computed by subtracting daily illness percentage rates of non-bathers from that of bathers, and termed 'excess illness'. Average daily excess illness percentage rates for GI, skin and acute febrile respiratory illness were 2.0 ± 3.3, 5.6 ± 4.7, and 1.2 ± 2.9%, respectively (Figure 2). Negative excess illness values represented days in which a higher illness percentage was reported in non-bathers when compared to bathers. Cumulative daily excess health effects (sum of percentages of GI, skin, and respiratory illness) ranged from −7% to 22%.

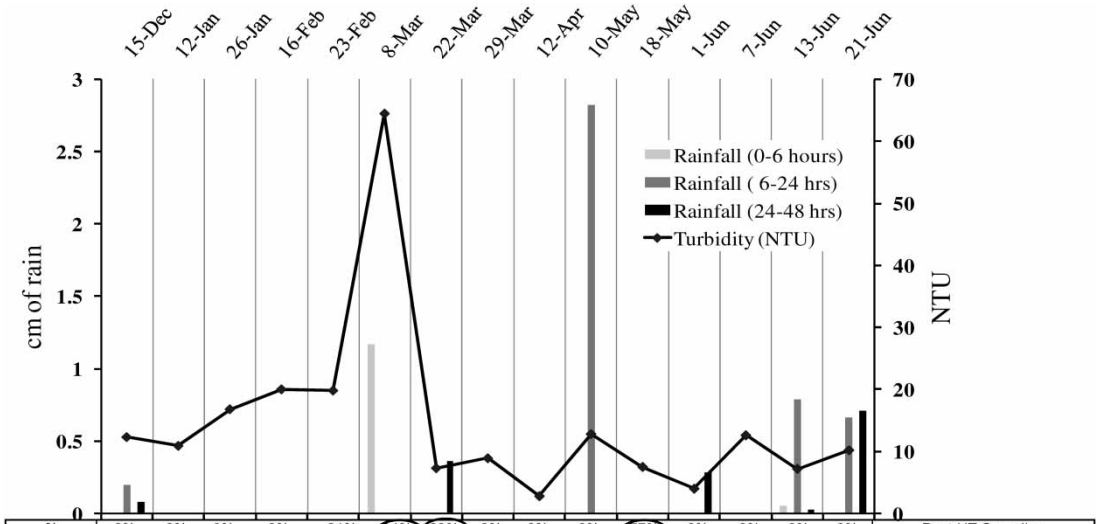
F− coliphage was detected on 3 of the 5 days characterized by the highest excess GI illness (see dotted rectangles on Figure 2). F+ coliphage was not detected in any of the samples, and hence no association was apparent in this study between this microbe and health conditions. The 3 highest levels of Bacteriodales BacHum-UCD human marker corresponded to the 3 days characterized by the highest excess skin illness. These 3 days also corresponded to days when enterovirus were detected; 6–24 hour rainfall was observed during 2 of the 3 days characterized by the highest excess skin illness (see rounded rectangles on Figure 2). The highest excess skin illness level on May 10th also corresponded with the highest values of enterococci measured by qPCR and CS, Bacteroidales DogBac marker, cumulative pathogens (*Cryptosporidium* spp., *Giardia* spp. and enterovirus), and the highest level (2.82 cm) of 6–24 rainfall (see solid rectangles on Figure 2).

Environmental parameters

The daily values for environmental parameters (pH, salinity, and temperature, turbidity and wind speed) for each of the 15 bather-collected study samples over the 15 study days can be found in the electronic supplement to this article (available at http://www.iwaponline.com/jwh/146.pdf). The average and standard deviation values for pH, salinity, temperature, and wind speed were 8 ± 0.3, 35.6 ± 1.4 psu, 26.4 ± 3.0 °C, and 6.7 ± 2.3 m/s, respectively. Solar radiation and tidal height yielded average values of 307 ± 92 W/m^2 and 0.35 ± 0.18 m, respectively (Figure 2). The turbidity varied significantly between study days, with a range of 2.8 to 64.4 nephelometric turbidity units (NTU) and an average of 14.5 ± 14.7 NTU. Rainfall was detected immediately prior to 7 of the 15 sampling dates, and reported as 0–6 h, 6–24 h, and 24–48 h rainfall prior to the mean sampling time (Figure 2).

Correlations were noted between water temperature and solar radiation, as well as water temperature and turbidity (Table 1). The increase in turbidity in the water with decreasing water temperature may be due to the presence of rainfall which occurs during cloudy conditions and hence decreases solar radiation and water temperature. Solar radiation was also found to correlate with enterococci (CS) (Table 1). Absolute tidal height was not associated with any microbes; however, enterococci (MF) values greater than the regulatory geometric mean value of 35 colony-forming units (CFU) 100 ml^{-1} occurred only when sampling was conducted immediately after peak high tide (i.e. post-HT sampling percentage greater than 0) (see circled areas on Figure 2).

Zero to 6 hour rainfall corresponded to elevated levels of turbidity and bacterial indicators: enterococci (MF), *E.coli*, fecal coliform, and *C. perfringens* (see light gray shading in Figure 2). Rainfall during the prior 6 to 24 hours had additional significance. The simultaneous presence of *Cryptosporidium* spp., *Giardia* spp. and enterovirus only occurred after 6 to 24 h rain events (see medium gray shading in Figure 2).



%	0%	0%	0%	0%	21%	84%	29%	0%	0%	0%	77%	0%	0%	0%	0%	Post-HT Sampling
m	0.41	0.50	0.60	0.05	0.47	0.45	0.57	0.15	0.27	0.03	0.53	0.33	0.28	0.19	0.45	Tidal Height
	rising	rising	rising	ebbing	rising	ebbing	rising	rising	rising	rising	ebbing	ebbing	rising	ebbing	rising	Tidal Stage
(W/m^2)	241	230	325	449	383	260	190	352	312	366	378	393	380	113	236	Solar Radiation
CFU/100 ml	<2	15	4	15	13	99	109	<2	3	29	50	13	<2	14	<2	Enterococci (MF)
	<2	<2	31	3	7	83	27	3	<2	3	23	25	<2	30	17	*E.coli*
	3	104	27	10	20	196	61	66	<2	5	9	127	7	159	147	Fecal coliform
	<2	<2	7	<2	1295	1653	402	13	4	5	34	24	6	159	16	*C. perfringens*
	55	52	61	18	13	69	100	23	13	124	43	15	11	48	68	Enterococci(CS)
GEU/100 ml	68	81	233	512	1647	483	89	124	46	2361	974	472	449	285	637	Enterococci (qPCR)
PFU/100 ml	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	F+ coliphage
	<0.3	**1**	**1.7**	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	**0.3**	**0.3**	<0.3	<0.3	F- coliphage
#organisms/ 100L	**1.6**	<0.6	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	**0.9**	<0.7	**1**	<0.5	<0.5	**0.5**	Cryptosporidium oocysts
	1.1	<0.6	<0.5	<0.5	<0.5	<0.5	**2.1**	<0.5	<0.5	**2.3**	<0.7	<0.5	**1.5**	<0.5	**1**	Giardia cysts
	1.6	<0.5	<0.5	<0.5	<0.5	<0.6	<0.6	<0.5	<0.5	**2.2**	**0.5**	<0.6	<0.5	**0.5**	**0.53**	Enterovirus
CFU/500 ml	pos	pos	pos	neg	pos	pos	neg	neg	pos	pos	neg	pos	pos	pos	pos	*V.vulnificus*
CFU/100 ml	11	16	24	11	4	<2	8	<2	11	<2	<2	19	14	2	13	*S. aureus*
TSC/100 ml	391	2090	1640	532	4738	120	463	<1	314	12900	1102	119	<1	516	538	Bacteroidales DogBac
GEU/100 ml	64	51	59	15	43	25	28	25	6	273	407	4	<1	37	28	Bacteroidales BacHum-UCD
	3	<1	5	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	Bacteroidales HF8
per 1 L	neg	pos	neg	pos	pos	neg	neg	pos	neg	neg	neg	neg	neg	neg	neg	*B. thetaiotaomicron*
	neg	neg	neg	neg	pos	pos	neg	pos	pos	neg	neg	neg	neg	neg	neg	Polyomavirus
per 500 ml	pos	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	pos	pos	neg	neg	Enterococci *esp* gene
% excess illness	0.0%	9.7%	4.8%	0.1%	2.2%	2.4%	5.8%	0.2%	0.5%	4.2%	1.9%	-0.1%	4.1%	0.0%	-4.9%	GI Illness
	10.3%	3.0%	4.8%	-2.3%	4.2%	8.6%	8.3%	7.7%	2.8%	15.6%	11.5%	5.1%	5.9%	0.0%	-1.1%	Skin Illness
	0.5%	0.0%	5.1%	-4.7%	0.0%	0.0%	5.9%	7.3%	2.2%	2.0%	2.0%	0.0%	-2.0%	0.0%	0.0%	AFRI

Figure 2 | Beach 'snapshots' including microbial indicators, pathogen levels, source tracking markers, and excess illness percentages between bathers and non-bathers rates, and their potential association with environmental parameters. Data highlighted to indicate possible influence of rainfall. Data circled or with a rectangle to indicate possible relationships between factors (see results). Excess illness percentage rates of 0% represent sampling days where there was neither bather nor non-bathers illness/infection while negative values represent days in which the number of cases in non-bathers exceeded the number in bathers. AFRI, acute febrile respiratory illness; GI, gastrointestinal.

The highest values of enterococci measured by qPCR and CS, Bacteroidales DogBac marker, cumulative pathogens (*Cryptosporidium* spp., *Giardia* spp. and enterovirus) occurred on May 10th which had the highest level (2.82 cm) of 6–24 rainfall (see solid rectangles on Figure 2).

Microbial indicators

Enterococci levels in water samples over the 15 sampling days ranged from <2 to 109 CFU 100 ml^{-1} using MF methods, 11 to 124 MPN 100 ml^{-1} using CS, and 46 to 2360 genome equivalent units (GEU) 100 ml^{-1} using qPCR (Figure 2). Measured concentrations of enterococci by qPCR were greater than MF ($p < 0.0001$) and CS ($p < 0.0001$) measurements; while measurements by CS were greater ($p = 0.005$) than those by MF. No significant ($p < 0.05$) correlations were noted between each of the enterococci measurement methods, qPCR, MF and CS. Enterococci levels measured by CS correlated with Bacteroidales BacHum-UCD levels and the inverse of solar radiation (Table 1). *Escherichia coli*, fecal coliform, and *C. perfringens* values ranged from <2 to 83, <2 to 196, and <2 to 1,653 CFU 100 ml^{-1}, respectively (Figure 2). The maximum values for these three indicators,

Table 1 | Correlation coefficients for significant ($p < 0.05$) correlations between indicator microbes, microbial source tracking markers and environmental factors

		R	p
Enterococci (MF)	*E. coli*	0.57	0.02
	C. perfringens	0.57	0.03
Enterococci (CS)	Bacteroidales (BacHum-UCD)	0.68	0.005
	Solar radiation	−0.56	0.03
E. coli (MF)	Fecal coliform	0.68	0.005
	C. perfringens	0.70	0.004
Water temperature	Solar radiation	0.53	0.04
	Turbidity	−0.58	0.02

Note: Parameters that did not meet normality requirements (such as rainfall, post-HT sampling %, pathogens, coliphage, and all of the source tracking markers except Bacteriodales BacHum-UCD), mostly due to being frequently below detection limits, were not evaluated statistically.

as well as the second highest value of enterococci (MF), were measured from the March 8 samples which had the highest level of 0–6 hour rainfall of 1.17 cm (Figure 2). Correlations were noted between *E.coli* and the remaining three indicator bacteria: enterococci (MF), *C. perfringens*, and fecal coliform (Table 1). *Clostridium perfringens* also correlated with enterococci (MF) (Table 1). Both F+ and F− coliphage were predominately below detection limits; however, F− coliphage was detected in 4 samples at low concentrations of 0.3 to 1.7 plaque-forming units (PFU) 100 mL^{-1} (Figure 2).

MST markers and bacterial pathogens

Polyomavirus, *B. thetaiotaomicron*, and *Enterococcus faecium esp* gene were detected on 4, 4, and 3 of the sampling days, respectively (Figure 2). Bacteriodales HF8 marker was predominately below detection limits with only two detects at (5 and 3 GEU 100 mL^{-1}) (Figure 2). Bacteroidales BacHum-UCD marker levels ranged from below detection to 407 GEU 100 mL^{-1} with an average of 71 GEU 100 mL^{-1} (Figure 2), and were found to correlate with enterococci (CS) (Table 1). Bacteroidales DogBac marker levels ranged from below detection to 12,900 target sequence copies (TSC) 100 mL^{-1} with an average of 1,700 TSC 100 mL^{-1} (Figure 2). *Staphylococcus aureus* levels ranged from <2 to 24 CFU 100 mL^{-1} with an average of 9.7 ±7 CFU 100 mL^{-1}; *V. vulnificus* was detected on 11 of the 15 sampling days (Figure 2).

Protozoan and viral pathogens

Using the bilayer concentration method (5 L filtration) and subsequent qPCR analysis, all bather-collected composite samples were below detection limits for both viruses and protozoa. However, for the large-volume investigator-collected composite samples, *Cryptosporidium* spp., *Giardia* spp., and enterovirus were detected on 4, 5, and 5, respectively, of the 15 sampling days (Figure 2). Levels of the organisms detected did not exceed 2.3 organisms per 100 L (Figure 2). The simultaneous presence of *Cryptosporidium* spp., *Giardia* spp. and enterovirus only occurred during 3 of the 15 sampling dates. Common environmental conditions during these 3 days were rainfall during the preceding 6 to 24 hours (Figure 2), and sample collection during rising tide. Also of interest was that during these 3 days, enterococci levels by MF were below regulatory thresholds (Figure 2). When enterococci levels exceeded the single sample regulatory standard of 104 CFU mL^{-1}, *Giardia* spp. was detected, although it was also detected on days when levels were below this regulatory standard.

DISCUSSION

Properly assessing the safety of recreational bathing waters is an evolving field of study which will continue to require further investigation. The complexity of the non-point source impacted beach system and its components poses a challenge to investigators attempting to make simple associations between environmental parameters, microbe presence, and human health risks in order to ultimately improve beach safety through source remediation and monitoring. The uniqueness of this study was in the multitude of different parameters that were analyzed along with human health effects, providing a wide 'snapshot' of the complex beach system that may be used to further our understanding of the system. To the authors' knowledge, this is the first study published that includes the analysis of such a wide range of varying parameters at a recreational beach. This is also the first study to measure pathogens concurrently

with human health effects in a subtropical beach environment. The results of this study should assist in directing future research, but are not sufficient (15 sampling days, beach 'snapshots') to make conclusions in regards to how the system functions or which beach monitoring practices should be taken.

Health outcomes

Average daily excess illness rates for bathers after subtracting the control group (non-bathers) provided an estimate for the daily health outcomes of bathers during this epidemiologic study (BEACHES) conducted in Miami, Florida. These daily average illness rates were therefore compared to the daily average water quality (by analyzing the composite sample made up of water aliquots from each sample collected by individual bathers). This approach does not assess the more precise exposure of the bather to the water in their vicinity at a specific time during the day (by analyzing each water sample collected by each bather during bathing (Sinigalliano *et al.* 2010; Fleisher *et al.* 2010). However, the analysis discussed in this study allowed the investigators to compare health outcomes with a wider range of pathogens, indicator microbes, and microbial source tracking markers, which were not available for the individual water sample collected by each individual bather.

Statistically significant correlations between health outcomes and water quality were not identified in this study. However, the data suggest relationships which could not be verified statistically given the limited number of times that samples were positive for many of the water quality parameters. The lack of relationship between health outcomes and pathogens that could potentially cause that particular illness may be due to the small samples size (15 samples), the fact that the pathogen causing increased illness in bathers may not be among those analyzed in this study, the inefficiency of the sampling techniques (as pathogen concentration and analysis methods may be subject to significant recovery loss), or that increased illness rates are not the result of one specific pathogen.

However, a relationship that was alluded to in this study but more strongly supported in Sinigalliano *et al.* (2010), was excess skin illness and prior 24 hour rainfall. The highest rate of skin illness (15.6%) was observed during the day (May 10) characterized by the highest quantity of 24 hour rainfall. This day was also characterized by the highest enterococci levels as measured by CS and qPCR, the highest levels of Bacteroidales DogBac marker, the second highest Bacteroidales BacHum-UCD human marker, and the presence of multiple pathogens (*Cryptosporidium* spp., *Giardia* spp. and enterovirus). The potential relationship between skin illness and rainfall may be due to the rainfall carrying skin pathogen(s), which were not analyzed for in this study, into the water column. These skin pathogen(s) (which may include unmeasured microbes such as pathogenic helminthes and yeasts detected in another study within the sand at this study beach (Shah *et al.* accepted)) may be transported in a similar fashion as the gastrointestinal protozoan and viral pathogens detected in this current study. However, 24 hour antecedent rainfall did not consistently result in increased skin illness rates, possibly due to impacts from other environmental factors such as tide and solar radiation or due to smaller amounts of rain which may have been insufficient to release the skin pathogens presumably residing in the beach sand. Also of interest is that on May 10, the day characterized by the greatest 24 hour rainfall, the GI illness rate was also elevated (4.2%) which is consistent with the detection of GI-related pathogens, *Cryptosporidium*, *Giardia*, and enterovirus. However elevated GI illness among bathers was not observed during other days where GI pathogens were detected (e.g. December 15 and June 21), thus further emphasizing the complexity between human health outcomes, microbial measures, and environmental parameters at this particular beach site.

Another potential relationship that was indicated in this study was that of F– coliphage and gastrointestinal illness (detected during 3 of the 5 days characterized by the highest excess GI). F– coliphage was only detected in 4 of the 15 samples, and therefore more sampling would be needed before determining whether or not this association truly exists. Nevertheless, this finding is interesting since F+ coliphage was the only indicator microbe which showed a correlation with bather GI illness in the only other published non-point source beach epidemiological study besides the BEACHES study, which was conducted in Mission Bay, California (Colford *et al.* 2007). Although F–

(somatic) and F+ (male-specific) coliphage are not the same, the fact that they both are bacterial viruses and both showed some (albeit weak) associations with gastrointestinal illness in two separate epidemiologic studies at non-point source study sites suggests that these indicators should be further evaluated. One reason for the lack of detection of F+ coliphage in this study may have been due to sample size. In this study, 100 mL samples were concentrated for F+ coliphage, while in the Colford *et al*. (2007) study, 1 L samples were used, thereby increasing detection limits.

Indicator microbes and pathogens

Comparison of the different enterococci enumeration methods showed that results from MF and CS methods were generally below values analyzed by qPCR. Previous studies have also shown that the MF method yields lower values than qPCR (Haughland *et al*. 2005; Sinigalliano *et al*. 2007; Abdelzaher *et al*. 2010); this might be explained by the fact that qPCR will detect target DNA from nonviable and non-culturable cells, whereas the culture-based methods will not. Additionally, enterococci values by MF were generally below values analyzed by the CS method, which may be attributed to the differences in the culture methods; MF requires the formation of a colony on agar-containing media, whereas CS requires the growth of bacteria in liquid media. Significant correlations were not found between these three analysis methods. These findings should be considered when updating regulations to include alternative methods for the analysis of this indicator microbe.

The current study did not demonstrate a consistent relationship between indicator microbes and pathogens. *Giardia* spp. and *S. aureus* were the only pathogens detected when enterococci by MF exceeded the single sample maximum (March 22). Plano *et al*. (2011) observed *S. aureus* including methicillin resistant *S. aureus* in the beach water and sand at this particular study site and attribute the source to shedding from human bathers. Abdelzaher *et al*. (2010) also observed detectable levels of *Giardia* during a set of prior sampling events at this same study site, with the detection of *Giardia* spp. only when enterococci by MF exceeded the single sample maximum of 104 CFU 100 mL^{-1}. However, in the current study *Giardia* spp. and other pathogens were also detected when enterococci levels were low. These inconsistencies can be attributed to different transport pathways, and possibly different sources of indicator and pathogens to the beach site. Although human bather shedding and dog and bird feces have been identified as intermittent sources of enterococci to the beach site (Elmir *et al*. 2007, 2009; Wright *et al*. 2009), the quantities of these sources are insufficient to account for the persistent elevated levels observed in beach water located near the shore line (Zhu *et al*. accepted). The cause of these persistent levels of enterococci to the beach study site has been firmly established as the inter-tidal zone at this study beach (Shibata *et al*. 2004; Wright *et al*. 2011). The sources of pathogens have not been as firmly established for this study site, although limited studies detecting pathogen presence in sand suggest that they too may originate from the shoreline as discussed below (Abdelzaher *et al*. 2010; Shah *et al*. accepted).

Bacterial indicators were elevated after 6 hour rainfall, when samples were taken immediately after high tide and during high turbidity events (which are likely due to the combined effects of rainfall and tide). This finding is consistent with other studies conducted on both this study beach (Shibata *et al*. 2004; Abdelzaher *et al*. 2010; Wright *et al*. 2011) and other non-point source study beaches (Solo-Gabriele *et al*. 2000; Coulliette & Noble 2008). High bacterial loads detected in runoff samples at the study beach (Wright *et al*. 2011) indicate that these indicator microbes are washed off from the surface of the beach sand into the water column in significant numbers which could warrant a bather swimming warning if analyzed by regulators within a few hours of the rain event. Elevated levels of indicator microbes also occur through tidal washing of the microbes from the beach sand into the water column, as is evident by elevated indicator levels after peak high tides at the study beach during this study and previous studies (Abdelzaher *et al*. 2010; Shibata *et al*. 2010; Wright *et al*. 2011).

In prior studies, pathogens have also been shown to increase in water bodies after rain events (Lipp *et al*. 2001). In this study, pathogens (detected less frequently than indicators, and therefore cautious conclusions should be made about associations with other parameters), seemed to associate with rainfall as well. However, pathogens showed a delayed response with rain, and were specifically detected

when rainfall occurred 6 to 24 hours prior to sampling. In a prior study (Abdelzaher *et al.* 2010), at this same study site, pathogens were most frequently detected during a sampling event characterized by significant rainfall during the previous 24 to 48 hours (3.3 cm) as opposed to rainfall during the previous 6 to 24 hours. Therefore more work is needed focusing on the pathogen response to the size and timing of antecedent rainfall events.

The findings from this study suggest that the pathogens detected in the water may *not* be mobilized by rainfall-runoff processes from the surface of beach sand as is the case with indicators, but rather are entering the water column via the rain through a different pathway potentially through sand/sediment as well. One possibility is that the pathogens are entering beach water through groundwater as has been suggested to be the case for indicator microbes (Boehm *et al.* 2004). Rainfall infiltration can induce an increased hydraulic gradient (increase in the groundwater table) which would encourage the movement of groundwater towards the intertidal zone and ultimately out into the water column along the shore. Pathogens present in groundwater could be transported towards the beach water column under these favorable hydraulic conditions. Response times of groundwater inflows are generally slower relative to surface water flow as shown for river systems (Solo-Gabriele & Perkins 1997), and similar effects could be observed along the coastal zone. Thus, if indicators are more abundant at the sediment surface, whereas pathogens are more abundant at depth, it would be reasonable to observe two different response times to storm events: a short response time for indicator microbes (0 to 6 hours) via surface runoff, and longer response times (24 hours) for pathogens via groundwater.

Unlike indicators, pathogens were also not associated with either peak or post high tide, but rather were more likely detected when the tide was rising. A review of the literature studying the exchange of water from the terrestrial environment to the sea describes that if the density of the ocean water increases above that of the pore water for any reason, pore water can float out of the sediment by gravitational convection in an exchange with denser seawater in a process termed 'floating' (Burnett *et al.* 2003). Such effects can occur through rainfall decreasing the density of the pore water, hence causing a buoyancy differential which would enhance the transport of the pore water potentially containing the pathogens into the water column. In addition, during rising tide, a similar effect would occur as less dense pore water floats out of the sediment in exchange with denser seawater.

Thus, the results of this study show that indicators and pathogens are present in the water during different time periods. These observations suggest that indicators and pathogens may enter the water column through different pathways characterized by different response times to rainfall events and tidal conditions. Enterococci was chosen as the recommended marine beach indicator based on prior epidemiologic studies at point source beaches which associated enteroccoci levels with gastrointestinal illness (Cabelli 1983). The assumption behind the existence of this relationship is that enterococci, itself non-pathogenic via waterborne routes, is related to a pollution matrix from human fecal sources that contains pathogens which are causing the detected health outcome. For the case of a beach dominated by point sources of pollution, it is reasonable to assume that the indicators closely track pathogens as their source and transport towards the beach (e.g. via currents) would be similar. However, for the case of non-point sources because of the different sources and possible different potential transport pathways, the indicator and pathogen signal may likely be decoupled (Goodwin *et al.* 2009), resulting in an inability of indicator microbes to track pathogens thus decoupling the relationship between indicators and health effects. This potential decoupling is supported since a relationship between enterococci levels and gastrointestinal disease has not been established in the limited non-point source beach epidemiologic studies conducted thus far (Colford *et al.* 2007; Fleisher *et al.* 2010).

Recommendations

The results of this study are intended to guide further research at non-point source beaches by providing a series of 'snapshots' of relevant parameters as opposed to establishing conclusive statements regarding associations between different water quality parameters and health outcomes. This research supports the fact that correlations between water quality and health outcomes (especially in non-point source beaches) are complex, lending support to

the need for more comprehensive approaches to beach monitoring and regulation strategies.

Such a comprehensive approach should include multiple-target monitoring (including the consideration of multiple microbial targets, measurements of physical–chemical parameters, and hydrometeorologic conditions). Through further sampling of the beach water quality, criteria may be developed, relevant to the study beach conditions, which are based on a combination of targets i.e., elevated enterococci levels plus positive detection for pathogens. In developing such criteria, standards may also reflect cumulative health effects (sum of GI, skin, and respiratory illnesses) as opposed to one specific illness. In addition to strengthening monitoring criteria by assessing multiple targets to reflect the nature of localized conditions, other factors should be taken into account including sampling in worst case scenarios. Along with improved monitoring techniques, improved source prevention of potential non-point sources at the study beach should also be implemented, including: re-nourishing sand, treating runoff or diverting it from bathing areas, installation of showers for bathers to use both pre and post bathing to limit bather shedding and infection after exiting the water, developing an upper limit for the amount of people allowed on the beach, properly sealing waste bins and limiting the access of dogs and other animals in areas where humans recreate. More flexibility should also be incorporated into monitoring and source prevention policies to take advantage of recent developments and discoveries such that study results can be used in a timely fashion to ultimately decrease bather health risk while limiting economic burdens.

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