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Spatial and temporal variation in indicator microbe sampling is influential in beach management decisions

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ABSTRACT

Fecal indicator microbes, such as enterococci, are often used to assess potential health risks caused by pathogens at recreational beaches. Microbe levels often vary based on collection time and sampling location. The primary goal of this study was to assess how spatial and temporal variations in sample collection, which are driven by environmental parameters, impact enterococci measurements and beach management decisions. A secondary goal was to assess whether enterococci levels can be predictive of the presence of *Staphylococcus aureus*, a skin pathogen. Over a ten-day period, hydrometeorologic data, hydrodynamic data, bather densities, enterococci levels, and *S. aureus* levels including methicillin-resistant *S. aureus* (MRSA) were measured in both water and sand. Samples were collected hourly for both water and sediment at knee-depth, and every 6 h for water at waist-depth, supratidal sand, intertidal sand, and waterline sand. Results showed that solar radiation, tides, and rainfall events were major environmental factors that impacted enterococci levels. *S. aureus* levels were associated with bathing load, but did not correlate with enterococci levels or any other measured parameters. The results imply that frequencies of advisories depend heavily upon sample collection policies due to spatial and temporal variation of enterococci levels in

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response to environmental parameters. Thus, sampling at different times of the day and at different depths can significantly impact beach management decisions. Additionally, the lack of correlation between *S. aureus* and enterococci suggests that use of fecal indicators may not accurately assess risk for some pathogens.

1. Introduction

Exposure to microbial pathogens poses a risk to swimmers in recreational waters through routes such as ingestion, inhalation, and skin contact (Boehm et al., 2009a). In order to reduce the risk of exposure, water quality at recreational beaches is assessed by regulatory agencies using indicator microbes. Enterococci is used as a fecal indicator at recreational marine beaches due to its historical associations with adverse health effects in humans bathing in point source (e.g. human sewage) impacted recreational waters (Cabelli et al., 1982; Fleisher et al., 2010). According to U.S. EPA guidelines, enterococci levels in marine waters should not exceed a monthly geometric mean of 35 colony forming units (CFU)/100 mL and a single sample value of 104 CFU/100 mL. States develop their own standards and sampling procedures based on these EPA guidelines (U.S. EPA, 1986). Many states (Table 1) issue advisories immediately after the single sample guideline is exceeded. Other states collect a follow-up sample, thus requiring two consecutive samples before an advisory is issued.

Differences in sample collection times and locations may affect enterococci measurements and resulting beach management decisions. Water quality samples in many states, including Florida, are generally collected in approximately waist-depth water and are often collected in the morning. Water samples in other states may be collected in knee-depth water or in ankle-depth water (Dorfman and Rosselot, 2010). Samples collected at different times of the day can result in significantly different enterococci levels due to a variety of environmental factors, such as solar radiation, rainfall, tide, and the presence of bathers (Wright et al., 2011; Whitman et al., 2004; Boehm et al., 2002). Prior research has also indicated that enterococci levels in water samples collected approximately 100 m offshore can be lower than

Table 1 – Fraction of days advisories would have been issued based on given sampling times and criteria.

	Standard One	Standard Two	Standard Three	Standard Four
Description	Knee-depth, no consecutive samples	Knee-depth, consecutive samples	Waist-depth, no consecutive samples	Waist-depth, consecutive samples
States[a]	AL, HI, MD, TX, VA	MS,WA	CT, GA, LA, ME, MA, NY, RI	FL
Time				
12:00 AM	9/9[b] (100%)	7/7[b] (100%)	1/10 (10%)	0/9 (0%)
1:00 AM	7/10 (70%)	4/9 (44%)		
2:00 AM	7/10 (70%)	5/9 (56%)		
3:00 AM	4/10 (40%)	1/9 (11%)		
4:00 AM	4/10 (40%)	1/9 (11%)		
5:00 AM	6/10 (60%)	3/9 (33%)		
6:00 AM	3/10 (30%)	0/9 (0%)	0/10 (0%)	0/9 (0%)
7:00 AM	2/10 (20%)	1/9 (11%)		
8:00 AM	2/10 (20%)	1/9 (11%)		
9:00 AM	2/10 (20%)	1/9 (11%)		
10:00 AM	3/10 (30%)	0/9 (0%)		
11:00 AM	2/10 (20%)	0/9 (0%)		
12:00 PM	0/10 (0%)	0/9 (0%)	0/9 (0%)	0/9 (0%)
1:00 PM	3/10 (30%)	1/9 (11%)		
2:00 PM	4/10 (40%)	0/9 (0%)		
3:00 PM	1/10 (10%)	0/9 (0%)		
4:00 PM	4/10 (40%)	2/9 (22%)		
5:00 PM	5/10 (50%)	3/9 (33%)		
6:00 PM	5/10 (50%)	3/9 (33%)	1/10 (10%)	0/9 (0%)
7:00 PM	6/10 (60%)	5/9 (56%)		
8:00 PM	5/9[c] (56%)	2/8[c] (25%)		
9:00 PM	5/10 (50%)	3/9 (33%)		
10:00 PM	6/10 (60%)	4/9 (44%)		
11:00 PM	6/10 (60%)	4/9 (44%)		

a States with marine beaches not included in this analysis have management criteria that differ from the four standard types listed (rainfall advisories, different enterococci levels, ankle-depth sampling, etc.).
b The sample on Day 3 was lost and therefore affected consecutive measurements for Days 2–3 and Days 3–4. Only 9 samples were available for this hour.
c The sample on Day 5 was not collected due to a storm.

those collected 10 m offshore at beaches not impacted by offshore point sources of pollution (Wright et al., 2011).

One of the limitations in using enterococci as the sole human health indicator at marine beaches is that it may not be able to account for non-gastrointestinal illnesses (Boehm et al., 2009a) caused by pathogens such as *Staphylococcus aureus*, a usual commensal colonizing bacteria that is capable of causing skin infections. Studies have indicated that waters with high *S. aureus* densities may be associated with higher risks of skin, eye and ear infections (Charoenca and Fujioka, 1995; Gabutti et al., 2000). Recent studies have also indicated that methicillin-resistant *S. aureus* (MRSA), an antibiotic-resistant strain of *S. aureus* that can lead to serious infections, is found in recreational beach sands (Goodwin and Pobuda, 2009; Soge et al., 2009; Tice et al., 2010; Shah et al., 2011; Plano et al., 2011).

In order to properly understand if enterococci levels are an accurate indicator of health effects, both environmental influences and pathogen presence in relation to enterococci levels should be thoroughly understood. The first objective of this study was to understand how spatial and temporal variance in sample collection may affect beach management decisions based on enterococci measurements. Numerous other studies have characterized spatial and temporal environmental variables that impact enterococci measurements, and these factors vary in importance at each study beach (Wymer et al., 2005). This study, however, specifically examines relationships between these variables and beach advisory scenarios that result from different methodologies in sample collection. The second objective of this study was to determine whether presence of enterococci can be predictive of a skin pathogen such as *S. aureus* including MRSA. This study presents an analysis of 238 sampling events over a 10-day period, providing a high-resolution analysis unique to this study.

2. Materials and methods

2.1. Site Description

This study was conducted over a ten-day period at Hobe Cat Beach in Miami, Florida, USA. This beach has been the subject of extensive, long-term study (Fleming et al., 2004; Shibata et al., 2004; Elmir et al., 2007; Wright et al., 2009, 2011; Abdelzaher et al., 2010; Sinigalliano et al., 2010; Fleisher et al., 2010; Abdelzaher et al., 2011; Shah et al., 2011) and has been characterized as a non-point source beach. The beach faces central Biscayne Bay, a semi-enclosed subtropical lagoon, and is characterized by poor water circulation. The water at the beach is relatively shallow, and the beach itself is narrow with a mean distance from the waterline to the inshore edge of the sand of 5 m. The length of the beach is approximately 1.6 km (Shibata et al., 2004). It is the only beach in Miami-Dade County that allows visitors to bring dogs. Twenty percent of samples from this beach exceeded microbial water quality guidelines in 2009, and the beach was placed under an advisory for 7 days during that year (Dorfman and Rosselot, 2010). The area surrounding the beach has been extensively evaluated for point sources of pollution, such as sewage outfalls and septic tanks, but no point sources have been found (Shibata et al., 2004).

2.2. Water and sediment sampling

Temporal differences in water quality were evaluated by collecting samples hourly over a 10-day period. Spatial differences were evaluated by collecting samples in both knee-deep and waist-deep water. Sampling was conducted from June 1st to June 10th, 2010, along 10 transects marked by poles located at the upper edge of the wrack line. All samples were collected aseptically into Whirl-pak® bags. Every hour over the 10-day period, water and subtidal sediment samples were collected at knee-depth (0.3 m). Knee-deep water samples were collected from the water surface (5–10 cm underwater). The sediment samples were collected aseptically into Whirl-Pak® bags using sterilized spoons from the upper 5 cm of the submerged sand in an area of about 20 × 20 cm.

Every 6 h, starting at 6:00 AM on June 1st, a surface water sample (5–10 cm deep) was collected from waist-deep water (1 m deep). Three additional shoreline sediment samples were also collected every 6 h in order to evaluate spatial variation in sediment enterococci levels. Supratidal sand samples, representing sand above the high tide line, were collected from the upper 5 cm of sand in a location 0.15 m shoreward from the transect poles. Fixed-location intertidal sands, representing sand between the high and low tide lines, were collected 2.4 m toward the water from the corresponding transect pole. Waterline sediment samples were collected 0.08 m above the water's edge and moved due to tidal action.

2.3. Environmental parameters

Every hour, salinity, pH and water temperature were measured near the knee-depth sample site (YSI model 650–01 m environmental monitoring systems; YSI, Yellow Springs, OH). Turbidity of the collected water samples was measured in the lab using a nephelometer (TD-40; Turner Designs, Sunnyvale, CA). The presence of humans, dogs, and birds on both water and sand 65 m to the right and to the left of the middle sampling transect was also recorded every hour. Rainfall, solar shortwave radiation, and wind data were recorded every 2 min at a measurement station less than 1 km from the sampling site (NSF-NIEHS OHH Center Remote Sensing Facilities Core: http://yyy.rsmas.miami.edu/etc/download-weatherpak.cgi). Tidal data were recorded by a wave and tide recorder every 32 s 160 m offshore (TWR 2050, RBR, Ottawa, Ontario). More details about data processing are available in the Supplemental Text.

2.4. Microbial analysis

Upon collection, samples were transported in coolers with freezer packs immediately to a lab located within 1 km for analysis of microbes. Enterococci were analyzed using EPA Method 1600 (U.S. EPA, 2006). *S. aureus* were extracted from samples via membrane filtration. Filters were placed on Baird Parker agar with Egg Yolk Tellurite Enrichment (Becton Dickinson, Sparks, MD) and incubated at 37 °C for 24 h. Colonies that were identified as presumptive *S. aureus* colonies were subjected to further confirmation testing as described by Plano et al. (2011). More details about *S. aureus* analysis methods are provided in the Supplemental Text.

Processing of sediment samples required extraction of enterococci and *S. aureus* from sediment into water by adding a measured amount of sediment (approximately 10 g) into a sterile plastic bottle with 100 mL of sterile phosphate buffered dilution water. These samples were shaken vigorously for 2 min to promote the transfer of bacteria into the water (Boehm et al., 2009b). Sediment was allowed to settle for 2 min, and then 3 and 25 mL of the supernatants were filtered. Any remaining sediment from each sample that was not used for microbe quantification was used to analyze water content, grain size and volatile organic compound percentage for each of the samples (Shah et al., 2011). This is further described in the Supplemental Text.

2.5. Statistical analysis

One-way analysis of variance (ANOVA) was used to determine significant differences among the means, with alpha set at 0.05 (i.e. 95% confidence limit). Pearson's χ^2 contingency test was used to test associations between categorical variables, with alpha also set at 0.05. Pearson correlation analysis was also performed in order to compare physical-chemical parameters, enterococci levels and *S. aureus* levels. Pearson correlation coefficients (r) greater than an absolute value of 0.45 and a p-value less than 0.05 were considered significant for this study. Averages are reported with standard deviations.

3. Results and discussion

3.1. Environmental parameters

Over the 10-day sampling period, the physical-chemical characteristics of the water samples were typical of subtropical marine water (average pH 8.04, salinity 33.5 practical salinity units (psu), water temperature ranging from 27 °C to 37 °C). Solar radiation levels ranged from <10 to 890 W per square meter (W/m^2) between the hours of 7:00 AM and 8:00 PM with near zero values outside this time. Tides were semidiurnal, and although the vertical tidal range was relatively small (0.7 m), the horizontal range was relatively large (7.3 m) due to the mild beach slope. Out of the 238 samples collected, 35 were collected during rainfall. The total rainfall for the 10-day study duration was 63.3 mm. The number of bathers (present only during the beach operating hours from 7:00 AM until 8:00 PM) was also typical for this beach site (Wang et al., 2010) with weekends showing the maximum number of bathers. Within 65 m of the sampling transect, the maximum numbers of humans in the water and on shore during a sampling event were 72 and 53, respectively, and the maximum numbers of dogs in the water and on the shore were 8 and 5, respectively.

3.2. Spatial variation

Enterococci measurements were affected by sampling location as observed from knee-depth versus waist-depth samples. Knee-depth water samples had enterococci levels ranging from below detection (2 CFU/100 mL) to above the detection limit (4000 CFU/100 mL) with an average of 270 CFU/100 mL and standard deviation of ±590 CFU/100 mL. Enterococci levels exceeded 1000 CFU/100 mL, a value that is nearly 10 times greater than the regulatory standard, in 16.5% of the knee-depth water samples. These exceedances occurred as 9 separate events during the hourly 10-day sampling program. These exceedances ranged in duration from 1 to 3 h in length (Fig. 1). Waist-depth samples ranged from below detection to 640 CFU/100 mL with an average of 32 ± 100 CFU/100 mL. The enterococci levels in the knee-depth samples were significantly higher than those of the waist-depth samples ($p = 0.02$). A comparison of median values and ranges of knee-depth and waist-depth enterococci levels is presented in Fig. 2. Forty-three percent (43%) of knee-depth water samples were above the enterococci regulatory guideline for single samples of 104 CFU/100 mL, while 5% of waist-depth samples exceeded the guideline. Retrieval of a water sample from waist-depth water resulted in a lower enterococci measurement than taking a sample at the same time from the knee-depth location in 89.5% of the samples. Thus, collecting samples from knee-depth would result in a higher number of advisories than collecting samples from waist-depth.

The likely source of spatial variation in water samples is wash-in of enterococci from the intertidal zone caused by tides and rainfall. Prior studies have indicated that shoreline sediment can be a non-point source of bacteria (Desmarais et al., 2002; Rogerson et al., 2003; Whitman and Nevers, 2003; Alm et al., 2006), including shoreline sediment at this study site (Shibata et al., 2004; Wright et al., 2011; Phillips et al., 2011a, 2011b). Although tidal height and knee-depth water enterococci levels did not correlate ($r = 0.21$), during non-rain conditions, knee-depth samples collected during outgoing tides had significantly higher enterococci levels than samples collected during incoming tides ($p = 0.04$). Additionally, 7 out of the 9 peak events occurred during outgoing tides, which is similar to observations in past studies (Abdelzaher et al., 2011). In addition to outgoing tides, rain events (defined in the supplemental text) were associated with an increase in knee-water enterococci levels. Knee-depth samples collected during rain events had higher enterococci levels than samples collected during non-rain events ($p = 0.004$). Sixty two percent (62%) of samples collected during rain events had enterococci levels above 104 CFU/100 mL, while 39% of non-rain event samples had enterococci levels above 104 CFU/100 mL. These results indicate that enterococci may be washing in from the shoreline sediments into the water through rainfall runoff and tidal action.

Analysis of sediment samples further support tidal wash-in as a source of spatial variation. Out of the four different types of sediment samples, supratidal sand had the highest enterococci levels with an average of 131 ± 210 CFU/g. The average levels for the fixed-location intertidal, waterline and subtidal samples were 18 ± 42 CFU/g, 19 ± 18 CFU/g, and 14 ± 23 CFU/g, respectively. Supratidal samples had significantly higher enterococci levels than fixed-location intertidal samples ($p = 0.001$), waterline samples ($p = 0.002$) and subtidal samples ($p = <0.0001$). No significant differences in enterococci levels were noted between the remaining sediment samples ($p > 0.1$). Possible causes of higher enterococci levels in supratidal sand include lack of tidal washing which allows enterococci to accumulate (Bonilla et al., 2007) and

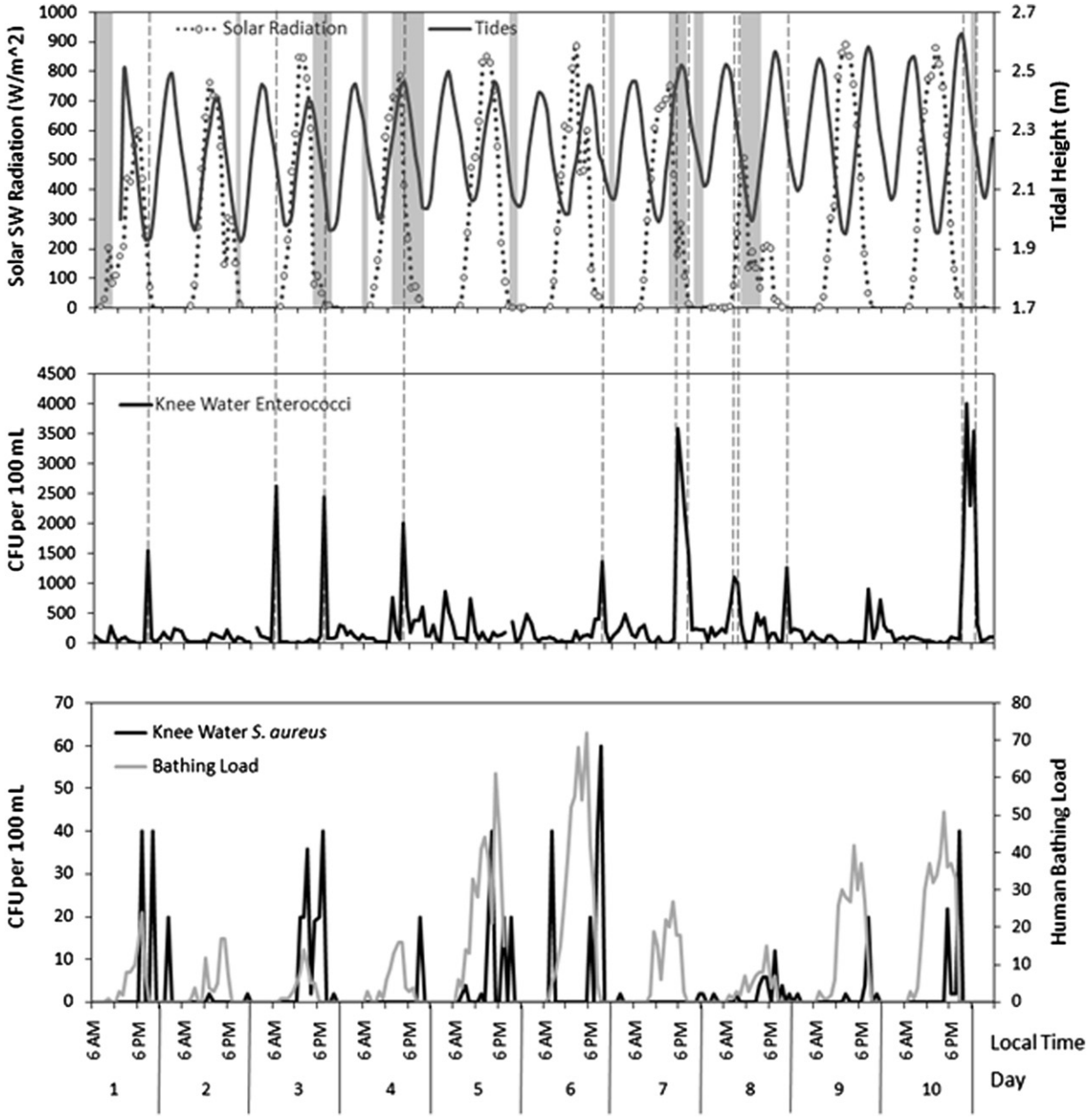

Fig. 1 – Comparison of physical parameters, knee-depth water enterococci, knee-depth water *S. aureus* and bathing load. The top graph displays tidal height (dark gray line), solar shortwave (SW) radiation averaged over the previous hour (dotted line with data points) and times of rainfall (gray rectangles). The middle graph displays knee-depth enterococci levels, with dashed lines aligning peak enterococci events (>1000 CFU/100 mL) to corresponding physical parameters. The bottom graph displays knee-depth water *S. aureus* levels and number of bathers present at each hour.

lower moisture content in which predators such as protozoa cannot survive (Boehm and Weisberg, 2005; Desmarais et al., 2002; Solo-Gabriele et al., 2000; Wright et al., 2011). Other sediment characteristics, such as water content, VOC percentage, and grain sizes were found not to correlate with water or sediment enterococci measurements.

3.3. *Temporal variation*

Time of day during which samples are taken can also impact results used for management purposes. Analysis of samples showed that elevated solar radiation was likely a major contributor to decreases in enterococci levels in water samples. This affected the results seen at different sampling hours in a manner consistent with other studies (Boehm et al., 2002).

Samples were grouped into morning (6 AM–12 PM), afternoon (1 PM–8 PM) and night (9 PM–5 AM) samples based on sunset and sunrise times during the 10-day sampling program. Morning knee-depth enterococci levels were significantly lower than night enterococci levels ($p = 0.04$). Variation is also shown between early morning and late morning

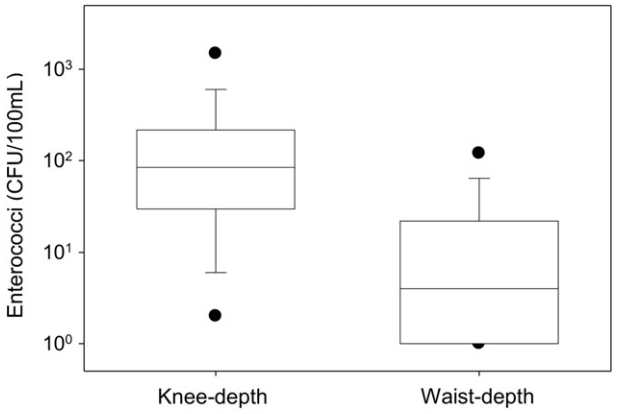

Fig. 2 – Box plot of knee-depth and waist-depth water enterococci levels ($n = 238$ for knee-depth samples, $n = 40$ for waist-depth samples). The center line in the boxes indicates the median value. Whiskers indicate 10th and 90th percentiles. Outliers (dots) indicate 5th and 95th percentiles. Ninety percent of the waist-depth samples had lower values than 50 percent of knee-depth values.

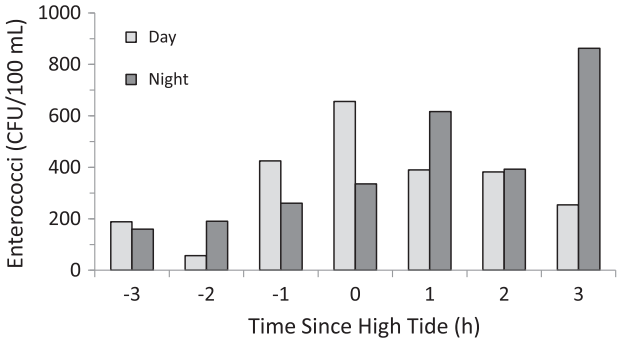

Fig. 4 – Daytime and nighttime mean enterococci levels grouped by number of hours before and after high tide.

samples (Fig. 3). No significant difference in enterococci levels was observed between night and afternoon ($p = 0.98$) or between morning and afternoon ($p = 0.06$) samples. Additionally, none of the 9 enterococci peak events (>1000 CFU/100 ml) occurred when solar radiation levels were at peak values. All enterococci peaks occurred when solar radiation levels were below 415 W/m^2, which is an approximate mid-range value for the solar radiation data (Fig. 1). Log-normalized enterococci levels in all knee-depth water samples inversely correlated with solar radiation ($r = -0.47$, $p < 0.0001$). Although humans and dogs are sources of enterococci (Elmir et al., 2007, 2009; Wright et al., 2009) and these sources are usually most abundant during times of high solar radiation, the effects of solar inactivation likely outweighed the contribution from humans and dogs during times of elevated solar radiation.

Patterns were also identified when daytime and nighttime enterococci levels were grouped by hours before and after high tide. Enterococci levels during the day generally peaked at high tide (Fig. 4). Enterococci levels at night, however, would continue rising after high tide occurred. We hypothesize that as the tide rises, enterococci is released from the sand. At night after high tide, solar radiation is not present to inactivate the enterococci, allowing levels to remain high as the tidal height decreases. After high tide during the day, however, solar inactivation causes die-off of released enterococci, causing levels to decrease after high tide.

Due to the influence of solar radiation on water enterococci levels, in combination with other factors that vary temporally, different management decisions could result from collecting samples at different hours of the day. One hundred percent of the samples taken at noon, for example, had enterococci levels below the single sample standard of 104 CFU/100 mL.

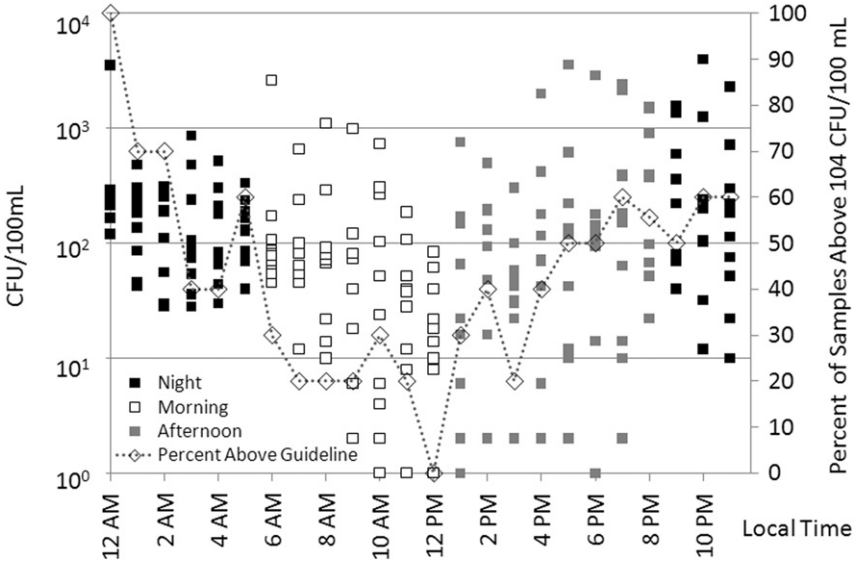

Fig. 3 – Knee-depth water enterococci levels grouped by hour. Black squares indicate night samples (9 PM–5 AM), white squares indicate morning samples (6 AM–12 PM) and gray squares indicate afternoon samples (1 PM–8 PM). The dotted line indicates the percentage of samples each hour above the swim advisory single sample guideline of 104 CFU/100 mL.

The geometric mean of all knee-depth noon samples was 16 CFU/100 mL, which is below the geometric mean used for swim advisories of 35 CFU/100 mL (U.S. EPA, 1986). Thus, no beach advisories would have been issued based upon the 12:00 noon samples. Samples taken at a later or earlier time, however, could have led to the issuance of swim advisories if collected at knee-depth. Samples collected at 6:00 AM exceeded this guideline on June 1st, 3rd and 8th. Fifty-percent of the 6:00 PM samples exceeded the single sample standard, and all 12:00 midnight samples exceeded the single sample standard (Fig. 3). As a result, beach advisories could have been issued during all consecutive days of this study based upon the 12:00 midnight knee-depth samples. For samples taken at 6:00 AM, 6:00 PM and 12:00 midnight, each set exceeded the recommended geometric mean level with geometric means of 112, 83 and 299 CFU/100 mL, respectively.

3.4. S. aureus

For the water samples, 45 out of 238 (19%) knee-depth samples and 5 out of 40 (13%) waist-depth samples were positive for *S. aureus*. In sediment, 4 out of 40 (10%) supratidal, 1 out of 40 (2.5%) intertidal, 1 out of 40 (2.5%) waterline, and 1 out of 239 (0.42%) subtidal samples were positive for presence of *S. aureus*. Two of the 238 (0.84%) knee-depth water samples tested positive for MRSA. None of the other samples (waist-depth or sediment samples) tested positive for MRSA.

Results show that *S. aureus* levels in the water were elevated following times characterized by high bather load (Fig. 1), with *S. aureus* levels higher when bathers were present than when bathers were not present ($p = 0.02$). These results support that bathers may be a source of *S. aureus* at beaches, which has been suggested by other studies (Gabutti et al., 2000; Elmir et al., 2007; Plano et al., 2011). *S. aureus* levels were also found to not correlate with enterococci levels in the knee-depth water samples ($r = 0.07$). The lack of correlation between *S. aureus* and enterococci is not unexpected. Although both *S. aureus* and enterococci can originate from human bather shedding, the numbers of organisms shed may be very different as shown by Elmir et al. (2007) where the number of *S. aureus* shed per bather was 10 fold greater than the number of enterococci shed. Additionally, the two organisms are associated with humans in very different ways. *S. aureus* are primarily skin colonizing organisms found in only 30–40% of all people, while enterococci species are associated with the gastrointestinal tract of all people. Therefore, the differences between *S. aureus* and enterococci levels are likely a combination of the different levels shed by humans coupled with possible higher sensitivity to solar radiation and a stronger shoreline source for enterococci. Therefore, both bacteria were observed at the study beach but each is subject to different inputs and different responses to environmental conditions, resulting in a lack of association between the two bacteria.

The two knee-depth water samples from which MRSA were isolated were collected on June 5th at 1:00 PM and June 7th at 8:00 PM. Consequentially, these two sampling times had considerably different bathing loads (41 and 0 respectively) and solar radiation levels (841 W/m^2 and 11 W/m^2). Of interest was that enterococci levels for both of the MRSA samples were above the single sample guideline (171 and 1360 CFU/100 mL), and thus a beach advisory could have been issued for both of these cases if such decisions were based upon knee-depth samples without confirmatory analyses. Due to the small sample size positive for the presence of MRSA, the correspondence between MRSA and enterococci could be entirely coincidental. More research is needed to further evaluate a possible relation between MRSA and enterococci at this beach.

3.5. *Comparison of different beach management standards*

Enterococci levels during this study were analyzed based on sampling procedures applied by different states. The majority of beach sampling procedures can be split into 4 standard types depending upon sampling depth and number of exceedances that trigger an advisory (Table 1). All states with marine beaches not included in Table 1 base their standards on different criteria, such as different enterococci levels, different sampling depths, and issuance of advisories based on rainfall. This analysis shows that issuing advisories based on one-time samples collected at knee-depth (Standard 1) results in the highest percentage of advisories. Requiring consecutive knee-depth samples to exceed regulatory guidelines (Standard 2) results in considerably less advisories with no advisories issued for samples collected between 10:00 AM and 12:00 PM. Standard 3 results in very few advisories (only 10% of the time for samples collected at 6:00 PM and at 12:00 AM). No advisories would have been issued at the study beach site based on Standard 4 (waist-depth, requiring confirmatory results). As described above, the data set shows that sample collection time affects percentages of advisories issued. Collection of samples during times of high solar radiation, for example, results in a lower percentage of advisories issued than collecting samples during times of lower solar radiation.

S. aureus presence was also compared to beach closure scenarios. Under Standard 1, 48% of water samples positive for *S. aureus* were collected during times that the beach would have been under an advisory (Table 2). Considering only the 137 sampling hours the beach would have been open under Standard 1, *S. aureus* was present in water samples during 26 of those hours (19%). Considering the 101 sampling hours the

Table 2 – Comparison of *S. aureus* presence and advisory issuance based on given sampling criteria.

	Standard One	Standard two	Standard Three	Standard four
Advisory when *S. aureus* is present	24/50 (48%)	12/50 (24%)	0/50 (0%)	0/50 (0%)
No advisory when *S. aureus* is present	26/50 (52%)	38/50 (76%)	50/50 (100%)	50/50 (100%)

beach would have been under an advisory according to Standard 1, 24 of those hours were positive for *S. aureus* (24%). Given the relatively even distribution of *S. aureus* between times the beach would have been open or closed, there was no significant association found between *S. aureus* presence and beach advisories (Pearson's χ^2 test, $p = 0.37$). Under the Standard 2 criteria, which relies on consecutive enterococci exceedances, 12 of the 50 (24%) positive water samples for *S. aureus* were collected during times that the beach would have been under an advisory. Under Standards 3 and 4, no advisories would have been in effect when *S. aureus* was present in knee or waist-depth water. Thus, management strategies based on shallower sampling locations and instant advisories provide more protection for exposure to *S. aureus* than other management strategies simply because more advisories would be issued. Results also suggest that the detection of enterococci does not necessarily indicate risks from potential *S. aureus* exposures.

Time lags in advisory issuance due to sample processing were also considered. In 52 of 98 knee-depth water samples with greater than 104 enterococci CFU/100 ml, exceedances were observed exactly 24 h later as well (5 exceedances on day 10 were removed due to no data being available the next day). Under a scenario in which beach advisories are issued exactly 24 h after sampling occurs, exposure to elevated enterococci levels the next day would have been prevented in these instances. A significant association was found for enterococci between exceedance status of one sample and exceedance status of the sample collected 24 h later (Pearson's χ^2 test, $p = 0.006$). This association was influenced by the effects of solar radiation on enterococci levels and the fact that solar radiation varies in cycles of 24 h. Also with a 24 h lag in advisory issuance, exposure to *S. aureus* would have been prevented in 20 cases. In 22 other instances, however, enterococci levels were too low to prevent exposure to *S. aureus* 24 h later. No significant association was found between enterococci exceedances and *S. aureus* presence 24 h later (Pearson's χ^2 test, $p = 0.79$).

Faster same-day qPCR-based technologies have been proposed to decrease inaccuracies associated with time lags. The use of same-day qPCR may require sampling earlier in the morning in order to obtain results in time for the peak bathing period. However because of the influence of solar radiation on enterococci, adjusting the sampling time earlier to facilitate same-day results may result in a shift in the baseline enterococci measures. Such potential shifts should be considered when making beach management decisions based upon same-day samples.

4. Conclusion

The results of this study showed that a fecal indicator such as enterococci may not be predictive of the presence of some pathogens such as *S. aureus*. Thus, additional human health risks may be present even when indicator levels are low, and additional indicators may be needed to protect bathers from pathogens that do not correlate with enterococci. At this beach site, an epidemiologic study (Fleisher et al., 2010; Sinigalliano et al., 2010) found that bathers were at higher risk than non-bathers in contracting skin illness, and the level of risk for skin illness was related to enterococci levels. The results of these prior studies in combination with this study suggest that enterococci may be indicative of a skin pathogen other than *S. aureus*. Further research should be conducted to identify other potential skin pathogens or other sources of skin irritation and infection at recreational beaches. Associations between MRSA and enterococci should also continue to be evaluated, due to both MRSA-positive samples being collected at times when enterococci exceeded regulatory levels.

Ultimately, enterococci levels are influenced by the interplay of several environmental factors, including solar radiation levels and release from suspected sand sources via tide and rainfall. The time and location at which a water sample is taken may greatly influence the measured levels of enterococci and the resulting beach management decision. To remove bias due to sampling location and time, observed levels can be scaled by the known temporal and spatial variability and thereby provide an approach to issuing beach advisories that more closely correlates with actual health risks to beachgoers.

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Appendix. Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2012.01.040.

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