Environmental Factors Influencing the Abundance of Enterococci in Gulf Coast Beach Waters

Kevin M. Chenier, E.I.[1]; M. Teresa Gutierrez-Wing, E.I., M.ASCE[2]; Zhi-Qiang Deng, M.ASCE[3]; and Kelly A. Rusch, P.E., M.ASCE[4]

Abstract: Enterococci concentrations in seawater samples collected in 2010 at a Gulf Coast beach in the afternoon were significantly lower (12 MPN/100 mL) compared with morning samples (172 MPN/100 mL). The factors affecting temporal differences of enterococci concentration in beach waters were studied through five laboratory experiments analyzing beach sands, solar radiation, salinity, and turbidity. Enterococci were found in beach sands at a geometric mean of 43 MPN per 100 g of sand, demonstrated the ability to persist for extended periods of time, and increased when incubated (geometric mean of 54 MPN per 100 g sand). Solar radiation inactivated large enterococci concentrations ($\geq 24, 196$ MPN/100 mL) in as little as four hours in salinities ranging from 0 to 25 parts per thousand (ppt). Increased turbidity (70 and 140 NTU) hindered the effect of solar radiation, suggesting that near-shore turbidity may promote higher enterococci concentrations. The results indicate that enterococci replenishment along Gulf coastal waters is not due to reactivation as found in other areas. This work illustrates that beach sands, solar radiation, salinity, and turbidity impact enterococci concentrations in Gulf Coast beach waters. **DOI:** [10.1061/(ASCE)EE.1943-7870.0000571](). © 2012 American Society of Civil Engineers.

CE Database subject headings: Public health; Sea water; Water quality; Beaches; Bacteria; Gulf of Mexico.

Author keywords: Public health; Water quality; Beaches; Bacteria.

Introduction

The Beaches Environmental Assessment and Coastal Health (BEACH) Act of 2000 requires states to monitor coastal recreation beach water bodies for fecal-indicator bacteria (fecal coliforms and enterococci) and notify the public when bacteriological criteria are exceeded. Human exposure to high concentrations of enterococci has been shown to coincide with an elevated risk of acquiring a gastrointestinal and respiratory illness, which makes enterococci a more adequate indicator in seawater water than fecal coliforms and *E. coli* (Wade et al. 2003; Cabelli 1983; Cabelli et al. 1982). Per regulatory criteria, enterococci concentrations in seawater shall not exceed a geometric mean of 35 MPN/100 mL and a single sample maximum of 104 MPN/100 mL [U.S. Environmental Protection Agency (USEPA) 1986].

Across the United States, of the 3,819 coastal beaches that were monitored by the corresponding state agencies, 1,642 (43%) had at least one advisory or closure during the 2009 beach recreation season (USEPA 2010). According to the USEPA, states and territories reported 6,203 notification actions during the 2009 swimming season. The monitoring results across the United States demonstrated that bacteriological criteria exceedences resulting in advisories were a common problem.

There are several factors impacting enterococci concentrations in seawater including solar radiation, salinity, turbidity, and beach sands. Solar radiation was a significant factor because enterococci concentrations in seawater could be drastically reduced in the presence of sunlight, whereas they were able to persist for days without exposure to sunlight (Fujioka et al. 1981; Davies-Colley et al. 1994). A study by Fujioka et al. (1981) determined that 90% of fecal streptococci were inactivated (T_{90}) within 60 to 180 min of sunlight exposure. Davies-Colley et al. (1994) found that insolation of 5.8 MJ $\cdot$ m^{-2} produces 90% inactivation (S_{90}) for sunlight-exposed enterococci. Davies-Colley et al. (1994) also state that enterococci require 2.3 times more insolation than fecal coliforms to reach 90% inactivation. The mean observed T_{90} for enterococci was found to be between 1.39 and 1.14 h. Alkan et al. (1995) determined that enterococci die-off rates in seawater were influenced by the variability of solar radiation intensity. Enterococci concentrations decreased as solar radiation increased, and inactivated enterococci did not reactivate after sunset as noted by Boehm et al. (2002).

Salinity and turbidity could also affect fecal-indicator bacteria concentrations. Sampling waters with a lower salinity were associated with higher levels of fecal-indicator bacteria (Lipp et al. 2001). Gulf of Mexico seawater was found to have an average salinity of 24 ppt over a 6-year monitoring period during seasonal recreation beach sampling in Holly Beach, Louisiana [Louisiana Department of Health and Hospitals (LDHH) 2009]. Suspended sands in the near-shore seawater attributable to turbulence caused an increase in turbidity and may be providing protection from the effects of salinity and sunlight. Beach sands provide osmotic protectors that could alleviate the effects of high salinities (Gerba and McLeod 1976). Die-off rates of fecal-indicator bacteria concentrations in seawater due to solar radiation decreased with increasing levels of turbidity (Alkan et al. 1995).

[1]Dept. of Civil and Environmental Engineering, Louisiana State Univ., Baton Rouge, LA 70803.

[2]Assistant Professor-Research, Dept. of Civil and Environmental Engineering, Louisiana State Univ., Baton Rouge, LA 70803 (corresponding author). E-mail: mgutie5@lsu.edu

[3]Associate Professor, Dept. of Civil and Environmental Engineering, Louisiana State Univ., Baton Rouge, LA 70803.

[4]Professor, Dept. of Civil and Environmental Engineering, Louisiana State Univ., Baton Rouge, LA 70803.

1130 / JOURNAL OF ENVIRONMENTAL ENGINEERING © ASCE / NOVEMBER 2012

J. Environ. Eng., 2012, 138(11): 1130-1137

Beach sand along coastal beaches was one possible source of enterococci. Coastal beach sands could contain and act as a reservoir for fecal-indicator bacteria (e.g., Yamahara et al. 2007; Ferguson et al. 2005; Alm et al. 2003; Oshiro and Fujioka 1995). Enterococci in the beach sands could also be transported to near-shore seawater through tidal effects (Yamahara et al. 2009; Boehm and Weisberg 2005). Boehm et al. (2009) found that the enterococci concentration in beach sand could exceed the enterococci concentration in the adjacent seawater on a per mass basis, often by orders of magnitude. Enterococci experience a long-term persistence and even regrowth in beach sands, according to Byappanahalli et al. (2006) and Hartel et al. (2005). Beach sand offers a more effective barrier to damaging ultraviolet (UV) radiation than water alone (Burkhardt et al. 2000). Enterococci may also persist in beach sands because they possess the ability to tolerate high salt concentrations (6.5% NaCl) in sands (USEPA 2002). If fecal-indicator bacteria are not reactivating overnight in seawater, then beach sands could be a potential source of enterococci that continuously replenish the near-shore seawater overnight.

Fecal-indicator bacteria are exposed to the beach environment (beach sands and water) in areas with known point sources, but at beaches with no known point source, fecal-indicator bacteria still exist. Therefore, water quality surrounding the beach may be directly impacted either by direct contamination of water or by enterococci in the beach sand. Heaney et al. (2009) conducted over 27,000 interviews with beachgoers at seven U.S. beaches and assessed the degree of sand exposure during the visit. Then, 10–12 days after the initial visit, beachgoers were interviewed again to answer questions about health symptoms experienced since going to the beach. Direct contact with beach sand (digging and being buried) was associated with an elevated risk of gastrointestinal illness and diarrhea, so exposure to beach sands may be as critical to beachgoer health as exposure to seawater (Heaney et al. 2009).

The Louisiana Department of Health and Hospitals collects recreational beach water quality data along the Louisiana Gulf Coast during the recreational season (April to October) since 2001 as part of the BEACH Act of 2000. Fig. 1 shows the yearly log-transformed mean enterococci and fecal coliform concentrations collected by the LDHH at Holly Beach. The LDHH has found that in each year since 2005, all swim advisories were attributable to

exceedances of enterococci criteria, with over 96% of advisory days in 2008 attributable to geometric mean criterion exceedance (LDHH 2009). In 2010, The group from Louisiana State University (LSU) gathered water samples in the afternoon during the recreation season and determined that geometric mean afternoon enterococci concentrations (12 MPN/100 mL) were significantly lower than in the morning (172 MPN/100 mL). Because the majority of swim advisories in Holly Beach are attributable to enterococci criteria exceedences, studies were conducted to better understand the effect of environmental factors on enterococci concentrations in Gulf Coast seawater.

The overall goal of this work was to determine the factors that affect enterococci abundance at Gulf Coast beaches. To accomplish the goal, three factors were considered: (1) the role of beach sands as a potential reservoir for enterococci; (2) the effect of sunlight on enterococci in seawater; and (3) the effect of salinity and turbidity on enterococci in seawater.

Methodology

Site Description

In this study, water and sediment (primarily composed of sand) samples were collected from Holly Beach along the Gulf Coast of southwest Louisiana during the recreational season of 2010. Holly Beach is a 5.5-km beach that is monitored by the LDHH on a weekly basis during the recreation season as part of the BEACH program (LDHH 2009). Before Hurricanes Katrina and Rita impacted the area in 2005, typical usage of the beach was 150 people per weekday, 1,000 people per weekend, and 6,000 people per holiday (LDHH 2003). The hurricanes caused the residential population and use of the beach to greatly decline, although usage is slowly returning to pre-hurricane levels (LDHH 2009) after the repair and construction of roads of access to the beach area. The beach is being continuously maintained by mixing and compaction of the sand. Since monitoring began in 2005, enterococci concentrations at Holly Beach routinely resulted in beach swim advisories (LDHH 2009).

Field Water Sampling

Water samples from Holly Beach were collected as described subsequently using techniques from the American Public Health Association (APHA) (2005) and ASTM (2009). Under the Louisiana BEACH Monitoring Program, LDHH collected seawater from six sampling stations (Holly 1 to 6) that were approximately 500 m apart from one another on a continuous beach segment. The LDHH samples were collected on Monday mornings between 7:00 a.m. and 8:00 a.m. To establish temporal differences of fecal-indicator bacteria during the day, LSU collected weekly samples from two stations (Holly 2 and 4) during the afternoon between 1:00 p.m. and 3:00 p.m. on days where the LDHH collected morning samples. Although there were no significant differences among individual sampling sites, Holly 2 and 4 were selected because they were the boundaries of the area where most beachgoers were found during initial site visits.

Water samples were collected in sterile plastic specimen containers. The samples were collected at a depth of 30 cm in areas with depths of close to 1 m. During sampling, care was taken that underwater sands were not disturbed and the water was flowing toward the sampler, to prevent contamination. The samples were stored on ice in an insulated container at approximately 1 to 4°C until they reached the laboratory and were analyzed within 6 h of collection according to USEPA guidelines (USEPA 2002).
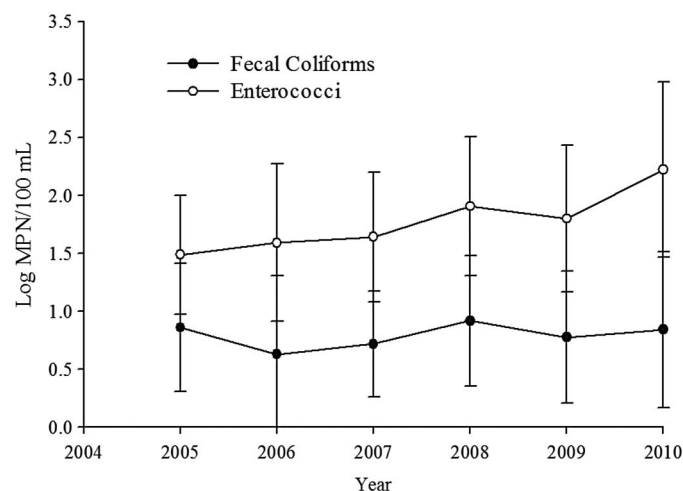

Fig. 1. Average log-transformed enterococci concentration in Holly Beach, Louisiana, from 2005–2010; the average enterococci concentration has increased over the 6-year span, while the fecal coliform concentration has remained constant

Field Sand Sampling

Sand samples were collected as described subsequently to determine if the beach sand could be a potential source of enterococci. Sand samples were collected from the beach site (see Fig. 2) as part of the weekly beach monitoring sample collection on the following dates: (1) March 31, 2010; (2) April 16, 2010; (3) May 3, 2010; and (4) May 10, 2010. Sand samples were collected above the saltwater line from various points and depths on the beach at LDHH sampling station Holly 4. The sampling points varied in distance from the water line and sampling depths. Sand subsamples were gathered from four coordinate points (0, 90, 180, and 270 degrees) from the wall of a 1-m-diameter hole to account for spatial variability of enterococci. Sand was collected using sterile 125-mL specimen jars placed perpendicular to the wall of the hole. The subsamples from the four coordinate points of the same depth in each sampling station were pooled together in a sterile plastic bag and placed on ice (1 to 4°C) for transport to the lab.

On March 31, 2010, sand samples were collected from nine points at Holly 4. The samples were collected at three depths (8, 38, and 76 cm) below ground surface. Samples were collected across three points parallel to the beach (center and 7 m to the left and right of center) as well as at three distances (1, 23 and 45 m) from the seawater (Fig. 2).

For the April 16, May 3, and May 10 sampling events, sand samples were collected from the five sampling points (Fig. 2). Two sampling points were 1 m above the seawater (D and E) and three were set 45 m from the seawater (A, B, and C), with lateral distances between the points of 25 m. Samples were collected at depths of 8 and 38 cm below ground surface.

Laboratory Analysis of Sands to Determine Presence and Persistence of Enterococci

To quantify enterococci in beach sand, the beach sand analysis was performed according to the simplest extraction method described by Boehm et al. (2009) using ASTM Method D6503-99 (IDEXX Enterolert Method) (ASTM 2009). Each sand sample was shaken in the collection bag for approximately 2 min to ensure complete mixing. For the March sampling event, 10 g of wet sand from the mixed sample was weighed out on sterile aluminum weigh boats with the weight recorded. In the April and May sampling events, 100 g of sand was used in the analysis. In all events the dry weight of the sand was $79.3 \pm 0.9\%$ of the wet weight, as measured by quintuplicate dry weight gravimetric determination. The sand was mixed in a ratio of 10 ml eluent to 1 g of sand with sterile deionized freshwater in a sterile 125 mL Erlenmeyer flask for the March event and a 1 L sterile reagent bottle on the other events. A quantity of 10 mL of the supernatant was diluted to 100 mL in another sterile 125 mL Erlenmeyer flask with Enterolert medium added. Once the medium was dissolved completely by mixing, the mixture was poured into a Quanti-Tray 2000 and sealed. The resulting positive wells were translated into an estimation of enterococci concentration using MPN Tables provided by IDEXX Laboratories, Inc. Enterococci concentration was reported as MPN per 100 grams of wet sand.

To explore the possibility of enterococci persistence in beach sands, the field sand samples demonstrating enterococci presence were incubated for one week at 21°C (the same temperature as that of the wet sand in the field) in the same plastic bag used for transport to the lab. After incubation, the sand was analyzed using the same process previously described.

Laboratory Experiments to Determine the Impact of Environmental Conditions on Enterococci Abundance

Laboratory experiments were conducted to determine the impact of salinity, turbidity, and solar radiation on enterococci. The preliminary experiments studied the influence of various salinities (0, 5, 15, 20, and 25 ppt), turbidity, and solar radiation on enterococci concentrations. On the basis of the results of the preliminary experiments, the impact of solar radiation on enterococci at uniform salinity and temperature was studied. Another experiment was conducted to examine the effect of varying levels of turbidity in water on enterococci concentrations when exposed to solar radiation. Finally, an experiment was conducted to determine if enterococci can be reactivated after solar inactivation. The experiments were
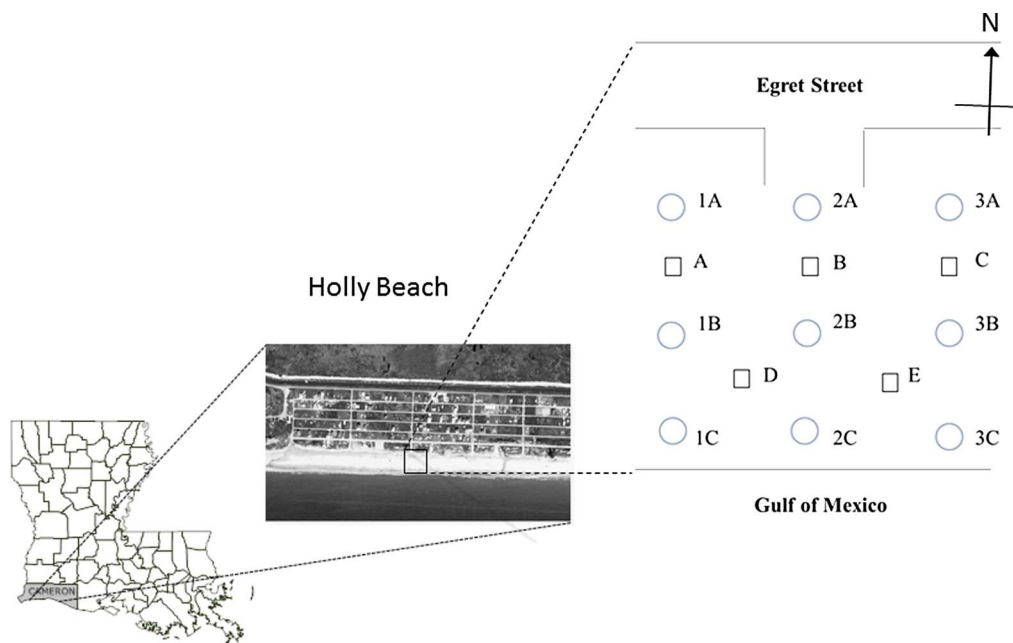

Fig. 2. Layout of sand sample collection points from Holly 4; the collection points for sampling event 1 on March 31, 2010, are represented with a circle; the collection points for sampling events 2 to 4 on April 16, May 3, and May 10, 2010, are represented with a square

planned to occur on days when similar temperature and no rainfall was predicted, to ensure the conditions for all experiments was as homogeneous as possible.

Preliminary Experiments to Determine the Effect of Salinity, Turbidity, and Solar Radiation on Enterococci

Preliminary experiments were completed to study the effects of solar radiation on enterococci concentrations in turbid water and under varying salinities. In the first preliminary experiment, plastic containers were filled with synthetic seawater at salinities of 5, 15, and 25 ppt. These salinities cover the range observed in the field. A platform was constructed that allowed containers to sit on top and be exposed to sunlight, whereas under the platform, containers were shielded from sunlight. The experiment was protected with a plastic barrier around it to reduce possible contamination. The underside of the platform was designed to allow airflow around the containers, but no sunlight. One set of three containers at each of the salinities was placed outside in direct sunlight, and a second set of three containers at each of the salinities was placed outside under the platform, completely in the dark. Negative controls were established in all experiments. Irradiance was measured at each sampling event with a LI-COR meter with a LI-190 quantum sensor. Although not discussed in this paper, the first preliminary experiment results suggested that similar solar inactivation could be achieved at each of the three salinities.

In the second preliminary experiment, triplicate containers of synthetic seawater were exposed to sunlight along with a single freshwater container. One control was placed in the dark. A single turbid water container was exposed to sunlight as a preliminary study to examine the influence of turbidity on the effect of solar radiation on enterococci concentrations. Turbidity was produced by addition of sterilized, dry sediment from the sampling site. The synthetic seawater (20 ppt) was mixed to bear a resemblance to the historical average salinity of Holly Beach during the recreation season.

In both preliminary experiments, each container was seeded using a 20-μL inoculum of the enterococci active culture in 3 L of sterile synthetic seawater. Samples were collected every 4 h from the seeding at 7:00 a.m. to 7:00 p.m. to span 12 h of sunlight exposure. The containers were shaken at every sample event. Enterococci concentration was measured in each container at each sampling time to determine if the concentration of enterococci was reduced.

Effects of Solar Radiation on Enterococci in Fieldlike Conditions

The first experiment was designed to test the effect of solar radiation on enterococci. For this experiment, triplicate containers with 3 L of synthetic seawater (20 ppt) were exposed to sunlight, while triplicate containers were placed under a cover in the dark. All six containers were placed in a single circulating water bath to ensure uniform temperature. The containers were inoculated using a 10-μL inoculum of enterococci concentrated culture, monitored, and analyzed for enterococci concentration at each sampling time (7:00 a.m., 11:00 a.m., 3:00 p.m., and 7:00 p.m.). The temperature showed a variation with a minimum of 21.5°C at 7:00 a.m. with a maximum of 32.7 ± 0.8°C at 3:00 p.m.

Effect of Solar Radiation on Turbid Waters

On the basis of the result of the turbidity container in the preliminary experiment, an experiment was completed to examine the influence of turbidity levels on the effect of solar radiation on enterococci concentrations. This experiment consisted of triplicate containers of synthetic seawater at 20 ppt with turbidity levels of 0, 70, and 140 NTU. The turbidity levels were chosen on the

basis of the 25, 50, and 75 percentile turbidity levels for ranked afternoon enterococci concentrations using recent beach recreation season data. Turbidity was produced by addition of sterilized, dried sediment, suspended in sterile synthetic seawater. Containers were inoculated using a 10-μL inoculum of enterococci concentrated culture and samples were collected every 2 h from 6:00 a.m. to 12:00 p.m. to span 6 h of sunlight. Enterococci concentration and turbidity were measured at each sampling time.

Reactivation of Enterococci after Solar Inactivation

An experiment was conducted to examine whether solar inactivated enterococci possessed the ability to reactivate during the overnight hours following inactivation. In this study, triplicate containers of synthetic seawater (20 ppt) were exposed to sunlight for 11 h, the total sunlight available on the day of the experiment. The containers were inoculated using a 10-μL inoculum of enterococci concentrated culture. Containers were seeded and samples were collected at the 7:00 a.m. (seeding time) and 6:00 p.m. to have initial and final bacterial concentrations representing solar inactivation. Once inactivated, the containers were placed in a 28°C water bath and incubated in the dark for 12 h to represent overnight field seawater conditions. At 7:00 a.m. the next morning, samples were collected and analyzed to determine if enterococci concentrations had increased, indicating reactivation.

Data Analyses

Enterococci concentrations were log-normal transformed for analysis purposes and transformed back to normal MPNs for reporting, because bacteria follow a log-normal distribution. A t-test was used to analyze the sand samples and experiments one and two results at the 95% confidence level. For analysis purposes, enterococci concentrations below the detection limit (10 MPN/100 mL) were assigned a value of 5 MPN/100 mL, whereas values over the upper quantitation limit (24,196 MPN/100 mL) were assigned a value of 24,196 MPN/100 mL, which is consistent with other studies.

Results and Discussion

Field Water Sampling

The enterococci concentration in the 2010 sampling season had a morning geometric mean of 167 MPN/100 ml, which is at least twice as high as the morning geometric mean concentrations observed between 2005–2009 (31 MPN/100 mL to 81 MPN/100 mL). Although the concentration followed an increasing trend over time, there was no clear reason for the steep increase in 2010. During the 2010 sampling season, the geometric mean afternoon enterococci concentration MPN was 12 MPN/100 mL. The geometric mean of the morning and afternoon enterococci concentrations were significantly different, which prompted experiments to determine the factors affecting enterococci abundance.

Field Sampling-sand

Sand samples were collected and examined for presence of enterococci and to determine if enterococci possessed the ability to persist for extended periods of time in the beach sands. The geometric mean enterococci concentration for each of the four individual sampling events can be found in Table 1. The geometric mean enterococci concentration for the sand sampling events combined was 43 (lower and upper standard deviation were −29.0 and +87,

JOURNAL OF ENVIRONMENTAL ENGINEERING © ASCE / NOVEMBER 2012 / **1133**

J. Environ. Eng., 2012, 138(11): 1130-1137

Table 1. Sand Sampling Results Showing Enterococci Concentrations in Holly Beach Sands for the Four Sampling Events

Date	Geometric mean (standard deviation range)[a]
March 31, 2010	91 (60.8, 182)
April 16, 2010	36 (30.4, 195)
May 3, 2010	12 (10.9, 95.8)
May 10, 2010	74 (56.4, 237)
Combined samples	43 (29, 87)

[a]Enterococci reported in MPN per 100 g wet sand; the numbers in parentheses are the lower and upper standard deviations.

respectively) MPN per 100 g wet sand. Enterococci concentrations were present and highly variable in the beach sands. Local beach officials use a tractor to rake the beach sands during the recreation season, which causes constant shuffling of beach sand several centimeters deep. Because of this shuffling, enterococci concentrations were not expected to exhibit any concentration gradients over the sampling points. The upper eight centimeter samples at the points furthest from the water line generated the most positive responses for enterococci. This is consistent with the work by Byappanahalli et al. (2006) that demonstrated similar results in freshwater backshore sand at Lake Michigan beaches. The finding of enterococci in the beach sands was consistent with studies conducted by Boehm et al. (2009) and Yamahara et al. (2007) that recognize beach sand as a potential reservoir of enterococci concentrations.

Laboratory Analysis of Sands to Determine Presence and Persistence of Enterococci

Eighteen of the 25 samples (72%) that were identified to have enterococci present in the initial analysis were again positive for enterococci. The geometric mean enterococci concentration after incubation for each of the four individual sampling events can be found in Table 2. The combined geometric mean enterococci concentration for the incubated sand samples was 57 (lower and upper standard deviation were -48.2 and $+463$, respectively) MPN per 100 g wet sand. Although the sand samples examined exhibited increases and decreases in enterococci after incubation, the overall geometric mean concentration of the reanalyzed samples was not significantly different than the overall geometric mean of the original enterococci concentrations ($p > 0.3456$). The enterococci concentrations were again highly variable. The results were consistent with other authors (e.g., Yamahara et al. 2009 and Hartel et al. 2005), demonstrating the ability for fecal enterococci to persist and even potentially regrow on sands.

The results indicate that beach sands along the Gulf Coast can act as a reservoir of enterococci for the near-shore beach waters. No point sources, such as sewage leaks similar to those found in Avalon Beach by Boehm et al. (2009) or human waste deposits that could explain the high enterococci concentrations were located at Holly Beach, further indicating the presence of nonpoint sources

Table 2. Enterococci Concentrations in Sand Samples after Seven Days Incubation at 21°C

Date	Geometric mean (standard deviation)[a]
March 31, 2010	115 (87.2, 362)
April 16, 2010	33 (28.8, 190)
May 3, 2010	384 (376, 17,329)
May 10, 2010	24 (18.5, 77.9)
Combined samples	54 (48.2, 463)

[a]Enterococci reported in MPN per 100 g wet sand; the numbers in parentheses are the lower and upper standard deviations.

of enterococci in the area, although an undiscovered point source may be possible. The results from the sand sampling events agree with studies by Boehm et al. (2009) and Yamahara et al. (2007) on California beach sands, Hartel et al. (2005) on Georgia, New Hampshire, and Puerto Rico sands, and Byappanahalli et al. (2006) on Lake Michigan beach sands demonstrating that enterococci exist and persist in beach sands.

Preliminary Experiments to Determine the Effect of Salinity, Turbidity, and Solar Radiation on Enterococci Abundance

The preliminary solar inactivation experiment was designed to test the effect of salinity and solar radiation on enterococci. In the preliminary experiment, enterococci were completely inactivated by 1:00 p.m. in the containers exposed to sunlight at 5, 15, and 25 ppt. The turbidity had no significant difference with time throughout the experiment. Several containers were discarded after the evaluation showed that the inoculation had been ineffective.

The second preliminary experiment was designed to determine the effect of solar radiation on enterococci in seawater and to explore three other parameters. Four conditions were tested: (1) one seawater container in the dark, (2) one seawater turbid container exposed to sunlight, (3) one freshwater container exposed to sunlight, and (4) three seawater containers exposed to sunlight. Initial seeding and sample collection at 7:00 a.m. for all containers yielded initial enterococci concentrations above the upper quantitation limit (24, 196 MPN/100 mL). At 11:00 a.m., the container in the dark and the turbid container were still above the upper quantitation limit, but the freshwater and seawater containers exposed to sunlight all had enterococci concentrations below the minimum detection limit (10 MPN/100 mL). This result is consistent with the estimated T_{90} between 60 and 180 min calculated by Fujioka et al. (1981) for streptococci and fecal coliforms under sunlight in seawater. The same result was found at 1:00 p.m. and 7:00 p.m. for all containers. There was a significant difference ($p < 0.0001$) in the decrease of the enterococci concentration in the containers exposed to light compared with the container in the dark over the course of the day.

Several observations were drawn from the preliminary experiments. For both the fresh (0 ppt) and seawater (5, 15, 20, and 25 ppt) containers, the salinity did not appear to be as significant an inactivating mechanism as solar radiation. In the second preliminary experiment, the enterococci concentration in both the freshwater and seawater containers fell below the detection limit within 4 h, indicating that solar radiation was a more dominating environmental factor than salinity. The preliminary experiments demonstrate that even across several salinities (0, 5, 15, 20, and 25 ppt), there was complete inactivation of enterococci in short time periods. The container in the dark and the turbid water container in the second preliminary experiment maintained the maximum detected level of enterococci throughout the day. The increased turbidity could have minimized the effect of the sunlight on enterococci by preventing the penetration of light as suggested by Alkan et al. (1995). Therefore, higher turbidity waters could have provided shelter for enterococci in beach waters. Further inactivation experiments were performed to enhance the initial experiments.

Effects of Solar Radiation on Enterococci in Fieldlike Conditions

Derived from the results of the preliminary experiments, an experiment was designed to analyze the effect of solar radiation on

Table 3. Results Showing the Difference in Enterococci Concentration in Containers Exposed to Sunlight and those Held in the Dark

Time	Condition	Mean ± standard deviation[a,b]
7:00 a.m.	Dark	≥24,196[c]
	Light	≥24,196
11:00 a.m.	Dark	≥24,196
	Light	≤10 ± 1.5
3:00 p.m.	Dark	16,967 ± 1.4
	Light	≤10
7:00 p.m.	Dark	15,988 ± 1.4
	Light	≤10
First 4 h in light	7:00 a.m.	≥24,196
	11:00 a.m.	≤10 ± 1.5
Initial versus final dark	7:00 a.m.	≥24,196
	7:00 p.m.	15,988 ± 1.4

[a]Enterococci reported in MPN per 100 mL.
[b]During analysis, enterococci concentrations below the detection limit (10 MPN) were assigned a value of 5 MPN, whereas values over the detection limit (24,196 MPN) were assigned a value of 24,196 MPN.
[c]No standard deviation was reported when there was no difference between replicates.

Table 4. Results Demonstrating the Effect of Turbidity on the Inactivation of Enterococci by Sunlight

Time	Turbidity (NTU)	Mean ± standard deviation[a,b]
6:00 a.m.	0	≥24,196[c]
	70	≥24,196
	140	≥24,196
8:00 a.m.	0	≥24,196
	70	≥24,196
	140	≥24,196
10:00 a.m.	0	≤10 ± 1.5
	70	13 ± 2.2
	140	89 ± 4.3
12:00 p.m.	0	≤10
	70	≤10
	140	49 ± 4.0

[a]Enterococci reported in MPN per 100 mL.
[b]During analysis, enterococci concentrations below the detection limit (10 MPN) were assigned a value of 5 MPN, whereas values over the detection limit (24,196 MPN) were assigned a value of 24,196 MPN.
[c]No standard deviation was reported when there was no difference between replicates.

enterococci alone at a constant salinity and uniform temperature. The results of the fieldlike experiment are shown in Table 3. All six containers had a salinity of 20 ppt with an initial enterococci concentration above the upper quantitation limit at 7:00 a.m. At 11:00 a.m., the geometric mean enterococci concentration for the containers exposed to sunlight was below the detection limit, compared with the containers in the dark which had a geometric mean enterococci concentration above the upper quantitation limit. At 3:00 p.m. and 7:00 p.m., the containers exposed to light continued to have an enterococci concentration below the detection limit. In contrast, the geometric mean enterococci concentration for the containers in the dark was 16,967 MPN/100 mL at 3:00 p.m. and 15,988 MPN/100 mL at 7:00 p.m. The results show statistically significant ($p < 0.0001$) inactivation of enterococci exposed to sunlight in as little as 4 h. Although there was a decline in the enterococci concentration, the containers in the dark did not show a significant inactivation over the course of the day ($p = 0.1161$).

The laboratory experiments support solar inactivation as a primary cause of lower enterococci concentrations in the afternoon than in the morning. As the day progresses and more sunlight infiltrates into the water (dependent on turbidity), enterococci undergo solar inactivation. These results concur with Ki et al. (2007) and Boehm et al. (2002) that found that bacterial inactivation in the beach surf zone was primarily caused by solar radiation. The solar irradiance levels in this study were higher than those reported by Noble et al. (2004) in southern California by approximately 30%. The higher solar irradiance levels (peak of 4,200 μmol · m^{-2} · s^{-1} ± 10% for all sampling dates) and temperature could account for the inactivation of the large initial enterococci concentrations in 4 h, compared with a T_{90} of 121.2 h obtained by Noble et al. (2004) at 20°C.

Effect of Solar Radiation on Turbid Waters

The preliminary turbidity experiment demonstrated that, although solar radiation can inactivate the enterococci, turbid waters could hinder the full effect. The results of the turbidity experiment are shown in Table 4. After initial seeding at 6:00 a.m., an almost complete inactivation of the enterococci was observed in the containers with no turbidity. This result was consistent with the previous solar inactivation experiments that showed that near-complete

inactivation occurred after 4 h of sunlight exposure. The inactivation rate for all the treatments was consistent with Fujioka et al. (1981), showing that the 90% of enterococci were inactivated (T_{90}) within 60 to 180 min of sunlight exposure. At 12:00 p.m., the containers with 0 and 70 NTU turbidity levels had complete inactivation of enterococci, whereas the containers with 140 NTU turbidity levels still had an appreciable enterococci concentration. The enterococci concentrations at 12:00 p.m. were consistent with the field samples gathered in the afternoon during the 2010 recreation sampling season. The results suggest that the sediment particles are either shielding enterococci from direct sunlight or lessening the effect of environmental stressors through attachment of enterococci to sediment particles.

The beach sand experiment demonstrated that enterococci exist and persist in coastal beach sands. The presence of enterococci in near-shore seawater could be explained by the inevitable transport of enterococci-contaminated beach sands and the hindrance to inactivation by solar irradiance by increased turbidity levels in near-shore seawater.

Reactivation of Enterococci after Solar Inactivation

Results of the reactivation experiment demonstrated that overnight reactivation of enterococci in seawater did not occur. After 11 h of sunlight exposure, with a peak solar irradiance of 4,200 μmol · m^{-2} · s^{-1} at approximately noon, the geometric mean concentration of enterococci in the three containers was reduced from above the upper quantitation limit (24,196 MPN/100 mL) to 13 MPN/100 mL. After incubation of all the containers at 28°C in the dark (overnight) for 12 h, the geometric mean enterococci concentration fell below the detection limit. There was no significant change ($p = 0.2508$) in the enterococci concentration overnight. Therefore, reactivation of enterococci was an improbable source of the replenishment in near-shore seawater, contrary to the findings of Ferguson et al. (2005). The inability for solar inactivated enterococci to regenerate overnight was also noted in a study by Boehm et al. (2002). In the field, the near-shore seawater must be replenished after every day to keep the concentrations elevated. The reseeding could be caused by other factors such as bird feces or by beach sands becoming suspended because of the tidal effect as noted by Yamahara et al. (2007) and Boehm and Weisberg (2005).

Additional Field Factors

Although this work focused on solar radiation, salinity, turbidity, and beach sands affecting enterococci concentrations along the Gulf Coast, it must be stated that other factors could have significant influence on enterococci concentrations. Frick et al. (2008) discuss wave height, rainfall, wind direction, wind speed, and temperature as factors affecting enterococci concentrations, especially in developing prediction models. These factors have been surveyed by LDHH and were found to impact the enterococci concentrations, although the magnitude of the effects could not be determined (LDHH 2009). Lyons et al. (2010) pose the theory that organic aggregates act as microscopic islands and provide substance for enterococci to latch on to for protection in beach waters. Signoretto et al. (2004, 2005) state that enterococci persist in the aquatic environment because of surface interactions with copepods and plankton. Recent sampling events noted the higher yield of enterococci in water that had larger amounts of seaweed nearby. A large number of seagulls, pelicans, and other wildlife in the area could have been contributing to the enterococci concentrations in the near-shore beach waters by adding feces to the water and transporting beach sands (Grant et al. 2001). However, recent samples collected by the LSU research group from ponded beach water where seagulls were found did not show appreciable concentrations of enterococci (unpublished data).

Conclusions

The laboratory experiments confirm the ability of solar radiation to inactivate large concentrations (>24,196 MPN/100 mL) of enterococci across a range of salinities (0, 5, 15, 20, 25 ppt) in as little as 4 h, which can partially explain the decrease in enterococci concentrations over the course of the day. With solar radiation having such a highly destructive impact on enterococci, the time that samples were collected for monitoring programs was very important. If samples were collected too late in the morning, solar radiation could have reduced the fecal-indicator bacteria concentration to a level safe for recreational beaches that otherwise would have been unsafe at an early sampling time. The experiments also established that there was a potential for higher turbidity water to buffer the enterococci from solar radiation effects. Increased levels of turbidity prevented complete inactivation of enterococci. This can explain why enterococci were still found in afternoon samples at coastal beaches.

While enterococci concentrations were inactivated largely in part by solar radiation, no appreciable reactivation of enterococci occurred overnight as suggested by other authors in other geographical regions. This outcome suggests that enterococci were replenished in seawater through some other source. One possible source could be the beach sands. Beach sands contained enterococci and could have been transported to near-shore seawater through various processes. Turbidity caused by turbulence in the surf zone could have included enterococci-attached sand particles. The addition of enterococci-attached sand to the seawater may have increased the enterococci concentration while preventing some of the harmful effects of solar radiation.

Gulf Coast beach sands contained enterococci, and this work along with results in literature showed that the enterococci in the beach sand persisted for extended periods of time. With enterococci persistence in beach sands, coastal beach sand must be considered a reservoir for enterococci. Other factors such as tides, wind, or animals could potentially provide transport routes for beach sand and elevate the enterococci concentration of the near-shore seawater. Windy days or post-rainfall runoff could be transporting enterococci into the near-shore seawater as well.

Solar radiation, beach sands, and surrounding water quality are factors that affected the complex nature of enterococci concentrations in Gulf Coast seawater. Solar radiation appeared to be the most significant of the factors examined, with beach sands demonstrating an important role in enterococci concentration persistence. Contrary to the findings in other regions, no enterococci regrowth after inactivation was observed.

Acknowledgements

References

Alkan, U., Elliot, D. J., and Evison, L. M. (1995). "Survival of enteric bacteria in relation to simulated solar radiation and other environmental factors in marine waters." *Water Res.*, 29(9), 2071–2081.

Alm, E. W., Burke, J., and Spain, A. (2003). "Fecal indicator bacteria are abundant in wet sand at freshwater beaches." *Water Res.*, 37(16), 3978–3982.

American Public Health Association (APHA). (2005). *Standard methods for the examination of water and wastewater*, 20th Ed., American Public Health Association, American Water Works Association, Water Environment Federation, Washington, DC.

ASTM. (2009). "Standard test method for enterococci in water using Enterolert." *D6503-99*, West Conshohocken, PA.

Boehm, A. B. et al., (2002). "Decadal and shorter period variability of surf zone water quality at Huntington Beach, California." *Environ. Sci. Tech.*, 36(18), 3885–3892.

Boehm, A. B. et al., (2009). "Faecal indicator bacteria enumeration in beach sand: A comparison study of extraction methods in medium to coarse sands." *J. Appl. Microbiol.*, 107(5), 1740–1750.

Boehm, A. B., and Weisberg, S. B. (2005). "Tidal forcing of enterococci at marine recreational beaches at fortnightly and semi-diurnal frequencies." *Environ. Sci. Tech.*, 39(15), 5575–5583.

Burkhardt, W., III, Calci, K. R., Watkins, W., Rippey, S., and Chirtel, S. (2000). "Inactivation of indicator microorganisms in estuarine waters." *Water Res.*, 34(8), 2207–2214.

Byappanahalli, M. N., Whitman, R. L., Shively, D. A., Ting, W. T. E., Tseng, C. C., and Nevers, M. B. (2006). "Seasonal persistence and population characteristics of *Escherichia coli* and enterococci in deep backshore sand of two freshwater beaches." *J. Water Health*, 4(3), 313–320. ⟨http://www.iwaponline.com/jwh/004/0313/0040313.pdf⟩.

Cabelli, V. J. (1983). "Health effects criteria for marine recreational waters." *Technical Rep. EPA-600/1-80-031*, U.S. Environmental Protection Agency, Office of Water, Washington, DC.

Cabelli, V. J., Dufour, A. P., McCabe, L. J., and Levin, M. A. (1982). "Swimming associated gastroenteritis and water quality." *Am. J. Epidemiol.*, 115(4), 606–616. ⟨http://aje.oxfordjournals.org/content/115/4/606.abstract⟩.

Davies-Colley, R. J., Bell, R. G., and Donnison, A. M. (1994). "Sunlight inactivation of enterococci and fecal coliforms in sewage effluent diluted in seawater." *Microbiology*, 60(6), 2049–2058. ⟨http://aem.asm.org/content/60/6/2049.full.pdf+html⟩.

Ferguson, D. M., Moore, D. F., Getrich, M. A., and Zhowandai, M. H. (2005). "Enumeration and speciation of enterococci found in marine

and intertidal sediments and coastal water in southern California." *J. Appl. Microbiol.*, 99(3), 598–608.

Frick, W. E., Ge, Z., and Zepp, R. G. (2008). "Nowcasting and forecasting concentrations of biological contaminants at beaches: A feasibility and case study." *Environ. Sci. Tech*, 42(13), 4818–4824.

Fujioka, R. S., Hashimoto, H. H., Siwak, E. B., and Young, R. H. (1981). "Effect of sunlight on survival of indicator bacteria in seawater." *Appl. Environ. Microbiol.*, 41(3), 690–696. ⟨http://www.ncbi.nlm.nih.gov/pmc/articles/PMC243761/pdf/aem00196-0142.pdf⟩.

Gerba, C. P., and McLeod, J. S. (1976). "Effect of sediments on survival of Escherichia coli in marine waters." *Appl. Environ. Microbiol.*, 32(1), 114–120. ⟨http://www.ncbi.nlm.nih.gov/pmc/articles/PMC170015/pdf/aem00006-0126.pdf⟩.

Grant, S. B. et al., (2001). "Generation of enterococci bacteria in a coastal saltwater marsh and its impact on surf zone water quality." *Environ. Sci. Technol.*, 35(12), 2407–2416.

Hartel, P. G. et al., (2005). "Survival and regrowth of fecal enterococci in desiccated and rewetted sediments." *Proc., 2005 Georgia Water Resources Conf.*, K. J. Hatcher, ed., Institute of Ecology, University Of Georgia, Athens, Georgia.

Heaney, C. et al., (2009). "Contact with beach sand among beachgoers and risk of illness." *Am. J. Epidemiol.*, 170(2), 164–172.

Ki, S. J., Ensari, S., and Kim, J. H. (2007). "Solar and tidal modulations of fecal indicator bacteria in coastal waters at Huntington Beach, California." *Environ. Manage.*, 39(6), 867–875.

Lipp, E. K., Kurz, R., Vincent, R., Rodriguez-Palacios, C., Farrah, S. R., and Rose, J. B. (2001). "The effects of seasonal variability and weather on microbial fecal pollution and enteric pathogens in a subtropical estuary." *Estuaries*, 24(2), 238–258.

Louisiana Department of Health and Hospitals (LDHH). (2003). *Louisiana's BEACH grant final report, grant year 2001*, Office of Public Health, Baton Rouge, LA.

Louisiana Department of Health and Hospitals (LDHH). (2009). *Louisiana BEACH grant report 2008 swimming season*, Office of Public Health, Baton Rouge, LA.

Lyons, M. M., Ward, J. E., Gaff, H., Hicks, R. E., Drake, J. M., and Dobbs, F. C. (2010). "Theory of island biogeography on a microscopic scale: Organic aggregates as islands for aquatic pathogens." *Aquat. Microb. Ecol.*, 60(1), 1–13.

Noble, R. T., Lee, I. M., and Schiff, K. (2004). "Inactivation of indicator bacteria from various sources of fecal contamination in seawater and freshwater." *J. Appl. Microbiol.*, 96(3), 464–472.

Oshiro, R., and Fujioka, R. (1995). "Sand, soil, and pigeon droppings—Sources of indicator bacteria in the waters of Hanauma Bay, Oahu, Hawaii." *Water Sci. Technol.*, 31(5–6), 251–254.

Signoretto, C. et al., (2004). "Adhesion of *Enterococcus faecalis* in the non-culturable state to plankton is the main mechanism responsible for persistence of this bacterium in both lake and seawater." *Appl. Environ. Microbiol.*, 70(11), 6892–6896.

Signoretto, C., Burlacchini, G., Pruzzo, C., and Canepari, P. (2005). "Persistence of *Enterococcus faecalis* in aquatic environments via surface interactions with copepods." *Appl. Environ. Microbiol.*, 71(5), 2756–2761.

U.S. Environmental Protection Agency (USEPA). (1986). *Ambient water quality criteria for bacteria—1986*, *U.S. EPA Rep. 440/5-84-002*, Cincinnati.

U.S. Environmental Protection Agency (USEPA). (2002). *Implementation guidance for ambient water quality criteria for bacteria*, EPA-823-B-02-003, US Government Printing Office, Washington, DC.

U.S. Environmental Protection Agency (USEPA). (2010). "EPA's BEACH report: 2009 swimming season." *EPA 820-F-10-003*. ⟨http://water.epa.gov/type/oceb/beaches/upload/report2009.pdf⟩.

Wade, T. J., Pai, N., Eisenberg, J. N., and Colford, J. M. Jr. (2003). "Do U.S. Environmental Protection Agency water quality guidelines for recreational waters prevent gastrointestinal illness? A systematic review and meta-analysis." *Environ. Health Perspect.*, 111(8), 1102–1109.

Yamahara, K. M., Layton, B. A., Santoro, A. E., and Boehm, A. B. (2007). "Beach sands along the California Coast are diffuse sources of fecal bacteria to coastal waters." *Environ. Sci. Technol.*, 41(13), 4515–4521.

Yamahara, K. M., Walters, S. P., and Boehm, A. B. (2009). "Growth of enterococci in unaltered, unseeded beach sands subjected to tidal wetting." *Appl. Environ. Microbiol.*, 75(6), 1517–1524.

JOURNAL OF ENVIRONMENTAL ENGINEERING © ASCE / NOVEMBER 2012 / **1137**

J. Environ. Eng., 2012, 138(11): 1130-1137