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Evaluation of Enterococci, an Indicator Microbe, and the Sources that Impact the Water Quality at a Subtropical Non-Point Source Recreational Beach

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EVALUATION OF ENTEROCOCCI, AN INDICATOR MICROBE, AND THE SOURCES THAT IMPACT THE WATER QUALITY AT A SUBTROPICAL NON-POINT SOURCE RECREATIONAL BEACH

By

Mary Elizabeth Wright

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Evaluation of Enterococci, an Indicator Microbe, and the Sources That Impact the Water Quality at a Subtropical Non-point Source Recreational Beach (May 2008)

Abstract of a thesis at the University of Miami.

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Beach advisories are issued at recreational beaches when the water quality exceeds regulatory limits for the indicator organism, enterococci. Elevated levels of enterococci have been observed at Hobie Cat Beach, the study marine beach site, located on Virginia Key, Florida. The study site represents a classic non-point source sub/tropical marine recreational beach area with high human and animal use, representative of many beaches worldwide in sub/tropical areas. The dissertation consisted of two separate but related studies: the first to identify environmental and geographic factors, and the second to evaluate the impact of known animal sources of enterococci.

The first efforts were made to identify the geographic location of the source of enterococci to the beach waters and to assess the environmental factors that impact the variation in concentrations observed at the site. These environmental factors and conditions include: proximity to shoreline, tidal changes, impacts of runoff, and sunlight intensity. Enterococci were enumerated by traditional membrane filtration or the chromogenic substrate method. Overall, results showed that the source of enterococci to the study beach was geographically located within the inter-tidal zone. These results suggest that the wash-in of sediments and accompanying pore waters (where the pore water is the water filling the spaces between grains of sediment) from the inter-tidal zone

play a major role in controlling enterococci levels within the water column. Wash-in occurs through both tidal fluctuations and runoff.

The second effort evaluated non-point sources, including animals, which are known to contribute to elevated levels of enterococci in recreational marine beach waters. Specifically, feces from dogs, birds, and shrimp mounds were collected from the beach; additional bird fecal samples were collected from both a local zoo and bird rehabilitation center. Fecal samples were weighed gravimetrically, and enumerated for enterococci using traditional membrane filtration method. The total numbers of animals which frequented the site were obtained through camera image analysis and in-field visual counting surveys. The highest enterococci concentrations were observed in dog feces (avg. 7.4×10^6 CFU/g dry feces), then birds (avg. 3.3×10^5 CFU/g dry feces) and the lowest measured levels of enterococci were observed in shrimp fecal mounds (2.0 CFU/g dry feces on average). A comparison of the microbial load (CFU per fecal event) showed that 1 dog fecal event was equivalent to 6,940 bird fecal events or 3.2×10^8 shrimp fecal events. Given the abundance of animals observed on the beach, these study results suggest that dogs are the largest contributing source of enterococci to the beach site (6.3×10^{11} CFU per day during weekends and 2.9×10^{11} CFU per day during weekdays), with humans (4.6×10^9 CFU per day during weekends and 4.8×10^8 CFU per day during weekdays) and birds (2.7×10^8 CFU per day) serving as secondary contributors. Shrimp served as an insignificant source (1.9×10^4 CFU per day). When maximum daily contributions were considered, dogs contributed the highest proportion of enterococci (99.2%) compared to humans (0.72%), birds (0.04%), and shrimp (<0.04%). Beach

management efforts at the study site should thus focus on requiring dog owners to properly dispose of dog feces deposited at the beach.

DEDICATION

This thesis is dedicated to my mother, Kathryn S. Audino who died on April 21, 2008 and my husband, Mark T. Wright. Their belief in me and their support has made this possible. My mother was always a tower of strength for me my entire life, her love and compassion during my undergraduate education was immense and she encouraged me to continue my education which helped me to have a Godly inspired drive to succeed. I thank God for each and every day I am able to spend with my two best friends, my mother and my husband.

I also dedicate this thesis to my siblings, Michael A. Durbin and Patricia K. Ruiz, who have listened and helped me see the light at the end of the tunnel.

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Specifically, I would like to thank Helena Solo-Gabriele and Lora Fleming for their encouragement and continued support throughout these studies.

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Chapter 1: INTRODUCTION

1.1 Background

Recreational beach water quality is important for the protection of human health, and when the standards are not meet, then there are a range of impacts on local communities. Many beach communities thrive on the economics which come from tourism; when there are unnecessary beach closures, it may result in heavy economic losses on various scales (Rabinovici et al. 2004). When beaches are characterized as unsafe to swim by closing the beach or posting signs that warn beach users against coming in contact with the water, then the public is rightly concerned about their environment, and their personal health and wellbeing. The focus of an engineer is on the health and safety of human populations, as well as the environment. The intervention taken for this protection is the regular monitoring of indicator microbes in the waters of beaches. Regulations are set as seen in Table 1.1; Florida has adapted the use of the indicator organisms, fecal coliforms, as part of their monitoring program.

Table 1.1: Federal and State Regulation for Microbes in Recreational Water

Indicator microbes	Regulatory Level	Regulator
Fecal coliform	A monthly geometric mean of 200/100 ml (at the minimum, 10 samples over a 30-day period), and 400 in 10% of the samples. 800 on any one day .	USEPA (1976) FDEP FDOH
Total coliform	A monthly geometric mean of 1,000/100 ml (at the minimum, 10 samples over a 30-day period). 2,400/100 ml in 20% of the TC single samples.	USEPA (1976)
Enterococci	35 CFU/100 ml, geometric mean 104 CFU/100 ml, single sample	USEPA (1986)
E. coli	Only for fresh water 126/100 ml, geometric mean 235/100 ml, single sample	USEPA (1986)

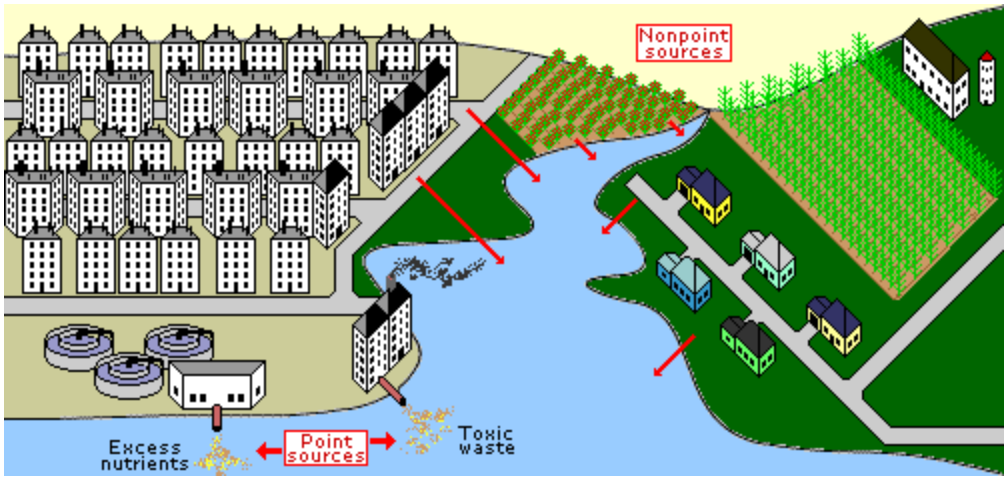
Indicator microbes are found in warm-blooded animals, and therefore, are meant to indicate the presence or absence of human or animal sewage or wastewater effluent or

runoff. The presence of the sewage would likely mean the presence of human pathogens, which can cause illness to swimmers and other beach users. Gastro-intestinal illnesses can come from contaminated recreational beach waters; signs and symptoms include: abdominal pain, vomiting, diarrhea, and fever. In addition, other illnesses associated with contaminated beach waters include skin, eye, ear, respiratory, and wound infections.

Traditionally, indicator microbes are considered an accurate assessment for conducting and managing recreational beach water quality when there is a known point source. Point sources are defined as wastewater treatment plant outfalls where sewage is discharged and outfalls from industrial sites (Figure 1.1). Indicator microbes work well when there are known point sources; inotherwords, they indicate the presence of pathogens which can cause disease in humans. Nonpoint sources are much more difficult to assess, and are not necessarily an accurate measurement of water quality because the correlation between poor water quality and potentially harmful pathogens or the occurrence of illness is weak (Fujioka et al. 1981; Griffin et al. 2003; Jiang and Chu 2004; Whitman et al. 2004; Colford et al. 2007). One overall concern with indicator microbes is that their presence or absence does not always mean the presence or absence of human sewage and hence pathogens.

Several nonpoint sources are seen in Figure 1.1. Other nonpoint sources to recreational beach waters can include beach sands and soil where microbes can re-grow within the environment or once deposited can continue to thrive in the natural environment. In addition, animals (including humans) participate in depositing microbes in recreational waters through several means. Humans shed microbes from the skin (Elmir et al. 2007), and animals (such as horses, dogs, reptiles, and birds) deposit fecal

matter. All of these contribute to poor water quality from the water column at recreational beaches.



Source: U.S. EPA, March 2008. http://omp.gso.uri.edu/doee/policy/orga1.htm

Figure 1.1: Nonpoint sources: heavy metals, oils from streets, and sewage from the city; fertilizers, pesticides, and sewage from farms and suburbs add excess nitrogen and phosphorus. All make their way directly and indirectly to the waterway. Point sources: the sewage treatment plant and factory introduce materials directly into the waterway.

1.2 Objectives

The following two chapters focus on two sets of sampling efforts which were designed to characterize enterococci sources to a local recreational beach, Hobie Cat Beach. This site is characterized by nonpoint sources of microbes to the water column. The first group of studies (Chapter 2) focuses on evaluating enterococci, an indicator microbe, and its frequency at the beach, while discovering locations which contained elevated concentrations and identifying environmental factors which influence the microbe levels. The second group of studies (Chapter 3) focuses on identifying potential sources of enterococci which frequent the site and perhaps contribute microbes to both the beach sands and water.

Chapter 2: INTER-TIDAL ZONE

2.1 Background

Traditionally, indicator organisms have been used to evaluate the safety of recreational waters. Indicators, such as enterococci, which are the EPA recommended indicator microbe for marine beaches (US EPA, 1986; FDOH, 1996), are inhabitants of the gastrointestinal tracts of humans and other warm-blooded animals, and are associated with the release of feces into the environment. The presence of indicator organisms themselves is not the concern, rather they are meant to indicate the presence of a pollution matrix (such as sewage) which is known to contain pathogens which adversely affect human health. Therefore, the presence of indicator microbes, such as enterococci, at elevated levels, is interpreted as a high likelihood for the presence of fecal contamination. Standards for indicators have been established through epidemiologic studies in areas impacted by known point sources of sanitary sewage primarily in temperate climates (Cabelli 1983). Beach advisories occur when indicator organism levels exceed these standards. Regulatory standards for enterococci are set for either the geometric mean over thirty days (35 CFU/00 ml), or for a single sample (104 CFU/100 ml) (US EPA, 1986; FDOH, 1996). This criterion is interpreted to represent a level of acceptable health risk to humans. However, many beach sites are impacted by non-point sources, and the relationship between human health and non-point sources of pollution has not yet been well established globally, particularly in sub/tropical environments. As indicated by Colford et al. (2007), there is a need for alternative indicators in areas impacted by non-point sources of fecal indicator bacteria.

Identifying the source of indicator microbes is especially difficult when the study site is impacted by non-point sources such as soils and beach sands (Fujioka et al. 1999; Hardina and Fujioka, 1991; Roll and Fujioka 1997; Whitman and Nevers 2003; Shibata et al. 2004). In particular, fine-grained sediments have been seen as a reservoir for enterococci (Sherer et al. 1992; Howel et al. 1996). In some cases, underlying sediments in recreational beach areas have been implicated as a source of enterococci (Indest 2003; Howell et al. 1996; Obiri-Danso and Jones 2000; Sherer et al. 1992). Specifically, in South Florida, river bank soils and beach sands have also been implicated as the source of indicator microbes to the water column (Desmarais et al. 2002; Rogerson et al. 2003; Shibata et al. 2004; Solo-Gabriele 2000). Studies have also shown that the re-suspension of sediments can increase the microbial loads in the water (Graczyk et al. 2007; Indest 2003; Brookes et al. 2004; US Geological Survey 2006a, b). Anderson et al. (1997), who found high densities of enterococci in marine sediments, suggested that the sources of contamination were either through persistence and/or replication in the environment. The ultimate source of indicators to soils and sands can include humans (Papadakis et al. 1997; Elmir et al. 2007) and animals (Williams et al. 2007), including birds (Jones et al. 1978; Lévesque et al. 1993, 2000; Fujioka 1988; Davies et al. 1995; Hatch 1996; Alderisio and DeLuca 1999; Jones and Obiri-Danso 1999; Obiri-Danso and Jones 2000), mammals (Kühn et al. 2003; Meals and Braun 2006), and reptiles (Harwood et al. 1999). These sources can persist for long periods and even multiply through regrowth, particularly in sub/tropical environments (Desmarais et al. 2002).

Environmental factors that have been shown to impact enterococci levels include rainfall, tides, currents, and sunlight. Indicators, which are deposited in the sediments or

originate from them, can subsequently be washed into a receiving water body through rainfall runoff (Roll and Fujioka 1997; Haack et al. 2003; Beversdorf et al. 2007) and tidal action (Solo-Gabriele et al. 2000; Boehm et al. 2002). As a result, many studies have found a correlation between fecal indicators and rainfall (Haack et al. 2003) and tides (Martin and Gruber 2005). Rainfall has been suggested as mobilizing the indicator sources and result in elevated microbes in a Florida watershed (Shehane et al. 2005). Martin and Gruber (2005) showed that wash-in was an important mechanism for organic debris (marine vegetation) such as kelp which was washed during high tide. The kelp in this study was intermittently impacted by dog feces, and the authors concluded that the tidal flushing of the kelp within the wrack line (area where organic debris deposits on the beach which then forms the wrack line) resulted in an intermittent contribution of indicator bacteria during high tide. Whether the source of the indicator bacteria came directly from the feces or from regrowth in the kelp was not fully explored in this study, but it is possible that the initial source or innoculum of bacteria came from animal sources. Solo-Gabriele et al. (2000) found elevated levels of *E. coli* which correlated with storm events and tidal cycles; the highest concentrations occurred during high tide and lower concentrations during low tide. Tidal effects were evaluated and seen on a seasonal scale; spring tide single samples of enterococci were twice as likely to exceed compliance than neap tide, suggesting that tide should be considered when assessing beach monitoring efforts (Boehm and Weisberg 2005). Wave action from currents has been shown to release sand or sediment borne *E. coli* into lake water (Ishii et al. 2007). Finally, sunlight effects were observed in Lake Michigan where the solar inactivation naturally reduced indicator bacteria (Whitman et al. 2004).

The study site, Hobie Cat Beach, has no known point source. Although there is a wastewater treatment plant ocean outfall located directly north and on the other side of the island, the currents at the outfall site do not allow for fecal contamination to migrate back towards the study site. The nearby Miami Seaquarium has waters that are drained directly into the Bear Cut to the east, and this water has been tested and found to be characterized by low levels of indicator bacteria as the Miami Seaquarium filters and chlorinates the waters used for its marine mammal exhibits (Shibata et al. 2004).

The overall focus of these studies was to identify the location of the enterococci source and to evaluate environmental factors that impact enterococci concentrations at a non-point source marine beach. This was accomplished through a series of sampling efforts aimed at analyzing the water column and sand both on the beach and below the water column. Enterococci was chosen for the study to be consistent with the local department of health beach monitoring program which uses enterococci for their evaluation of the beach. Environmental factors which were targeted as part of this investigation included antecedent rainfall, tidal height, and sunlight intensity. Bathing load and seasonal impacts were also evaluated as part of these studies.

2.2 Materials and Methods

2.2.1 Site Description. The beach chosen for this study, Hobie Cat Beach, is located on Virginia Key, a small island on Biscayne Bay which is immediately east from the coast of Miami, Florida, U.S.A. (Figure 2.1). Previous research has shown that this site is characterized by intermittant enterococci exceedences (Fl. Dept. of Health, 2007). Hobie Cat Beach is also called Dog Beach as it is the only beach within Miami-Dade County

that allows dogs. The beach is utilized year round with visitors varying from 276 (avg. 55) visitors during the weekdays to 2,644 (avg. 1,088) visitors on weekends. The ratio of dogs to humans is roughly 1 to 7 for any given day of the week. The climate of Miami, Florida, is characterized as tropical with an average ambient temperature of 21.1 ºC (27.6^{o}C during the summer months of May through October and 22.0 ^{o}C during the winter) and annual average rainfall of 149 cm (total of 109 cm during the summer months and 39 cm during the winter months).

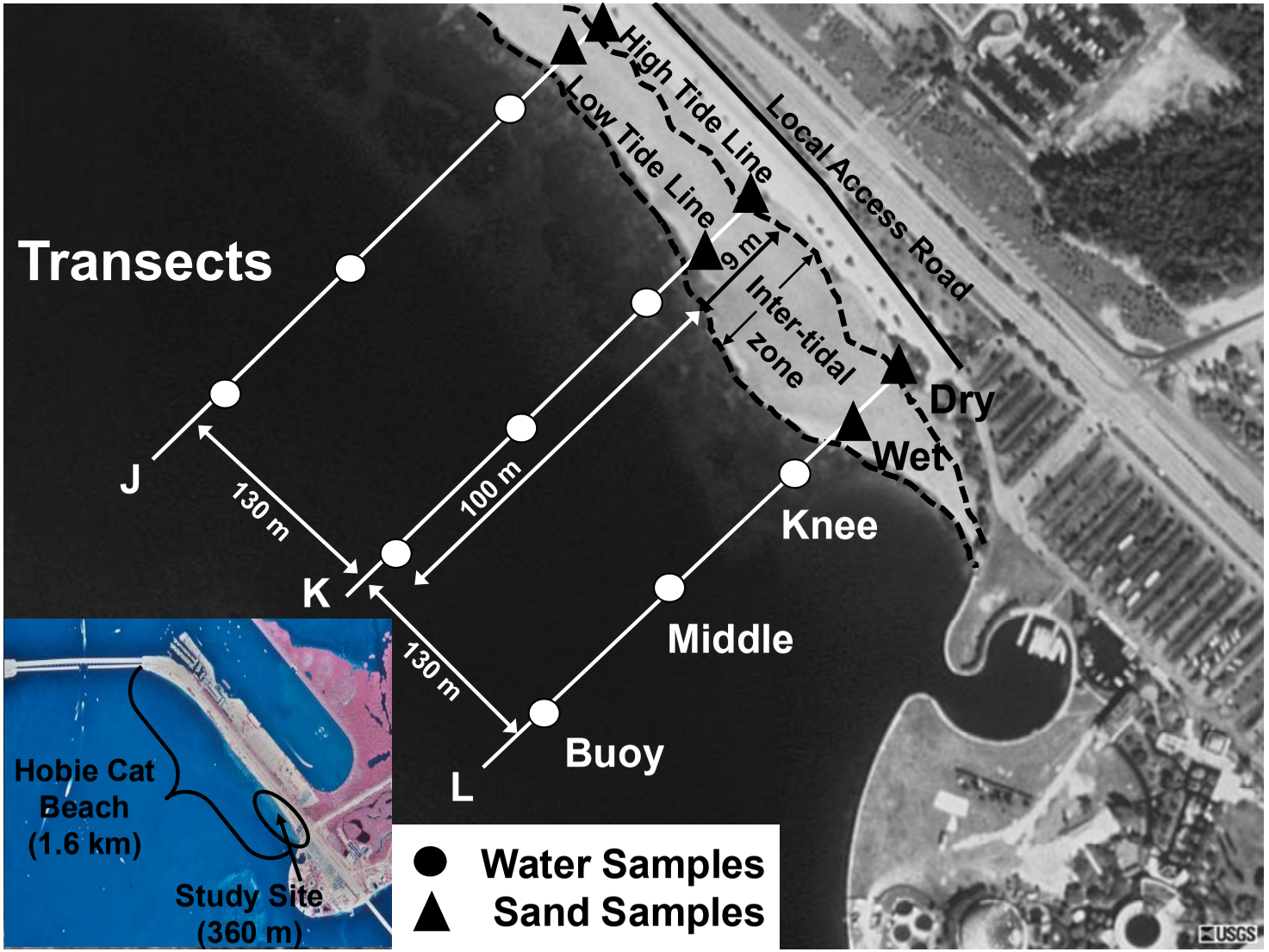


Figure 2.1: Hobie Cat Beach: Study Site is located on the southeastern portion of Hobie Cat Beach. Transects (J, K, and L) are indicated by the lines, with the inter-tidal zone shown as the area between the dashed lines.

Although the beach is approximately 1.6 km long, this study focused on the south-eastern 360 meters of the beach, as previous research (Shibata et al. 2004) indicated that this area was characterized by elevated indicator levels (Figure 2.1). A local access paved road (6 m wide) runs parallel to the beach at a distance of 13 meters from the mean high tide. The beach is characterized by relatively poor water circulation because it is shallow and located within a cove. Movement of water near the shoreline is controlled by tidal action as opposed to wave action. The average fluctuation in tidal height at the site is 58 cm. Due to the relatively shallow slope (0.06 m/m) near the shoreline (defined as the interface between the water and the exposed beach sand), this fluctuation in tidal height results in a 5 to 12 meter horizontal distance between the respective shoreline between high and low tide. The sand area, which is periodically wetted and dried within this 5 to 12 meter band of the beach, is referred herein as the "inter-tidal zone."

The waves at the site are extremely fetch-limited, resulting in waves or ripples which rarely exceed a height of 10 cm and a wavelength of 1 to 2 m. The winds are usually offshore or parallel to the shoreline which contributes to the low energy wave climate. The water content for the sand varies from 1 to 30% depending on the location of the sand. Sand within the backshore zone, because it is not influenced by the tidal action, is termed herein as "dry" sand, and is typically characterized as having a water content of 3.8%. Sand within the inter-tidal zone is termed as "wet" sand, and is typically characterized by a water content of approximately 22%. Beach sand is characterized as calcium carbonate gravel to mud with a mean grain size of 0.5 mm. Volatile solids (organics) content of the sand is low at 0.85 ± 0.020 %.

2.2.2 Sample Collection. Several sampling efforts were conducted; these included: tidal studies, hourly sampling, 10-minute sampling, runoff sampling, and spatially intensive sediment sampling. Sampling sites were identified with respect to a series of 3 transects which were marked by buoys (called J, K, and L approximately 130 m apart from one another) located about 100 m offshore in 5.4 meter depth of water (Figure 2.1). All samples were collected aseptically using pre-sterilized Whirl-Pak® sampling bags. Water samples were collected from the upper most layer of the water column from 0 to 25 cm. Sample collection occurred carefully to minimize the disturbance of the underlying sediments. Sand samples were consistently collected from the upper 1 to 3 cm layer of sand. Pre-sterilized metal spoons were used to place sand into Whirl-Pak® sampling bags. All samples were transported from Hobie Cat Beach to the laboratory in an iced cooler, and analyzed within 4 hours.

In addition to water and sand sampling, physio-chemical parameters measured during each sampling event included salinity, temperature, pH (YSI Model 650-01m Environmental Monitoring Systems, Yellow Springs, OH, 1999), and dissolved oxygen (HACH Dissolved Oxygen Test Kit, Model OX-2P). The turbidity of water samples and sand filtrates were also measured (Model 66120-200, VWR, Newark, DE). Rainfall data were available from 7 tipping bucket rain gauges located within 1 km of the site (P. Minnett, University of Miami, Coral Gables, FL, personal communication). Sunlight intensities (solar radiation levels) were evaluated using idealistic solar radiation and sky cover data,i.e. solar radiation equals idealist solar radiation multiplied by (one minus the sky cover) .Idealist solar radiation is solely determined by the relative angle between the

sun and the location of interest, without taking into consideration of atmospheric influence. (Dynamic Hydrology, P.S Eagleson, Page 30) Sky cover is defined as the percentage of the sky obscured and can be found from National climatic Dater center website. The sky cover data is acquired for Miami International Airport to the north of our site (http://www5.ncdc.noaa.gov/cgi-bin/script/webcat.pl?action=ALL). Tidal height information was obtained for Bear Cut located immediately south east of the site from *Tides and Currents* website (NOAA 2004 http://tidesandcurrents.noaa.gov/tide_pred.html). Confidence limits were evaluated using the student t-test. All ranges correspond to a 95% level of confidence.

2.2.2.1 Tidal Studies. These efforts focused on evaluating enterococci levels in water and sand at two to three locations along transects J, K, and L, during both low and high tides. Sampling occurred primarily during the summer months (11 events), with two additional sampling events during the winter. Summer sampling included six high tide events and five low tide events. Water and sand samples collected during the winter were only evaluated during high tide.

Water collection sites along each transect included: a sample in knee deep water; a sample midway between the shoreline and the buoy; and a sample near the buoy. The "knee" water sample was located roughly 10 m from the shoreline, and was collected during high and low tides. The "middle" sample was approximately 53 m from the buoy, and collected at high tide only. The "buoy" sample was located approximately 13 meters from the buoy towards the shoreline, and collected during both tides. One exception to this general water sample collection strategy was the collection of "ankle" deep water

samples (top 0 to 15 cm of water column within 1 m of the shoreline) during a high bather load event described in the Results Section.

"Dry" sand samples were collected during both high and low tides in an area 1 meter above the seaweed wrack line (i.e. shoreward above the recent high tide line). "Wet" sands were collected from the center of the inter-tidal zone, and were "inundated" during high tide and "exposed" during low tide.

2.2.2.2 Hourly Sampling. The hourly sampling effort spanned two semidiurnal tidal cycles (48-hours), and included the collection of water and sand samples at transect K. Water samples were consistently collected in "knee" deep water. As such, the location of the water sample varied along the length of transect K, with samples collected farther from the paved road during low tide and closer during high tide. Sand samples were collected from a consistent location as marked by a pole placed in the middle of the inter-tidal zone. Sand samples were collected in a clockwise fashion from around the pole, each hour correlating to a different location. Samplers approached the pole from a large perimeter to minimize the disturbance of the sand.

2.2.2.3 10-Minute Sampling. In order to further evaluate the impacts of tide, water samples were collected every 10 minutes for a total of four hours, two hours before and two hours after high tide. Sampling occurred during two days in the winter. During the first day, samples were collected in "knee" deep water at transect K. As the tide changed, the locations along transect K were changed accordingly. During the second day, the experiment was conducted as described above plus additional water samples were

collected simultaneously at a location marked by a stationary pole which was located in the inter-tidal zone midway between J and K transects.

2.2.2.4 Runoff Sampling. Natural channels are formed within the backshore zone after rainfall events. These channels route the flow of rainwater runoff towards the ocean. Runoff samples were collected from the delta or outflow from these natural channels located between transects J and L. A total of 36 samples were collected during nine rainfall events. Of the nine rainfall events, three events occurred in July, during the hourly sampling effort described above, where the number of samples collected per event was 4, 2, and 1, respectively. Four of the remaining rainfall events occurred in August 2004 and the last two occurred during December 2004 and February 2005. The number of runoff samples collected during each event in August was 6, 7, 7, and 3. Three samples were collected during the event in December and another three samples were collected during the event in February.

2.2.2.5 Spatially Intensive Sediment Sampling. Sand samples were collected along the K transect. The first sand sample was collected in the backshore zone, 15 m away from the shoreline behind the paved road. The remainder of the samples were collected on the beach side of the paved road and continued towards the buoy. A total of 12 sand samples were collected, each approximately 4 m apart for "dry" sand and 15 to 20 m apart for "wet" sand.

2.2.3 Laboratory Methods. Enterococci were evaluated for both water and sand samples. All water samples were enumerated using the standardized membrane filtration (MF) method (U.S. EPA 2002, Method 1600), except for the hourly sampling event which utilized the chromogenic substrate technique. In brief, the MF method was based upon a selective medium (mEI agar, Becton Dickinson, Sparks, MD) and incubation of filters at 41°C for 24 hours. All colonies that were blue or characterized by a blue halo were recorded as "enterococci colonies." The chromogenic substrate method used a reagent commercially known as Enterolert™ (IDEXX, Atlanta, GA) that was mixed with the sample, and then poured into a series of wells (Quanti-Tray®, 2000), which were sealed and incubated at 41°C for 24 hours. Fluorescent wells were counted using an ultraviolet light, and reported as a most probable number (MPN). Water sample volumes utilized were 50 ml and/or 100 ml. Blank samples were included, and triplicates of each sample were evaluated throughout all experiments. Results from replicate analysis showed that 95% confidence limits ranged from 14-35% for water and 20-30% for sand depending on the location of the sand.

For sand analysis, the "washable" enterococci were extracted from the soil using a modified version of the procedure outlined by Van Elsas and Smalla (1997). To enumerate the organisms in the sand samples, two preliminary steps were performed. The first step was to measure the water content of sand. Water content was determined by measuring the weight difference of sand before and after drying (110°C for 24 hours) approximately 18 g of sample on pre-weighed weighing dishes.

The second step required the extraction of organisms from the sand particles to a predefined volume of sterile water. In order to accomplish this, approximately 7 g of un-

dried sand were aseptically removed from the sampling bags and placed into sterile pre-weighed jars. Approximately 30 to 50 ml of sterile phosphate buffer dilution water (PBS) were then added to each jar. The jars were manually shaken for 30 seconds, and the liquid samples were then filtered using pre-sterilized 30 µm pore size nylon net filters (Type NY30, Millipore, Bedford, MA). An additional 50 to 70 ml of PBS was used to remove the sand from the jar. All of the additional liquid and sand was filtered through the same 30 µm pore size nylon net filters. The final volume of filtrate was recorded, and this filtrate was analyzed for enterococci. The mEI agar method (U.S. EPA 2002, Method 1600) was used to enumerate enterococci for all sand filtrates, except for those collected during the hourly sampling event which were enumerated using the chromogenic substrate method described above. Sand filtrate volumes analyzed ranged from 0.01 to 50 ml. All sand results are reported on a per gram of dry sand (g sand) basis as all reported enterococci levels have been corrected for the moisture content of the sand

2.3 Results

2.3.1 Tidal Studies. One of the 13 tidal study sampling events was characterized by an exceptionally large bather load with 595 human bathers (377 on the sand and 18 in the water) and 44 dogs (25 on the sand and 19 in the water). This high bather load event was preceded by dry conditions. All of the remaining sampling events were characterized by low bather loads (<30 bathers with an average of eight bathers within the study site).

In addition, three of the 13 events were characterized by antecedent wet conditions and occurred during the summer (two) and winter (one). Both summer events were characterized by 4.4 cm of rainfall during the prior 24 hours. The summer events

occurred on the same day, the first one corresponding to low tide with 3.1 cm of rainfall measured during the previous six hours. Rainfall during the preceding six hours (0.01 cm) during the second sampling event was much lower compared to the first event on that day. The winter event had an antecedent rain amount of 2.1 cm during the 24 hours prior and no rain during the preceding six hours. For the remaining 10 events, the preceding 24 hour rainfall was less than 0.06 cm with a majority of the cases characterized by a zero rainfall quantity.

No significant correlations were seen between enterococci concentrations found in water and water temperature (avg. 30°C, range 21 to 36°C) or turbidity (avg. 3.8, range 0.6 to 19 NTU) or dissolved oxygen (avg. 3.7 mg/L, range 0.7 to 8.3 mg/L). Measured salinity (avg. 36‰, range 33 to 37‰) and pH (avg. 7.9, range 6.8 to 8.5) were consistent with those seen in marine recreational beach waters.

Tidal studies showed that enterococci concentrations in the water samples consistently decreased with distance from the shoreline. On average for all events (excluding the event characterized by rainfall during the preceding six hours and the event characterized by extremely high bather load), "knee" samples were measured at 47 ± 36 CFU/100 ml; "middle" samples were at 4.1 ± 2.0 CFU/100 ml; and the "buoy" samples were 1.8 ± 0.61 CFU/100 ml ($p<0.05$). The trend of decreasing enterococci concentrations as one proceeds away from the shoreline was observed consistently during high and low tides, regardless of season. Specifically, during high tide, enterococci levels were observed at 71 ± 48, 4.1 ± 2.0, and 2.1 ± 0.8 CFU/100 ml, on average, from "knee" to "middle" to "buoy" samples, respectively. During low tide, "knee" samples (4.3 ± 3.5

CFU/100 ml) were higher than "buoy" samples (1.5 ± 1.0 CFU/100 ml) on average, but this difference was not statistically significant ($p>0.05$).

The observed trend of decreasing water column concentrations with distance from shore was also apparent for the event characterized by immediately preceding wet conditions (3.1 cm of rain during the previous six hours). In this event, all three "knee" samples were above the detection limit (>500 CFU/100 ml). For samples collected at the buoy line, one was above detection limit (>500 CFU/100 ml), while the other two "buoy" samples were relatively low at 2.0 and 17 CFU/100 ml (avg. 9.3 ± 4.4 CFU/100 ml) with an overall average of (>173 ± 321 CFU/100 ml) (Table 2.1). Comparing these results with the results from the remaining two events which were characterized by significant quantities of rain during the preceding 24 hours but either no or very small amounts of rain in the preceding six hours, demonstrated that enterococci levels in the "knee" samples (51 ± 58 CFU/100 ml and 30 ± 22 CFU/100 ml) were similar to that measured during dry conditions.

Table 2.1: Tidal Studies: Individual results for the water and sand samples. Environmental factors are also indicated. Red indicates the colonies were above detection limit and are also indicated by a greater than sign.

Environmental Factors							Water Samples				Sand Samples		
					Antecedent Rain (cm)								
Date	Tide	Antecedent Rain	Bather Load	Season[a]	6 h	24 h	"ankle"	"knee"	"middle"	"buoy"	"dry"	"exposed"	"inundated"
							CFU/100 ml				avg. CFU/g dry sand (avg. CFU/100 ml pore water)		
22-Jun-04	Low	Wet	Low	S	3.14	4.44	-	>500	-	>173	>1,090 (>1.5 x 10^6)	>158 (>6.8 x 10^4)	-
22-Jun-04	High	Wet	Low	S	0.01	4.45	-	51	1.0	1.0	49 (4.5 x 10^4)	-	11 (2.6 x 10^3)
28-Jun-04	Low	Dry	Low	S	0.00	0.03	-	9.7	-	1.0	>103 (>1.6 x 10^6)	>40 (>1.9 x 10^4)	-
29-Jun-04	Low	Dry	Low	S	0.00	0.06	-	2.0	-	1.0	73 (3.3 x 10^5)	27 (5,520)	-
30-Jun-04	High	Dry	Low	S	0.00	0.00	-	31	7.0	2.3	304 (3.4 x 10^6)	-	43 (1.1 x10^4)
1-Jul-04	High	Dry	Low	S	0.00	0.00	-	9.2	5.8	1.0	>229 (>4.8 x 10^6)	-	7.1 (1.6 x 10^4)
6-Jul-04	High	Dry	Low	S	0.00	0.00	-	53	2.8	2.0	>609 (>1.1 x 10^7)	-	12 (2.4 x 10^4)
7-Jul-04	Low	Dry	Low	S	0.00	0.00	-	2.3	-	1.3	>285 (>2.0 x 10^6)	18 (8.9 x 10^3)	-
12-Jul-04	Low	Dry	Low	S	0.00	0.00	-	3.3	-	3.0	204 (4.2 x 10^6)	6.3 (3.3 x 10^3)	-
14-Jul-04	High	Dry	Low	S	0.00	0.00	-	146	2.8	2.3	795 (2.7 x 10^6)	-	55 (1.1 x 10^4)
3-Feb-05	High	Wet	Low	W	0.00	2.09	-	30	7.8	3.7	661 (9.44 x 10^5)	-	4.8 (1.7 x 10^3)
10-Feb-05	High	Dry	Low	W	0.00	0.00	-	180	1.6	-	12 (1.2 x 10^5)	-	22 (8.9 x 10^4)
30-May-05	High	Dry	High	S	0.00	0.00	150	>210	-	1.3	3,630 (6.9 x 10^6)	-	20 (1.0 x 10^4)

[a]Season: S = Summer, W = Winter

The high bather load event showed variable results. The "ankle" samples were 280, 144 and 19 CFU/100 ml resulting in an average of 150 CFU/100 ml (Table 2.1). The "knee" samples were characterized by one sample which measured at above detection limits (>580 CFU/100 ml) while the other two samples showed 34 and 13 resulting in an average of >209 CFU/100 ml (Table 2.1). The "buoy" results were consistent with results from the other sampling events, with levels measured at an average of 1.3 CFU/100 ml. In general, a decrease in enterococci concentrations with distance from the shoreline was observed, however, this trend was not consistent for each sample along each transect.

Overall results for sand, excluding the first antecedent rainfall event and high bather load event, showed the highest levels in the "dry" sand averaging >302 ± 159 CFU/g (>2.9 ± 1.9 x 10^6 CFU/100 ml of pore water) with lower yet elevated levels within the "wet" sand from the inter-tidal zone (>22 ± 10 CFU/g or >6.9 ± 3.3 x 10^3 CFU/100 ml pore water, combined "exposed" and "inundated" sand samples). Four out of eleven "dry" sand sampling events were above the detection limit. High tide "dry" sand results (>380 ± 230 CFU/g or 3.2 ± 2.9 x 10^6 CFU/100 ml) were higher than low tide "dry" results (>167 ± 95 CFU/g or 2.0 ± 1.6 x 10^6 CFU/100 ml). For the "wet" sands from the inter-tidal zone, similar counts were observed in the "exposed" sand (23 ± 14 CFU/g sand or 9.2 ± 6.9 x 10^3 CFU/100 ml pore water) relative to the "inundated" sand (22 ± 1.4 CFU/g sand or 5.6 ± 3.4 x 10^3 CFU/100 ml pore water).

Antecedent wet conditions showed the highest levels in "dry" sand samples. The first antecedent wet condition sampling (low tide during the summer) contained results with above detection limits, and the concentrations in "dry" sand were at >1,090 CFU/g sand or >1.5 x 10^6 CFU/100 ml pore water (Table 2.1). Two of the three "dry" samples

were above the detection limit (>2,580 and >662 CFU/g sand or >4.1 and 0.47 x 10^6 CFU/100 ml pore water), while the third sample measured at 34 CFU/g sand or 4.4 x 10^4 CFU/100 ml pore water. "Exposed" wet sand samples collected during the first antecedent wet conditions had one sample above detection limit (>244 CFU/g sand or >1.0 x 10^5 CFU/100 ml pore water), and the other two samples were also above but lower (118 and 110 CFU/g sand or 6.4 and 4.0 x 10^4 CFU/100 ml pore). The second set of sand samples collected during antecedent wet conditions (same day as the first at high tide) had higher concentrations in the "dry" sand (49 CFU/g sand or 4.5 x 10^6 CFU/100 ml pore water) than the "inundated" sand (11 CFU/g sand or 2.6 x 10^3 CFU/100ml pore water) (Table 2.1). The sand collected during the third event characterized by antecedent wet conditions (winter during high tide) also had higher levels of enterococci in "dry" sand (661 CFU/g sand or 9.4 x 10^5 CFU/100 ml pore water) than "inundated" sand (4.8 CFU/g sand or 1.7 x 103 CFU/100 ml pore water). Overall, sand from the first event (the event characterized by 3.1 cm of rainfall during the prior six hours) had the highest levels of enterococci.

The sand collected during the one sampling event characterized by high bather loads showed enterococci levels in "dry" sand samples at 3.6 x 10^3 CFU/g sand or 6.9 x 10^6 CFU/100 ml pore water (Table 2.1). The enterococci levels measured in the "inundated" sand during this event were lower at 20 CFU/g sand or 1.0 x 10^4 CFU/100 ml pore water (Table 2.1).

2.3.2 Hourly Sampling. Enterococci varied throughout the hourly sampling effort with levels in the water ranging by at least three orders of magnitude, from below detection

limits (<10 MPN/100 ml) to 5,790 MPN/100 ml with an average of 610 MPN/100ml (Figure 2.2). No rainfall was recorded during the first 11 hours of monitoring. At the beginning of this period, which was initiated at 10:30 am (Eastern Daylight Time, EDT) in the morning, the site was dry (zero antecedent rainfall within the previous six hours and 1.04 cm of rainfall within the previous 24 hours). In addition, the sampling during the first two strong daylight hours showed a decrease in enterococci concentrations. The enterococci levels during the intense daylight hours (defined as 10:00 am to 4:00 pm (EDT)) were generally low (avg. 30 MPN/100 ml).

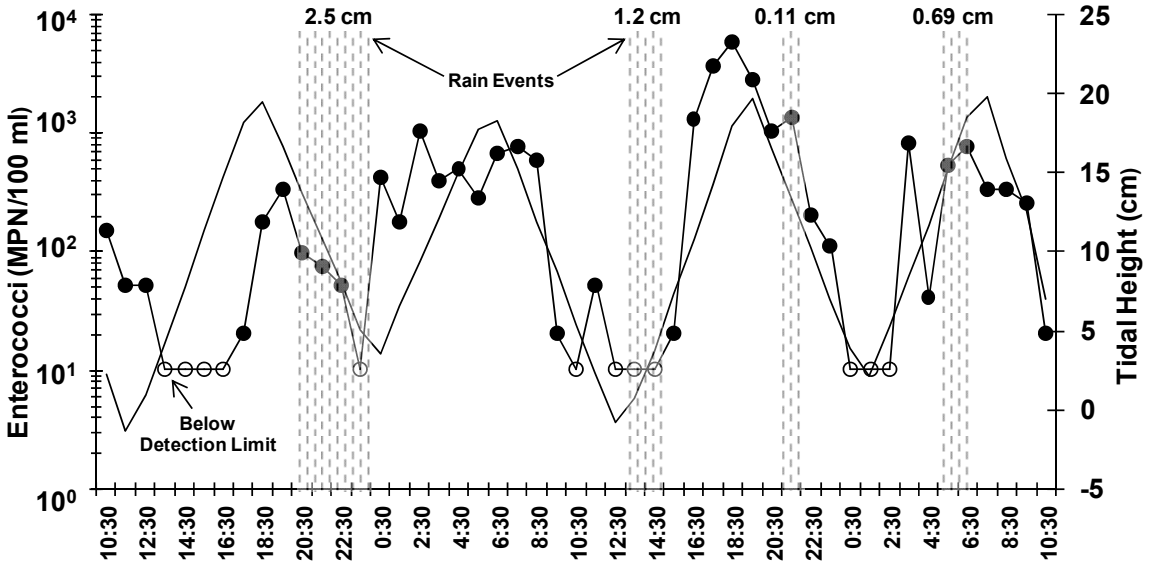


Figure 2.2: Hourly Sampling: Enterococci levels from water samples collected hourly over 48 hours. Tidal height is superimposed on the plots and rain events are highlighted in shading that represents the duration of the rain event along with the amount of rain as indicated by the number above the shaded region. Samples which measured at below detection limits are plotted at the detection limit and are shown by open symbols.

After 11 hours of monitoring, the first of four different rain events occurred during the sampling effort. These rain events included: 2.5 cm from hour 9:30 to 11:30 pm; 1.22 cm from hour 1:45 to 2:30 pm; 0.114 cm at 10:00 pm; and 0.686 cm from 5:45 to 6:45 am. Throughout the hourly sampling event, enterococci levels were generally

observed to increase during or immediately after the rainfall events. This was especially apparent for the first, third and fourth events. A two hour delay in the enterococci increase was observed for the second event (1.2 cm). This rainfall event occurred during a cycle characterized by a very low tide.

Enterococci concentrations in the sand fluctuated from below detection limit to 2.5×10^6 MPN/g (4.8×10^8 MPN/100 ml pore water) (Figure 2.3). The overall average for the duration of the 48 hours was 2.8×10^5 MPN/g (5.5×10^7 MPN/100 ml pore water). Sand remained "inundated" during the duration of the sampling except during low tidal periods (tidal height < 0.76 cm) and averaged $1.3 \pm 0.7 \times 10^5$ MPN/g while the "exposed" sand averaged $3.0 \pm 1.2 \times 10^5$ MPN/g. Although the average value for the "exposed" sand was higher than the average value for the "inundated" sand, the difference was not significant at a level of 95% confidence. However, these differences are significant at the 90% confidence level. When comparing the pore water concentration for the sand with the water column concentrations, the sand samples (5.5×10^7 MPN/100 ml pore water) were on average five orders of magnitude higher than the concentrations observed in the water (610 MPN/100 ml).

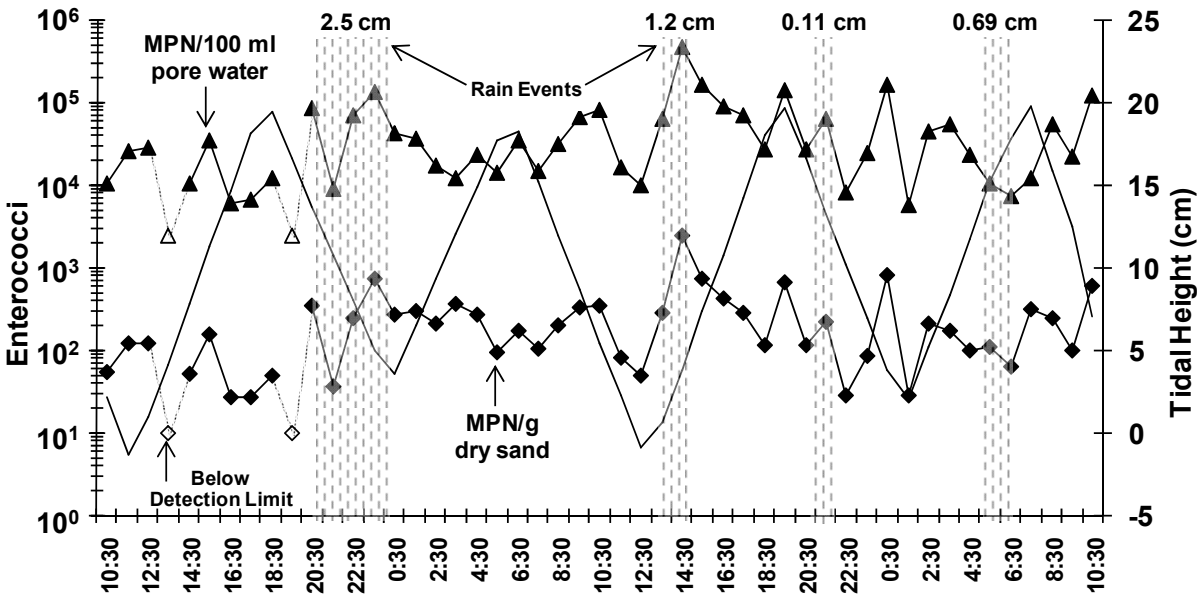


Figure 2.3: Hourly Sampling: Enterococci levels from sand samples collected hourly (units in terms of grams and pore water volume in the denominator). Tidal height is shown concurrently. Rain events as shown by shaded regions represent the length of the rain event along with the amount of rain as indicated by the numbers located over the shaded region. Samples which measured at below detection limits are plotted at the detection limit and are shown by open symbols.

2.3.3 10-Minute Sampling. Distinct differences were observed between the three, 10-minute sampling efforts. The overall average enterococci concentration during the first effort in the summer was 140 ± 4.8 CFU/100 ml. In the subsequent two efforts, which occurred on the same day during the winter, the average enterococci concentration was measured at 12 ± 1.7 and 23 ± 3.8 CFU/100 ml, respectively. The first sampling effort showed a distinct increase in enterococci concentrations with incoming tide, followed by a steady decrease with outgoing tide. For the first effort, enterococci concentrations were 90 ± 4.0 CFU/100 ml before high tide; concentrations measured above 500 CFU/100 ml for a time period of 45 minutes after the high tide. The following hour after high tide, concentrations remained elevated (203 ± 11 CFU/100 ml), and then decreased to an

average of 56 ± 6.9 CFU/100 ml. The results from this day showed a correlation with tide (Figure 2.4).

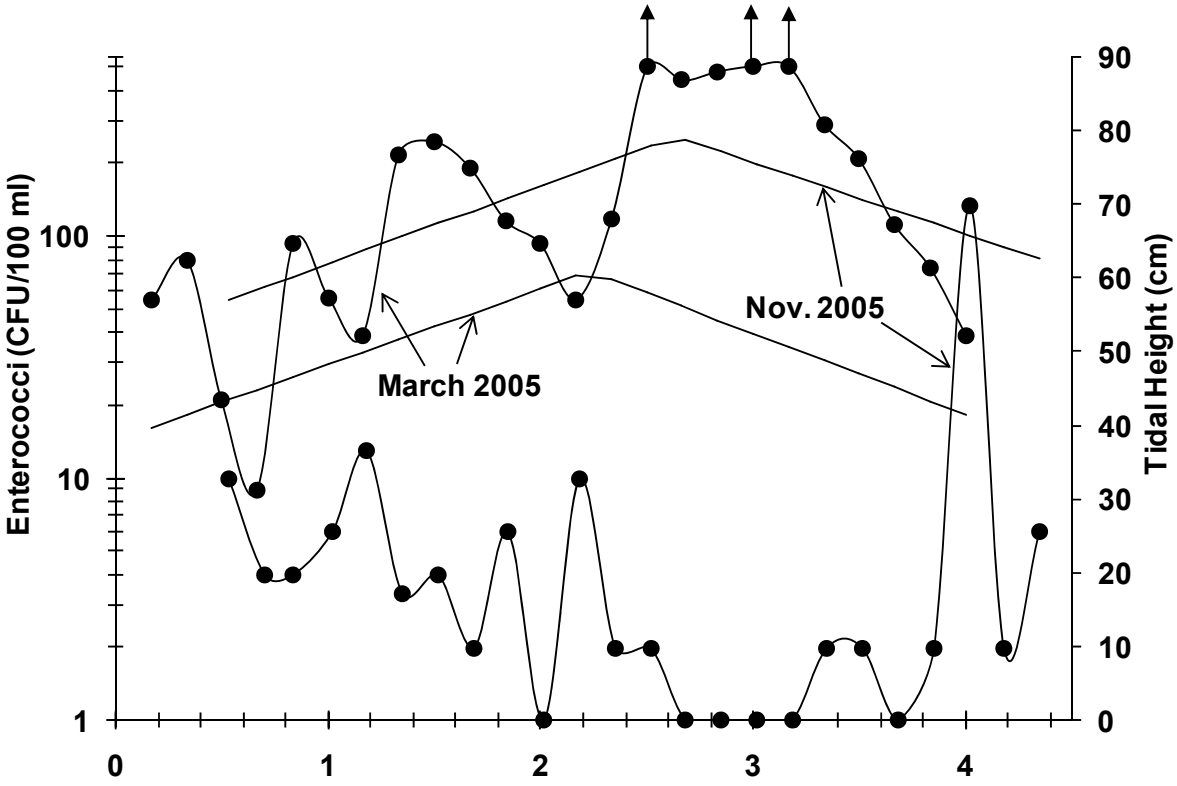


Figure 2.4: 10-Minute Sampling: Enterococci results from water samples taken during the 10-minute sampling efforts in March and November. Tidal cycle results are superimposed for those sampling events. Data points that are overlaid by an arrow going upwards indicate that samples were above detection limits.

However, a subsequent attempt to replicate these results did not demonstrate such significant variation in enterococci concentrations. During the second sampling date in the winter (for "knee" samples which followed the tide and for samples collected at one stationary location), enterococci levels remained low (4.8 ± 0.95 CFU/100 ml), with the exception of one elevated sample (134 ± 8.4 CFU/100 ml) which occurred 1 hour 23 minutes after high tide, at the very end of the sampling effort. Levels then immediately dropped to 4 ± 2.3 CFU/100 ml. The samples collected at the stationary pole showed

steady results (7.4 ± 0.97 CFU/100 ml) prior to the peak, and (291 ± 8.7 CFU/100 ml) 1 hour 48 minutes after the high tide.

2.3.4 Runoff Sampling. The overall combined results including all samples showed an average at >15,100 CFU/100 ml with a range from 690 to >32,600 CFU/100 ml. Of the nine runoff events (each with multiple samples ranging from n=1 to n=7) there were four events, which contained samples above detection limits (avg. >19,200 CFU/100 ml). The average enterococci concentration for the five events with countable colonies was 11,700 CFU/100 ml and >9,930 CFU/100 ml when all samples (including above detection limits) were averaged. The runoff samples collected during the hourly sampling event in July showed an average enterococci concentration of >22,500 ± 5,460 MPN/100 ml. Hourly runoff samples were two orders of magnitude higher than knee deep samples (avg. 204 MPN/100 ml) collecting during rain events of the hourly sampling. The runoff samples collected in August were measured at 14,000 ± 5,350 CFU/100 ml, while the winter results were more variable. The results for December were 690 CFU/100 ml and the February results were above detection limits at >1,000 CFU/100 ml. Overall, summer samples including above detection limit samples (>19,100 CFU/100 ml) demonstrated higher concentrations than winter (845 CFU/100 ml).

2.3.5 Spatially Intensive Sediment Sampling. Enterococci concentrations in the sand were the highest just above the shoreline in the dry sand (backshore sand area). This location showed on average 53 CFU/g sand (130,000 CFU/100 ml pore water). The next area that showed elevated levels was within the inter-tidal zone and averaged 34 ± 4.8

CFU/g sand (46,000 ± 270 CFU/100 ml pore water). Enterococci concentrations decreased with distance from this zone of elevated enterococci levels, in both seaward and landward directions (Figure 2.5). The seaward average results (2.8 ± 1.5 CFU/g sand or 570 ± 25 CFU/100 ml pore water) were not significantly different then the landward sand results (6.5 ± 1.8 CFU/g sand or 12,000 ± 130 CFU/100 ml pore water).

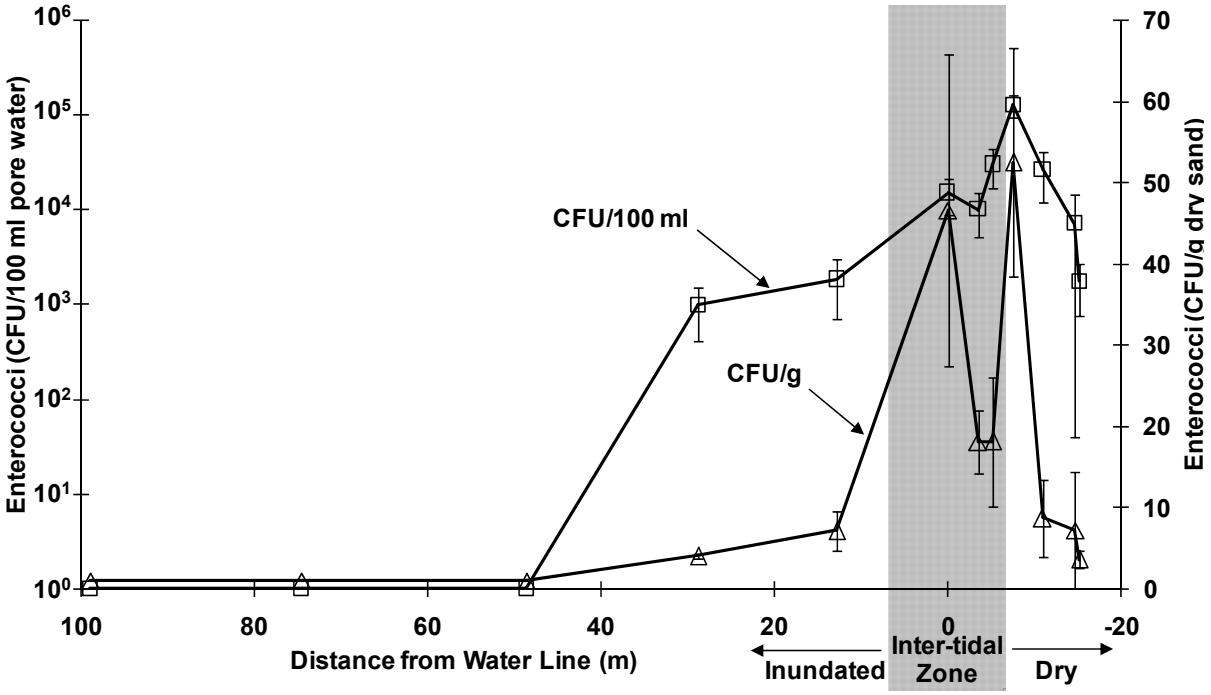


Figure 2.5: Spatially Intensive Sediment Sampling: Enterococci levels in water and sand samples. Shaded area represents the inter-tidal zone. The area to the right corresponds to the backshore zone or the location of the dry sand. The area to the left corresponds to permanently inundated sediments.

2.4 Discussion

In the first study, environmental factors played a significant role in changing enterococci concentrations at the beach. Antecedent rainfall was a dominating factor increasing bacteria levels found in the water column. This was evident from the tidal studies, hourly sampling, and runoff sampling events with the immediate impacts to the

water column following rain events. Elevated levels of enterococci (>500 CFU/100 ml) were observed during the tidal studies when rain occurred during the prior six hours. Such elevated levels were not observed for other events characterized by rain during the prior 24 hours, and no rain during the prior six hours. Apparently, the impacts from rainfall events were relatively short-lived and likely lasted six hours or less for this particular site.

The effects of rainfall were also supported from the results of the hourly sampling effort which showed increases in enterococci concentrations to values of 10^4 MPN/100 ml immediately after the two largest storms. The most likely source of enterococci during storms was runoff. Runoff contained greater than three orders of magnitude higher levels of enterococci (>25,300 CFU/100 ml) in comparison to measurements within the water column (47 CFU/100 ml) (all combined "knee" averages from tidal studies). The influence of rainfall and subsequent runoff has been frequently observed in other studies (Roll and Fujioka 1997; Haack et al. 2003). Beversdorf et al. (2007) indicated that *E. coli* levels in the water column increased by 100-fold after rain events.

In addition to rainfall, tides were also observed to significantly impact enterococci levels. These impacts were observed from the tidal studies, hourly sampling, and 10-minute sampling efforts. During the tidal studies "knee" water samples (71 ± 48 CFU/100 ml) collected during high tide were higher than "knee" samples (4.3 ± 3.5 CFU/100 ml) collected during low tide. Hourly sampling also showed a strong tidal influence on the enterococci concentrations in water samples since levels at high tide were elevated and remained elevated for up to one hour afterwards, avg. 1,700 CFU/100 ml (ranging from 173 to 5,800 CFU/100 ml). During low tide, results were <10 CFU/100 ml, although one

sample showed 52 CFU/100 ml. Results from the 10-minute sampling effort showed variable results. For one event enterococci concentrations were elevated during high tide and continued to >500 CFU/100 ml for up to one hour after high tide. Levels were significantly lower before and after high tide showing the tidal influence. When the experiment was replicated, the results did not show a tidal influence. Different responses during repeated sampling events suggest that the tidal signal was intermittent and dependent upon additional environmental factors.

Intermittent signals are commonly observed at other beaches (Alm et al. 2006; Boehm and Weisberg 2005; Boehm et al. 2002). Haugland et al. (2005) indicates that these intermittent signals area due to high levels of spatial and temporal variability. For Hobie Cat Beach, we suspect that the intermittent signal may have been due to periodic flushing of sand from the beach. During the first effort, it was possible that the source of enterococci was from the upper edges of the inter-tidal zone where the enterococci may have been thriving. Between the first and second 10-minute sampling effort, there were several large storm events (including two hurricanes) which likely eroded beach sands containing elevated microbe levels. With the erosion of the sand, the microbes within the beach sands were lost from the upper edges of the beach, thereby resulting in the loss of the tidal signal.

Another environmental factor could have accounted for the decrease in enterococci concentrations: possible sunlight impacts. Sunlight may influence the enterococci in the water samples. Liu et al. (2006) found that light-dependent inactivation was apparent when modeling. Hourly sampling results showed that samples during noon on both days were below detection limits (<10 CFU/100 ml). Hourly sampling suggests

that perhaps sunlight decreases the enterococci levels in the water, this occurred during the first two cycles of sunlight hours. This could indicate the possibility of a sunlight effect which should be further evaluated.

There was a slight seasonal variation in the enterococci concentrations observed in the water samples from the tidal studies, with slightly higher levels observed during the summer versus the winter. There was no significant difference seasonally for enterococci levels in the sand samples from the tidal studies. Seasonal variation needs to be further evaluated as there were only two sampling events that occurred during the winter which did not allow for an appropriated comparison.

Several components of the current study indicate that the inter-tidal zone was the geographic location for the source of enterococci within the study site. Ferguson et al. (2005) also identified the inter-tidal zone as the geographic location of elevated levels of enterococci in water and sediments, and identified re-suspension as the mechanism of transferring microbes from the sediments to the water column. The tidal studies presented heredemonstrated that the levels of enterococci were elevated within the inter-tidal zone, consistently showing a decrease in enterococci concentrations as they were collected further away from the shoreline. Sand samples evaluated during the tidal studies also showed elevated levels within the inter-tidal zone with an average of 22 CFU/g; no significant difference was observed between the "exposed" and "inundated" samples ($p>0.05$). Results from the spatially intensive sediment sampling also showed increased enterococci concentrations within the inter-tidal zone, where the concentrations at the "knee" location were observed at 47 CFU/g, while the concentrations at the "middle" and "buoy" locations were much lower (<1 CFU/g).

In addition to observing elevated enterococci levels in the sands from the inter-tidal zone, even higher levels of indicator bacteria were observed in the backshore "dry" sand samples (>302 CFU/g) within a couple of meters of the seaweed or wrack line. The significance of this area as a potential source of enterococci was further supported from the spatially intensive sediment sampling which again showed that elevated concentrations of enterococci were found in the inter-tidal zone with the highest located just above the shoreline. Bonilla et al. (2007) also showed demonstrated highest bacteria levels at the same study site within the upper beach sand. The high, yet variable results, of the dry sand can be attributed to the infrequent wetting of the area near the water. The lack of continuous or frequent inundation limits the degree to which inputs from humans, animals, or perhaps from regrowth, are distributed within this area, resulting in "patchiness" in the elevated levels of enterococci observed in this region.

Beversdorf et al. (2006) also indicated that the moisture content of the sand may affect the persistence of the bacteria, and that bacteria may be able to replicate and possibility colonize within the beach sand. Solo-Gabriele et al. (2000) suggested that the intermittent wetting and drying plays a role in this persistence and regrowth potential of indicator bacteria with indicator bacteria multiplying during the drying cycle. The sands within the inter-tidal zone are regularly wetted and dried in 12 hour cycles due to tidal action. However, at the study site, the sands in the backshore just above the wrack line experience less frequent wetting (only during wave action from a passing boat or during extreme tide events (e.g. spring tide)), and it is possible that the lower moisture content within this area that is less frequently wetted was ideal for enterococci persistence, resulting in the highest levels of enterococci observed within the "dry" sand.

When considering the sand results in terms of its pore water concentrations, the levels of indicator microbes in sand on a pore water basis were orders of magnitude higher than the levels observed in the water column. This difference was observed during the tidal studies from the inter-tidal zone (>6.9 x 10^3 CFU/100 ml pore water in sand versus 47 CFU/100 ml in the water, on average) and during the hourly sampling effort (5.5 x 10^7 CFU/100 ml pore water in sand versus 610 MPN/100 ml for water). The spatially intensive sediment sampling (4.6 x 10^4 CFU/100 ml pore water) showed higher enterococci concentrations by two to three orders of magnitude when compared to water from the tidal studies (avg. 47 CFU/100 ml). Bonilla et al. (2006) showed similar results where wet sand contained 10 times more bacteria than water and dry sand was 100 times greater.

The extremely high concentrations observed in the sand pore waters suggests that the pore water was the primary reservoir of enterococci, and that enterococci can then be released from this reservoir through wash-off via runoff events or through tidal action. Wash-off implies the wash-in of the pore water and not necessarily the wash-in of the sand grains. The sand grains (along with its corresponding pore water) serves as the matrix within which enterococci reside, but the sand grains themselves may not necessarily serve as the means of transport, as re-suspension of sand grains cannot account for the elevated levels observed in the water column as the suspended solids concentrations are not high enough.

The significance of the inter-tidal zone as the pathway for enterococci inputs to the shore was on occasion confounded by the combined effects of rainfall and tidal action. During the hourly sampling, the enterococci signal did not always respond

immediately to the rainfall event. This was especially evident when the rainfall events occurred during low tide (an hour delay for the 2.5 cm storm event and a two hour delay for the 1.2 cm storm event). During low tide, the runoff from the rainfall ditches could possibly have been contained, and was therefore not able to flow towards the ocean because of the larger horizontal distance to the ocean. As the tide rose (after a period of one to two hours), the shoreline approaches the runoff ditches, and the runoff from the ditches could have then been able to reach the shoreline water which then may have resulted in a very rapid increase in enterococci levels due to the combined effects from runoff and increasing tides. Overall, the results suggest that the inter-tidal zone itself seemed to be the main source of enterococci, which was further magnified by the effect of runoff. However, the effects of rainfall tended to be much stronger than the effects of tides, since as noted above, runoff exacerbated the wash-in process of the bacteria from the inter-tidal zone as well as carrying bacteria from other sources.

As shown, enterococci may have been significantly influenced by environmental conditions such as rainfall, tide, and sunlight intensity. The different studies conducted here all suggest that despite the environmental conditions, the geographic source of enterococci was the inter-tidal zone. Through tidal action and runoff, enterococci were washed into the water column. This has significant health and regulatory implications. Since enterococci, the indicator microbe studied, may be impacted by environmental conditions and uses the inter-tidal zone as a location for re-growth and entrance into the beach water, it may no longer be an adequate indicator of the presence of human fecal pollution, and hence pathogens and risk to human health. Further studies need to be

completed to determine whether pathogens are also affected by similar conditions, and whether these indicators are adequately serving as surrogates of pathogens.

The ultimate source of the elevated levels of enterococci found in both the sand and water near the shoreline can possibly be attributed to the activities that impact this location of the beach, i.e. presence of human and animal activity. The high bather load sampling from the tidal studies showed levels >210 CFU/100 ml at the "knee" water location. In addition, the "dry" sand samples were the highest (3.6×10^3 CFU/g or 6.9×10^6 CFU/100 ml) during the high bather load event than any other "dry" sand collected during the tidal studies. As mentioned earlier, humans, dogs, and birds are common at the beach. Wright et al. (2008) estimates that dogs are the major contributor with a daily enterococci load of 1.2×10^{11} CFU during weekends and 3.6×10^{10} CFU during weekdays. Further research to determine the sources of enterococci within the inter-tidal zone should also be conducted. In addition, rain events impacted water concentrations of enterococci, therefore further research to determine the rain water runoff impacts and the release of enterococci from the pore waters in sand which are washed into the water should be performed.

Chapter 3: SOURCES

3.1 Background

Marine beach advisories are recommended by the United States Environmental Protection Agency (EPA) when enterococci levels exceed 35 colony forming units (CFU) per 100 ml of water (geometric mean from a minimum of five samples over a 30 day period), or when enterococci levels exceed 104 CFU per 100 ml for a single sample (U.S. EPA 1986). These criteria can be exceeded through either point sources of sewage (e.g. ocean outfalls from a wastewater treatment plant) or from non-point sources of enterococci. Non-point sources are generally distributed throughout the contributing watershed, and, in many cases, enter the water column through the shoreline via direct or indirect fecal inputs through wash-in via runoff and tidal action. For beaches which are impaired, strategies to improve water quality should be based upon an evaluation of the microbial loads for the particular beach. For beaches impacted by non-point sources, quantification of the microbial loads is difficult due the excessive variety of possible sources, and the fact that the contributions from these sources can vary dramatically in time and space.

Humans and other animals have been identified as possible non-point sources of enterococci to recreational beach waters. Previous studies (Robinton and Mood, 1966; Hanes and Fossa, 1970; Smith and Dufour, 1993) concluded that high bacterial densities were shed by bathers into the water column. Gerba (2000) reported that the amount of fecal material shed per bather was estimated at 0.14 g. Elmir et al. (2007), during a study conducted at the study site in Miami (FL), demonstrated that adult humans shed on the

order of 5.8 x 10^5 enterococci from their skin into marine water per 15 minute bathing period.

Animal sources can come from animal pasture runoff, wildlife, waterfowl, and domestic animals. For example, farm run-off water contained enterococci in 100% of the samples collected in four European countries (Kühn et al. 2003). Meals and Braun (2006) reported concentrations of *E. coli* from dairy manure runoff to be within 10^4 to 10^6 organisms per 100 ml. These findings indicate a potential bacterial influence from these types of runoff into water bodies. Additionally, wildlife contributes to the degradation of water quality. For example, *Giardia* spp. was detected in beaver fecal samples from East Texas (Dunlap and Thies 2002). Mundt (1962) studied several mammals and reptiles that live in the wild, and found that 71.3% of the mammal fecal samples and 85.7% of the reptiles fecal samples tested positive for enterococci. Harwood et al. (1999) found that feces from Diamond Back turtles contained total and fecal coliforms in 100% and 80% of the samples collected, respectively.

Waterfowl (such as geese, ducks, seagulls and heron) are known to contribute microbial pathogens and fecal indicators. Graczyk et al. (1998) found that migratory Canada geese contribute *Cryptosporidium* sp. oocysts and *Giardia* cysts to surface waters. Several studies have reported an association between the presence of feces from shore birds and elevated numbers of fecal bacteria in water reservoirs, beach sediments and coastal waters (Jones et al. 1978; Lévesque et al. 1993, 2000; Fujioka 1988; Davies et al. 1995; Hatch 1996; Alderisio and DeLuca 1999; Jones and Obiri-Danso 1999; Obiri-Danso and Jones 2000). Recent studies have indicated that bird feces may be a primary cause for elevated fecal indicator levels, specifically within some recreational waters

(Boehm et al. 2005; Haack et al. 2003; Wither et al. 2005) as well as beach sands which influence recreational waters (Bonilla et al. 2007). For example, Bonilla et al. (2007) indicated that a single gull dropping can influence a 3 m^2 area of shoreline sand. Calci et al. (1998) reported that seagull feces contributes up to $>10^4$ PFU/g of male-specific bacteriophage (MSB), a recommended fecal indicator (Havelaar et al. 1993; Stetler 1984). In addition, shrimp within the sea bed and their fecal pellets (Ziebis et al. 1996; Manning and Kumpf 1959) can represent an as yet-unexamined potential microbial contribution from their release into the water. In addition to examining shore birds, Calci et al. (1998) found male-specific bacteriophage (MSB) in fecal samples from domestic animals (cattle, chickens, dairy cows, dogs, goats, hogs, horses, and sheep). According to Calci et al. (1998) horses and hogs excreted the highest MSB, on order of 10^7 PFU/animal/day. Cox et al. (2005) studied fecal coliforms in Sydney (Australia) and measured concentrations of 1.8×10^5, 3.8×10^4, 7.1×10^6, and 3.1×10^7 CFU/g wet feces, for adult cattle, horses, pigs, and dogs, respectively. Dog feces deposited within decomposing marine vegetation were shown to have elevated enterococci concentrations within the wrack line on the beach shore (Martin and Gruber 2005).

Soils, beach sands, and runoff have also been documented as non-point sources of fecal indicators (Fujioka et al. 1999; Hardina and Fujioka 1991; Roll and Fujioka 1997; Whitman and Nevers 2003; Shibata et al. 2004); in particular, fine-grained sediments have been demonstrated as a reservoir for enterococci (Sherer et al. 1992; Howel et al. 1996; Durbin et al. 2005). The ultimate source of indicators to soils and sands can include natural regrowth of indicators (Desmarais et al. 2002), deposition of animal feces, and possibly microorganisms shed from humans (Papadakis et al. 1997; Elmir et al. 2007).

These indicators can be subsequently washed into a receiving water body through rainfall-runoff (Roll and Fujioka 1997, Haack et al. 2003: Beversdorf et al. 2007) and through tidal action (Solo-Gabriele et al. 2000; Boehm et al. 2002). As a result, many studies have found a correlation between fecal indicators and both rainfall (Haack et al. 2003) and tides (Martin and Gruber 2005). Wash-in, the entrainment of microbes from a surface into water that comes into contact with the surface, is the mechanism of enterococci transport; the soil and sands are the reservoir. The ultimate source of fecal indicators from these non-point sources (wash-in from soils and sands) can include fecal deposits, natural persistence and regrowth (Davies et al. 1995; Solo-Gabriele et al. 2000; Anderson et al. 2005; Alm et al 2006).

The current study specifically addressed the fecal deposit contribution in a subtropical recreational marine beach. These fecal deposits can be directly inputted within the water body; they can be transported to the water body through wash-in; or they can be incorporated into the soil and sand reservoir, and washed in through soil and sediment rinsing and re-suspension. The primary objective of this study was to quantify the direct enterococci inputs from non-point sources at a beach site. Direct inputs corresponded to the initial load from animal sources, not indirect loads through wash-in mechanisms. Specifically, input loads were evaluated by enumerating the animals (dogs, birds, and shrimp mounds) that frequented the site, and the measurement of feces from these animals for their enterococci load. For comparative purposes, the number of humans that frequented the beach was also enumerated. Human counts were combined with human enterococci shedding numbers from Elmir et al. (2007) at the same study site, to estimate the contribution from human swimming activities in comparison to that

from animal feces. This information was used to identify beach management strategies which would improve the microbial water quality for the study site.

3.2 Materials and Methods

3.2.1 Site Description. The study site was located at Hobie Cat Beach, within a subtropical climate characterized by an average ambient temperature of 24.8ºC (27.6°C during the summer months and 22.0°C during the winter) and annual average rainfall of 149 cm (total of 109 cm during the summer months and 39 cm during the winter months). The site is located on Virginia Key, a small island on the eastern edge of Biscayne Bay that is immediately east from the coast of Miami, Florida, U.S.A. (Figure 3.1). This site was chosen because 10 swimming advisory/warnings have been issued for the beach since the inception of the Florida Healthy Beaches Program in August 2000 (Fl. Dept. of Health, 2007), and previous research had shown elevated levels of enterococci (Shibata et al. 2004, Durbin et al. 2005). Hobie Cat Beach is also called Dog Beach or Hobie Beach, as it is the only beach within Miami-Dade County where people are allowed to bring their pets including dogs. In addition to dogs and humans, birds had been observed to gather near the shoreline, in particular during the early morning hours, and shrimp fecal mounds were readily observable in the water throughout the study site.

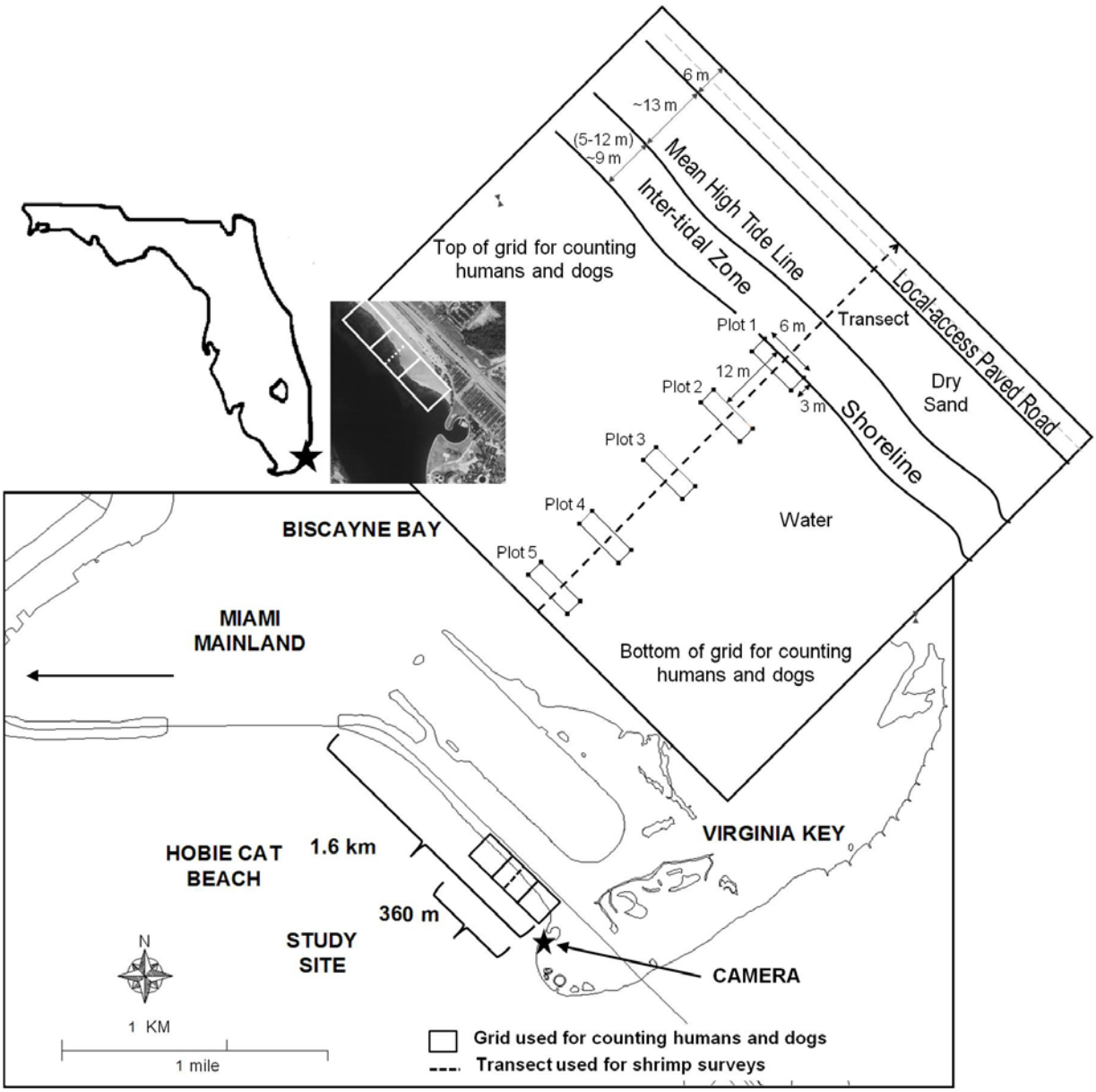


Figure 3.1: Hobie Cat Beach is located within Biscayne Bay on Virginia Key, east of Miami, Fl. The Study Site is identified as the eastern most 360 m of Hobie Cat Beach and the camera is further southeast. Top right shows the study transect and plots used for the in-field visual counting survey method for enumeration. The overlay shows where humans and dogs were counted both in the water and on the beach.

Hobie Cat Beach is approximately 1.6 km long; the focus area of this study was the eastern 360 m of the beach that faces towards the west, on the bay side of the island. The width of the beach was defined by a local-access paved road that runs parallel to the beach at a distance of approximately 13 meters from mean high tide. There were no storm

drains at the site and runoff from the paved road flows directly to the beach. The beach was characterized by relatively weak water circulation because the bottom slope is small and headline features are found at both ends. Movement of water near the shoreline was dominated by tidal action while wave action is mostly low energy due to prevailing offshore winds from the east. The average fluctuation in tidal height at the study site was 58 cm. The beach slope steepens significantly at the shoreline resulting in a relatively shallow slope (approximately 0.06 m/m) where this fluctuation in tidal height resulted in a 5 to 12 meter horizontal translation of the instantaneous distance between the respective shoreline between high and low tide. The sand area that received periodic wetting and drying during the tidal cycles is referred herein as the "inter-tidal zone."

Virginia Key, where the study site was located, is also home to a wastewater treatment plant. The outfall from this plant is located approximately 3.25 km further out in the Atlantic Ocean. Observations show, however, that remote sources, such as this outfall do not impact enterococci levels at the study site; prior studies have consistently shown a decreasing gradient offshore from the site with mostly below detection levels of enterococci in chest deep waters, whereas the shallow water near the shoreline is characterized by higher levels. In general, study data showed that the shallower the water, the higher the enterococci levels. Thus, a major source of enterococci to this site has been shown to be from the inter-tidal zone (or swash zone). Of note, sand at this site has been shown to have high levels of enterococci within the inter-tidal zone (39 ± 20 CFU/g dry sand) and within the dry zone (380 ± 200 CFU/g dry sand) located 1 m above the inter-tidal zone (Durbin et al. 2005). The cause of the elevated indicator levels in the sand is not yet fully understood. Suspected sources to the sand include direct and indirect animal

and human inputs and potential survival and regrowth of enterococci along the shoreline in the sand.

3.2.2 Sample Collection. Feces collection occurred over a period of 8 months between June 2005 and January 2006. Specifically, fecal samples from dogs, birds, and shrimp were analyzed for enterococci concentration and total fecal mass; the product of the two provided the total enterococci load per animal fecal event. During collection, all samples were aseptically transferred into pre-weighed Whirl-Pak® sampling bags or pre-sterilized tin containers using scoops or spoons. Sample collection occurred carefully to minimize the inclusion of non-fecal matter (such as sand, twigs and rocks). All samples were transported from their respective collection sites to the laboratory in an iced cooler and analyzed within 4 hours.

Prior to the enterococci enumeration, the total mass was determined for each sample, where the weight of the container was subtracted from the total mass of the container plus feces. After weighing, an aliquot of the fecal sample was used for enterococci enumeration, and a separate aliquot was used for water content analysis to allow reporting of enterococci numbers in units of colony forming units (CFU) per gram of dry feces.

Dog feces were collected by asking a dog owner: 1) if a fecal event occurred; 2) the time it occurred; and 3) the location of the event or by following the dog until a fecal event occurred. Once the fecal event was identified, the sample was then collected. Dogs were identified as either large (> 9 kg) or small (< 9 kg). Dog owners were asked if the dog had any medical condition or was currently taking any medications. No participant

dogs were known to have medical conditions, and none were taking medications. Information about the dog's age, weight and breed were also documented. A total of 9 samples were collected from the study site. In addition to the above mentioned samples, the total mass of a dog fecal event was measured for two dogs, one large (23 kg) and one small (3.2 kg), each of which were monitored for a week. During this week, each dog fecal event was collected and then weighed to obtain the total mass of feces produced per day by each dog.

Bird feces were collected from the beach, zoo, and a bird rehabilitation center within Miami-Dade County. The bird diets from the zoo and bird rehabilitation center were similar to the wild bird diets, i.e. fish but the birds from the zoo also had a grains in their diets. The sample collected at the beach was obtained by watching birds early in the morning at sunrise, and waiting for a fecal event to occur. Only one sample from an Ibis (*Eudocimus albus, E. ruber, Plegadis falcinellus, or P. chihi*) was collected after several attempts of sample collection over a span of two weeks. Due to this difficulty, sampling efforts were expanded to include birds from a local zoo and a local bird rehabilitation center. The native bird exhibit at the local zoo consisted of an artificial lake that included a small island located in the center of the lake and a concrete border that surrounded the lake. Samples were collected from a plastic tarp that was placed under a tree on the island. In the afternoon once the zoo closed to the public, the plastic tarp was sanitized, and the concrete border was repeatedly scrubbed and washed. The following morning six feces samples from birds known to congregate overnight in the tree on the island, Ibis and Heron (*Ardea herodias, Butorides striatus, Egretta caerulea, Egretta tricolor, Nycticorax nycticorax and N. violaceus*), were collected from the tarp. In the morning

three fecal samples from the previous night were collected from the concrete border corresponding to an Ibis, a Heron, and a duck (unidentified species). Throughout the following morning, four additional bird fecal samples were collected from the concrete border by observing the fecal event (Ibis and Coot (*Fulica americana*)) and immediately collecting the sample. For the bird rehabilitation center, fecal samples were collected from a) cages located indoors which housed Pelicans (*Pelicanus occidentalis and carolinensis*) (4 samples, n=4), b) a concrete pad located outdoors where Pelicans congregate (n=2), and c) dock facility where Gulls (*Larus atricilla and delawarensis)* and Pigeons (*Columba leucocephala*) frequent (n=6). Indoor cages were sanitized and lined with newspaper and a plastic mat prior to housing the birds. The concrete pad was washed with water prior to sample collection; the dock had no prior cleaning. A total of 26 bird fecal samples were collected and analyzed from the 3 study sites.

Ghost shrimp fecal pellets (sand mounds which contained shrimp (*Callianassa californiensis*; Murphy and Kremer 1992) were collected by scooping the sand directly into a Whirl-Pak® bag underwater to minimize the loss of fine sediments. Preliminary studies showed no statistical difference when only a portion of the mound (with fecal pellets) or entire mound was collected, resulting in the entire mound being used for analysis. Nine mounds were collected from the study site out to a distance of 50 m within the inter-tidal zone where colonies of shrimp live on the ocean floor. Mounds were easily identified above water due to the shallow and clear water; turbidity for the samples was approximately 3.6 Nephelometric Turbidity Units (NTU).

3.2.3 Laboratory Methods. Enterococci were extracted from the fecal samples using a modified version of the procedure outlined by Van Elsas and Smalla (1997). The method requires two basic steps. The first step was to measure the water content of the feces. Water content was determined by measuring the weight difference of feces before and after drying (110°C for 24 h). The mass of feces used for water content analysis was approximately 21 g for dog feces, 3.5 g for pelican feces, 0.7 g for all other bird feces, and 29 g for shrimp mounds. The second step required the extraction of enterococci from the feces to a predefined volume of sterile water. In order to accomplish this, approximately 2.0, 2.6 and 4.6 g for dogs, birds, and shrimp, respectively, of fresh, un-dried feces (wet fecal matter) were aseptically removed from the sampling bags and placed into sterile pre-weighed jars. Approximately 30 to 50 ml of sterile phosphate buffer dilution water (PBS) was then added to each jar. The jars were manually shaken for 30 seconds, and then placed in a sterile graduated cylinder and raised up to a volume of 100 ml with PBS. This solution was then analyzed for enterococci concentration using a standardized membrane filtration (MF) method (U.S. EPA 2002, Method 1600). In brief, the MF method was based upon a selective medium (mEI agar, Becton Dickinson, Sparks, MD) and incubation of filters at 41°C for 24 hours. All colonies that were blue or characterized by a blue halo were recorded as enterococci colonies.

3.2.4 Animal Enumeration. Two methods were used to enumerate the animals (humans, dogs, birds, and shrimp mounds) that frequent the study site during daylight hours, camera image analysis, and in-field visual counting surveys. The camera image

analysis was based upon the use of a digital camera (C-8080WZ, Olympus) with pan, tilt and zoom capabilities.

This camera was placed in an environmental housing and mounted near the top of a pole, approximately 3 m from the ground. The pole was located across the bay on private property, approximately 440 m from the study site (Figure 3.1). The camera took images of the beach area every 15 minutes and created a set of 3 images along the study site. The 3 images were subsequently assembled into one panorama for disk storage. Fifty images were analyzed over a period of 16 months (beginning April 2005 and ending July 2006) with the addition of 16 images analyzed during Labor Day weekend (September 2005).

The study camera database contained the following animal counts: people within the backshore zone of the beach; people in the water; small and large dogs in the backshore zone and in the water; and birds (Crows, Ibis, Gulls, Anhingas, Pelicans, Herons and Vultures). Direct counts were established for the study site (360 m eastern portion of the beach (6,380 m^2)), and these direct counts were scaled up to estimate the total numbers at Hobic Cat Beach (28,300 m^2 in area).

Two general approaches were used for in-field visual counting surveys. The first method was used to count humans and dogs, and the second method was used to count shrimp mounds. Humans and dogs were enumerated at one instant during the day using a grid system within the study site (Figure 3.1). Humans and dogs within each grid were surveyed and counted to find the total number of people both in the water and on the beach. The numbers from each of the three grids were added together giving the total numbers within the 6,380 square meter area of the three combined grids. Humans and

dogs were counted a total of 13 days during June 2004 and May 2005. All days were non-holiday weekday counts, except for Memorial Day (Monday) 2005.

The second in-field visual counting survey method, used for counting shrimp fecal mounds, was based upon establishing a set of five 6 m by 3 m plots which extended out from the shoreline (Figure 3.1). Shrimp mounds were then counted during high tide and during low tide within each plot during two consecutive days (two high tide counts and two low tide counts during the 2-day period). The entire procedure was conducted twice, once during the summer (August 2 and 3, 2006) and once during the winter (December 21 and 22, 2006).

3.3 Results

3.3.1 Enterococci. Highest enterococci concentrations on average were found in dog feces (7.4×10^6 CFU/g dry feces), then bird feces (3.3×10^5 CFU/g feces), with the lowest were in shrimp fecal mounds (2.0 CFU/g feces). All averages include only those samples that did not exceed the detection limit. The microbial load from dog feces, bird feces, and shrimp fecal mounds were measured at 3.2×10^9 CFU per dog event, 4.7×10^5 CFU per bird fecal event, and 10 CFU per shrimp fecal mound. These results indicated that one dog fecal event has the same enterococci loading as 6,940 bird fecal events and 3.2×10^8 shrimp fecal mounds.

Of the nine dog fecal samples collected, two were above the detection limit (>4.9×10^7 and >2.8×10^8 CFU/g dry feces). The range for the samples that were within detection limits was from 5.7×10^4 to 2.4×10^8 CFU/g dry feces. The median concentration (which included the samples that were above detection limits) was 1×10^7

CFU/g feces (Figure 3.2). The two samples that measured above the detection limit were observed to contain greater than 4.9 x 10^7 CFU/g feces. The average enterococci concentration for large dogs was 6.4 ± 0.38 x 10^7 CFU/g feces (n=4), while the average for small dogs was 5.9 ± 0.19 x 10^6 CFU/g feces (n=3). The mass of dog feces in terms of dry weight per event was 79 g for large dogs and 28 g for small dogs. Given these values, the microbial load was computed at 4.4 x 10^9 CFU/event for large dogs and 1.5 x 10^8 CFU/event for small dogs. For large and small dogs evaluated in this study, the computed microbial load was 3.2 x 10^9 CFU/event overall.

The results from the collection and weighing of dog feces over a period of a week showed fecal masses within the same order of magnitude. The total mass (grams of dry feces) produced per day from a large dog was 52 g while the small dog produced 7.6 g daily.

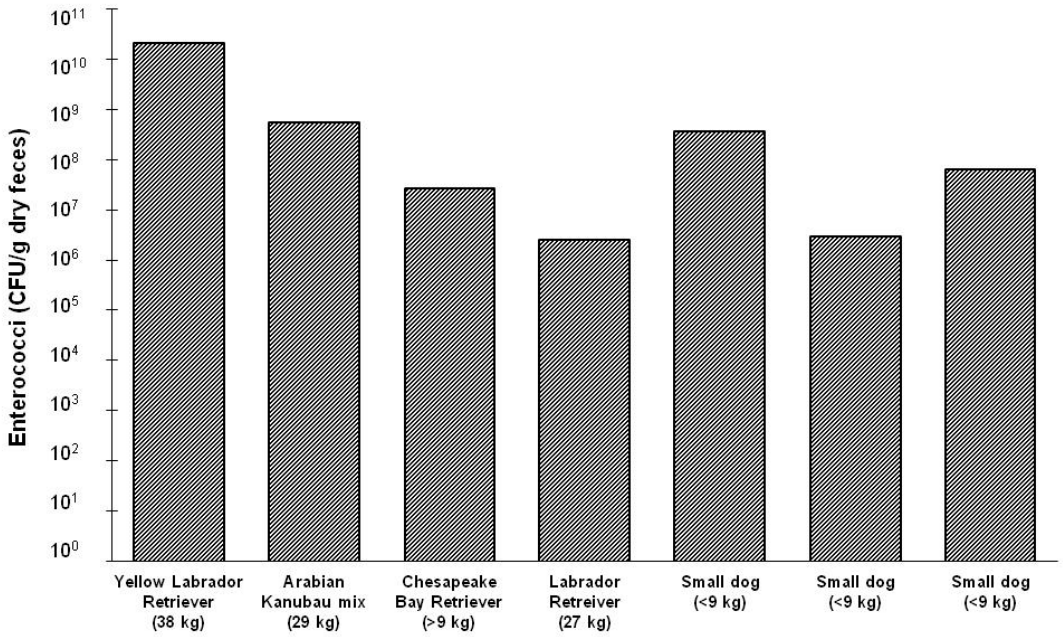


Figure 3.2: Enterococci concentrations (in units of CFU per gram of dry dog feces) for individual types of dogs along with the weight of each dog shown in parenthesis.

Bird feces showed variable concentrations of enterococci ranging from 347 to 3.1 x 10^6 CFU/g dry feces, with a median value of 3.3 x 10^5 CFU/g feces for the entire 26 sample data set. 26 samples were processed; eight were above the detection limit, which resulted in a total sample size of 18 within detection limits. Enterococci concentrations in bird group ranked from lowest to highest included Ibis, Gull or Pigeon, Coot, Duck, Heron, and Pelicans respectively (Figure 3.3). The combined average concentration for Ibis, Coot, Duck, Gull and Pigeons was 8.0 x 10^3 CFU/g feces; the combined average for Heron and Pelicans was 9.7 x 10^5 CFU/g feces. The average mass of a bird fecal event was 0.82 g with the smallest mass of 0.027 g for a Gull or Pigeon and the largest for a pelican (1.7 g). The smallest contribution in terms of microbial load was from a Gull or Pigeon (53 CFU/event), and the largest contribution was calculated from Pelicans (5.1 x 10^6 CFU/event). The average microbial load from all types of birds was 4.7 x 10^5 CFU/event.

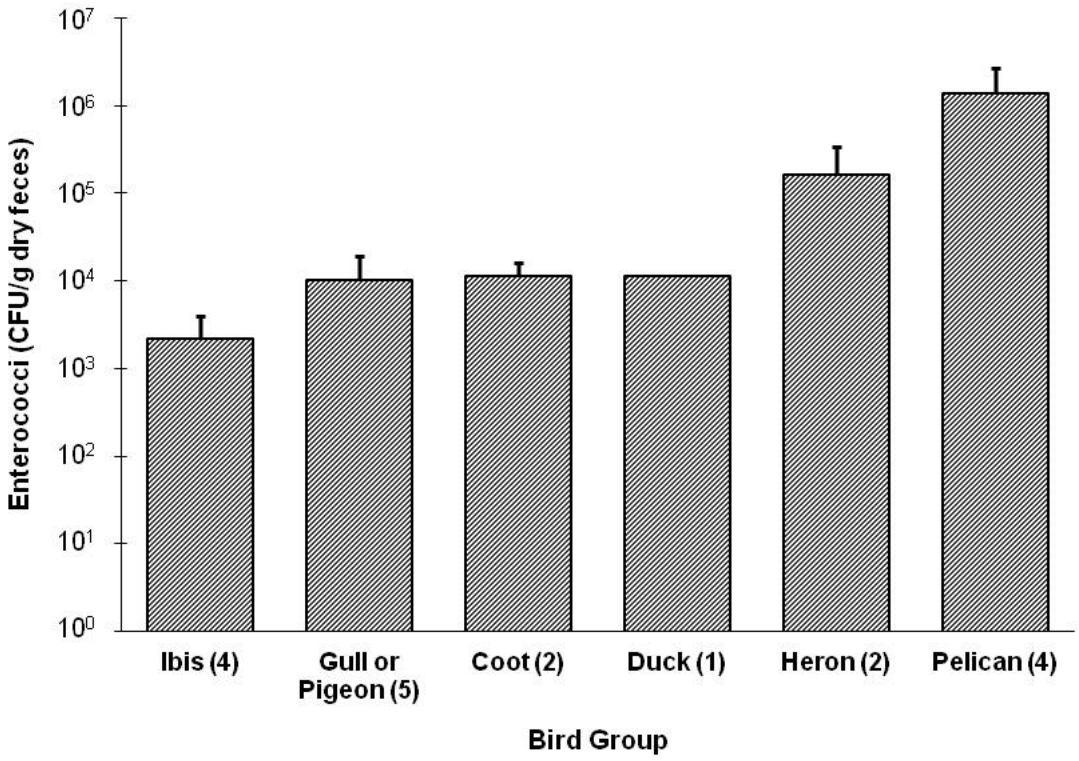


Figure 3.3: Enterococci concentrations (per gram of dry feces) from feces from different types of birds and the respective sample size in parenthesis. Error bars represent the standard deviation for the respective type of bird within that sample size.

Shrimp fecal mound concentrations were < 7.7 CFU/g feces, with a median value of 0.95 CFU/g feces and a minimum concentration of 0.65 CFU/g feces. The shrimp mounds contained fecal pellets mixed with sand. Durbin et al. (2005) measured the enterococci levels of sand within the inter-tidal zone sand at the study site as 39 ± 20 CFU/g dry sand, thus the material used by the shrimp in developing their mounds appeared to contain less enterococci than the surface sediment within the inter-tidal zone. The average mass of shrimp mound samples was 6.9 g, resulting in an average microbial load of 10 CFU per mound.

3.3.2 Animal Enumeration. The averages below correspond to the days non-zero counts were observed. Results for human counts (using both camera image analysis and

in-field counting surveys) included a non-holiday weekend, two holiday weekends, and several weekdays. Of the 52 camera images analyzed, nine days had zero observed humans, while the remaining 43 days contained counts for people. All (n=13) in-field counting surveys included counts for people.

3.3.2.1 Humans. Human enumeration results from both camera image analysis and in-field counting surveys demonstrated that the highest human count during the weekdays was 276 (avg. 55) and during the non-holiday weekend day was 351. The largest number of people was observed during holiday weekends (2,644 people maximum, with an average of 1,333). The maximum population of 2,644 people corresponded to a density of 1 person per 10 square meter of beach. The overall average number of people during weekends on the beach (considering both non-holiday and holiday weekends) was 1088. During the weekends, more people were observed along the shore out of the water (695 on average) compared to in the water (392 on average). For data collected during weekdays, the distribution between people who were in the water versus on dry land was available only from the camera image analysis. Camera images indicated that the average number of people at the beach during weekdays was 46 people, with 33 people on the shore and 13 people in the water. The number of people that typically frequented the beach on weekdays was determined to be within the range of 1 to 25 (Figure 3.4).

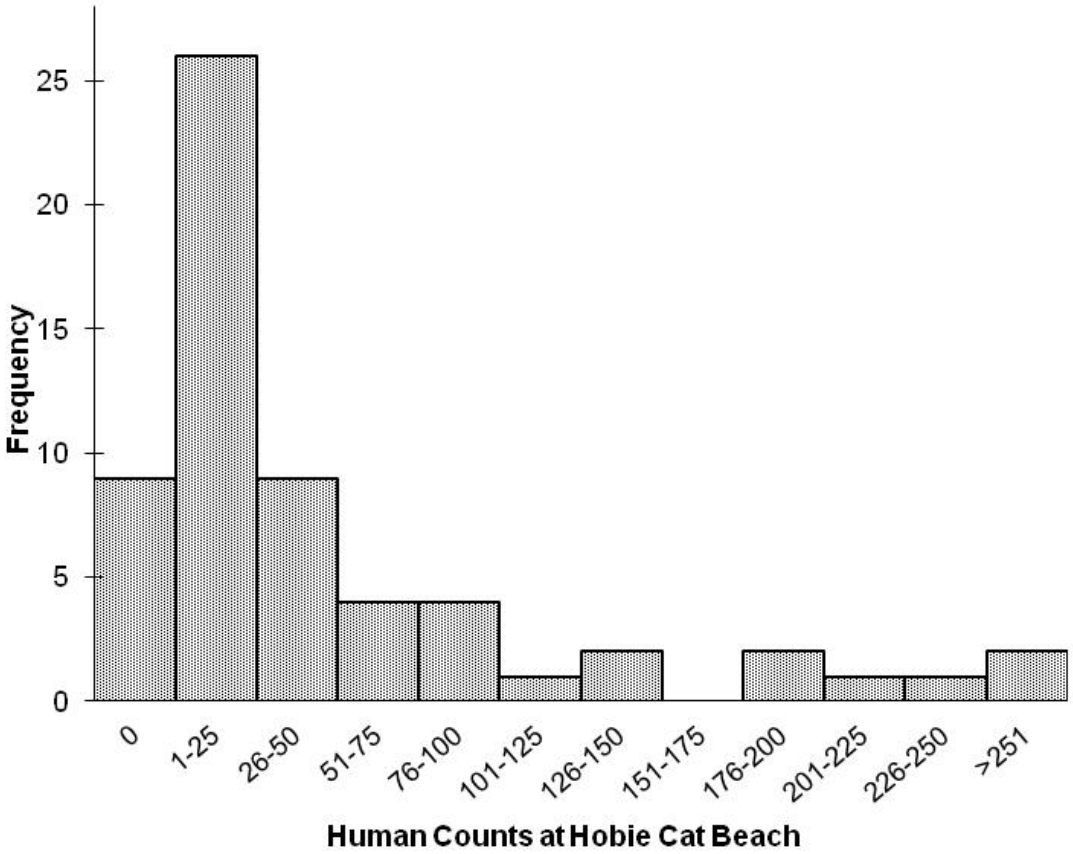


Figure 3.4: Enumeration of humans observed through the daylight hours during the weekdays using camera image analysis and counting survey. Maximum number was 276 people.

3.3.2.2 Dogs. Of the 65 days of data (from both camera image analysis and field counting surveys), 36 days corresponded to no dogs. The overall average number of dogs during weekdays was found to be 11 dogs, with the highest number at 89. During the one non-holiday weekend, 13 dogs were observed at the beach; during holiday weekends, the maximum number observed was 196, with an average of 83. For weekends as a whole (including non-holiday and holidays), 66 dogs were observed on average. The number of dogs observed during any random day of the week generally ranged from 0 to 10 (Figure 3.5).

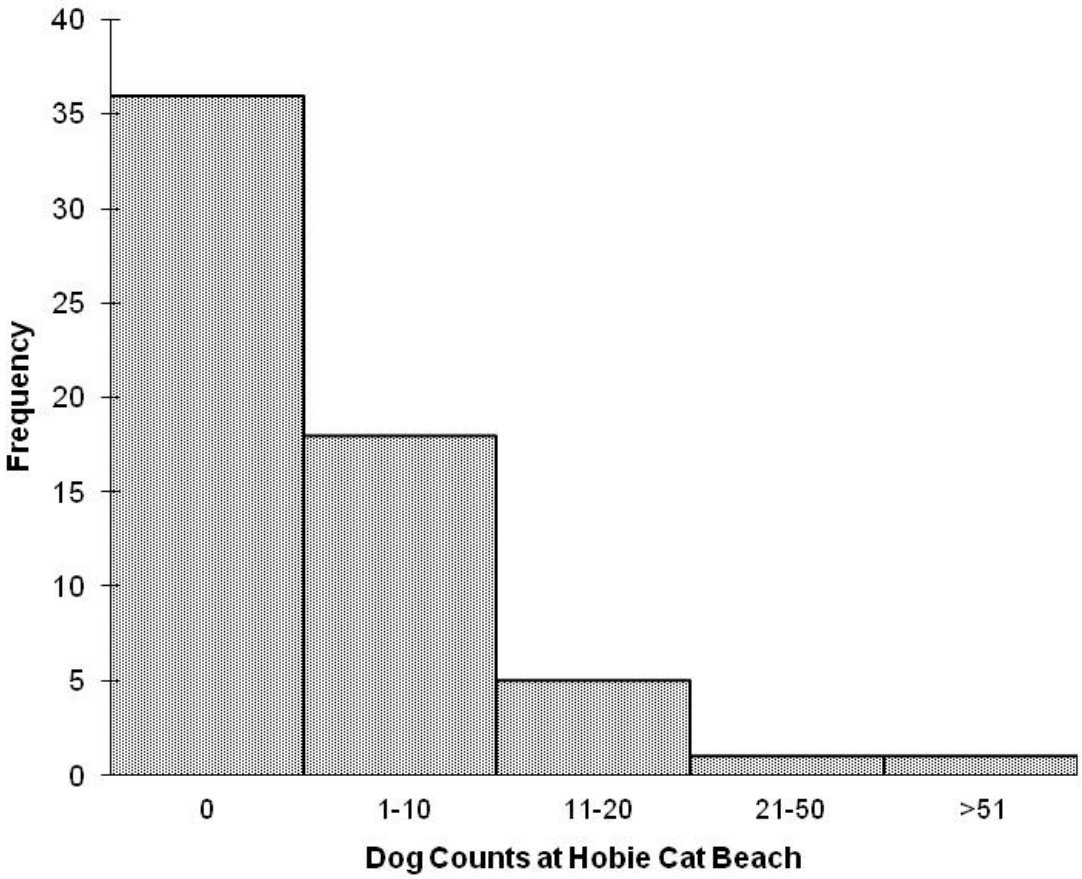


Figure 3.5: Enumeration of dogs through the daylight hours during the weekdays using camera image analysis and counting survey. Maximum number of dogs observed any weekday was 89.

Dog counts were separated by size for camera image analysis only. From the camera images, large dogs were observed to represent the majority of the population, with their proportion varying from 60% to 90%. The location of dogs was documented from the visual counting survey conducted during a holiday weekend. This survey showed roughly 57% of the dogs on shore, whereas the remaining dogs were in the water.

3.3.2.3 Birds. Thirty-three of the 53 days of camera images evaluated contained countable birds. No counts were taken visually in-field. The average number of birds (combined species) documented per camera image over the 53 days was 150, with a

maximum number of 587. The number of birds (when present) was typically in the 1 to 50 range (Figure 3.6). An additional peak of bird frequency was observed within the range of 301 to 350, suggesting that birds frequented the site predominantly as individuals and in flocks of about 300. Gulls were observed most frequently (31 days), and were characterized by the highest counts (maximum of 587); the average count was 151 per day during these 31 days. Pelicans and Anhinga (*Anhinga anhinga*) were seen four days, and averaged 22 and 4 per day, while Vultures were observed three days and averaged 12 per day when present. Herons were observed two days, and had an average count of 13. The bird species observed the least were Crows and Ibis which were documented only one time each, with counts of 89 and 9, respectively.

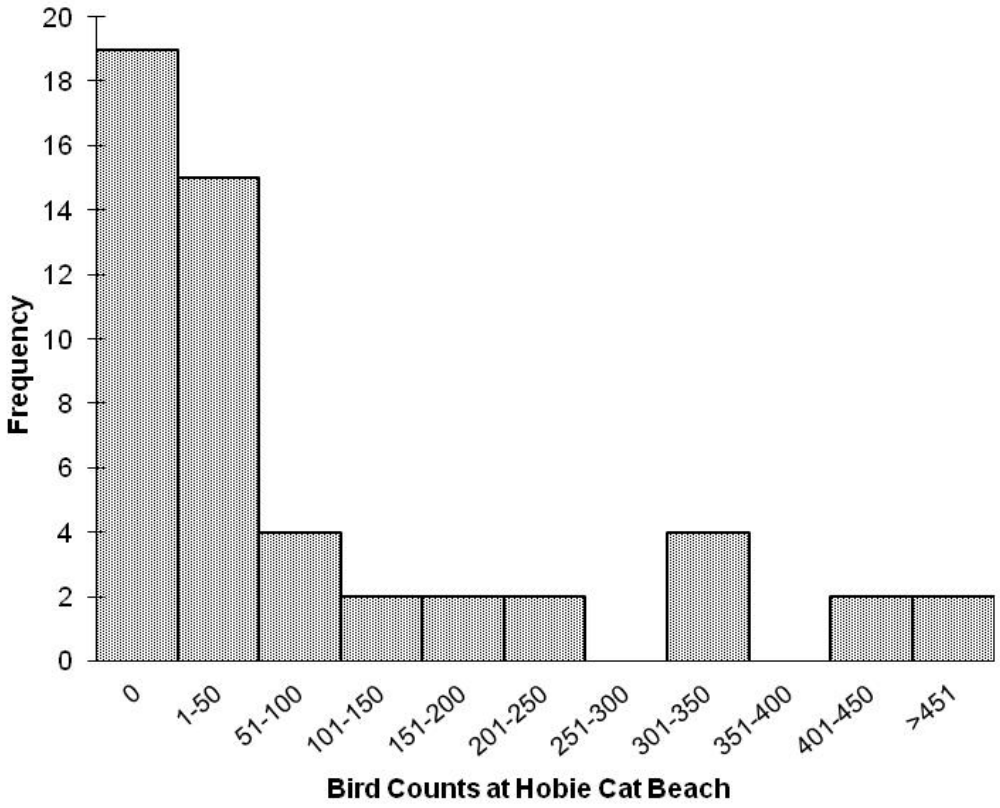


Figure 3.6: Enumeration of birds throughout the week using camera image analysis only. The highest number of birds observed was 587.

3.3.2.4 Shrimp. Shrimp fecal mounds, as estimated from in-field counting surveys, showed an overall average of 984 mounds with a median value of 919. The overall maximum count occurred during high tide (1,837 mounds). The average number of mounds observed during high tide was 1,181, while during low tide the average was 787 mounds. The maximum number of mounds observed during low tide was 1,312.

3.4 Discussion

3.4.1 Comparative Contributions. In order to compare contributions between non-point sources, the assumption was made that the presence of an animal represented a fecal event, meaning one dog had one fecal event while at the beach and one bird had one fecal event, etc. Humans were assumed to contribute enterococci three times per day from skin shedding directly into the water while bathing. This assumption was based upon an average duration at the beach of two to four hours, and 3 bathing events during this period.

In order to evaluate the worst possible situation, the highest numbers observed during the enumeration process for all animals (including humans) was combined with the mean microbial load to provide the potential contribution of enterococci to the beach from non-point sources evaluated as part of this study (Table 3.1). This worst case scenario suggested that during the weekend the enterococci contribution to the beach per day could be as high as 6.4×10^{11} CFU/day. Calculating an average (average enumeration numbers multiplied by the mean microbial load) for the weekend resulted in a mean weekend contribution of 2.1×10^{11} CFU/day. The highest weekday enterococci contribution (2.9×10^{11} CFU/day) and average weekday contribution (3.6×10^{10}

CFU/day) were between a factor of 3 to 10 times lower than the corresponding weekend values.

Table 3.1: Results for the enterococci load per event, animal enumeration, and total contribution for people, dogs, birds, and shrimp mounds. Maximum contributions per day correspond to the product of the maximum enumeration and the average value of the enterococci contribution per event.

Source		**Enterococci**	**Enumeration**		**Daily Enterococci Load (CFU/day)**	
Type	Day[a]	(CFU/event)	Maximum	Average	Maximum	Average
People	W	1.7×10^{6} [b]	2,644	1,088	4.6×10^{9}	1.9×10^{9}
	D		276	55	4.8×10^{8}	9.6×10^{7}
	A		2,644	129	2.5×10^{9}	9.9×10^{8}
Dogs	W	3.2×10^{9}	196	66	6.3×10^{11}	2.1×10^{11}
	D		89	11	2.9×10^{11}	3.6×10^{10}
	A		196	19	4.6×10^{11}	1.2×10^{11}
Birds	A	4.7×10^{5}	587	150	2.7×10^{8}	7.0×10^{7}
Shrimp	D	1.0×10^{1}	1,837	984	1.9×10^{4}	1.0×10^{4}

[a] W = Overall weekend (non-holiday weekend and holiday weekends), D = Weekday daylight hours, A= All 7 days of the week

[b] Refers to one person swimming 3 times during a beach visit

A human bather study conducted at this site (Elmir et al. 2007) showed that one bather contributed on order of 5.8×10^{5} CFU enterococci per 15-minute bathing event. The contribution of enterococci comes from the shedding from the bather's body into the water column. The results from this human bather study were used for comparison with the animal fecal contributions at the study site. This direct analysis and comparison provided an overall picture of combined nonpoint sources as they contributed enterococci to the beach waters. Under the assumption that one human was assumed to swim 3 times during each visit, resulting in a total contribution of 1.7×10^{6} CFU per visit or per event. Elmer et al. (2007) showed that after four 15 minute exposure events in the water, the enterococci contribution on average was 3×10^{5} CFU.

When comparing the amount of enterococci shed from humans to enterococci concentrations in animal feces, the results (CFU per event) indicated that one dog feces

equated to 1,872 people, 6,940 bird feces, and 3.2 x 10^8 shrimp fecal mounds. The enterococci contribution from dog feces far exceeded the contribution from human shedding or from bird feces. This observation was especially significant as people, birds, and shrimp substantially outnumbered the quantity of dogs. The enterococci contribution from dog feces was most significant because of the high concentration of enterococci in the feces coupled with the large mass, resulting in an exceedingly large contribution per dog event.

The microbial load for dog feces could be highly variable. The literature is very limited with respect to documenting the enterococci concentration associated with dog feces. Although data were available with respect to total bacterial and fecal coliform levels (Calci et al. 1998), and data existed concerning the characterization of enterococci isolates (Rodriques et al. 2002; Leener et al. 2005; De Graef et al. 2005; Delgado et al. 2007), only one such study had measured total enterococci counts for dog feces and reported in one sample 1.13 x 10^4 CFU/g feces in wet weight (Anderson et al. 1997). The US EPA (2001) documented that fecal coliform contributions from dogs and cats were 5 x 10^9 CFU/day. The current study found that enterococci concentrations from dogs can be variable, with an average of 7.4 x 10^6 CFU/g dry feces and an average daily load of 1.2 x 10^{11} CFU/day, which are two orders of magnitude higher than the estimated fecal coliform load from the US EPA (2001) study.

In the current study, the mass of dog feces used to estimate the total daily load corresponded to fecal masses that were collected and analyzed at the study site. This was an accurate assessment in terms of the contribution of enterococci from those dogs present during that sampling event. However, the mass of dog feces was subjective and

directly corresponded to the size of the dog, the dog's food consumption, and whether the fecal event occurred on the beach or not. The literature suggests that a large-sized dog (weights ranging from 19 to 32 kg) produces approximately 40 g per day of dry feces (range of 32 to 49 g) (Spears et al. 2004, Murray et al. 1997). Weekly weights combined with a National Research Council (NRC) formula (NRC 1985) showed that the fecal masses for large and small dogs were 52 g and 7.6 g, with a dry feces mass of 26 g (large dog) and 5.2 g (small dog). With this variation of dry mass weights, the actual microbial load is variable within an order of magnitude. Furthermore, the enumeration of dogs observed from the camera images may not be completely representative of the actual number of dogs that frequent Hobie Cat Beach. As this beach is the only beach within Miami-Dade County that allows animals, dogs frequent this beach all times of the day and throughout the year, both during daylight times and in the evenings after dusk. Therefore, the number of dogs that frequent the beach could be higher than the number observed exclusively during daylight hours; however, this uncertainty is much less than that associated with feces mass.

Enterococci concentrations in bird fecal samples from the current study (1.0×10^4 CFU/g dry feces for gulls or pigeon and 1.2×10^4 CFU/g dry feces for ducks) were conservative when compared to the literature. Oshiro and Fujioka (1995) found that pigeons contribute 4.0×10^5 CFU/g (unspecified whether dry or wet weight) feces; Roll and Fujioka (1997) reported 1.4×10^6 CFU/g dry feces for ducks. While Haack et al. (2003) indicated that bird enterococci concentrations were 1.4×10^7 CFU/g wet feces for gulls and 5.0×10^7 CFU/g wet feces for ducks. These values converted to enterococci per gram dry feces using the water contents measured in this study resulted in much higher

concentrations by two orders of magnitude (3.3×10^6 for gulls and 9.8×10^6 CFU/g dry feces for ducks). Fogerty et al. (2003) found that gull feces from the Great Lakes region contain between 10^4 to 10^8 CFU/g dry feces, the maximum being much higher than concentrations reported in previous research and in the current study. The enterococci concentrations on average (3.3×10^5 CFU/g dry feces) observed in bird feces from this study appeared to be in the lower range and contributed somewhat to the decreased significance of bird fecal contributions at this particular study site.

The overall mass of bird feces observed in this study ranged from 0.027 to 1.6 g, dry weight. Kushlan (1977, 1979) suggested that an overall daily average mass of fecal matter on dry basis for an Ibis is approximately 10 g. The current study measured 1.7 g for an Ibis for one event suggesting that an Ibis has on the order of 5 to 6 fecal events per day. Bedard et al. (1980) found that a housed ring-billed gull produced 8.3 g of fecal matter, and Gould and Fletcher (1978) found that the average amount of feces excreted by a gull per day ranged from 11.2 to 24.9 g. The current study documented 0.11 g per fecal event for gulls, which suggests many fecal events per day for gulls. The assumption made, i.e. one observed bird equates to one fecal event, is potentially incorrect. It is possible that birds may deposit more than one fecal event per visit. However, given the difficulty in finding bird fecal samples on the beach, the assumption of one event per bird is assumed adequate.

Shrimp fecal mounds were found to be negligible, but human and dog contributions indicate an overall concern. The results (combined weekdays and weekends) showed that the average contribution (overall enteroccoci per day given the study enumeration) from humans to be 9.9×10^8, while the average contribution from

dogs was 1.2 x 10^{11}. Enterococci load from the bird feces was smaller (avg. of 7.0 x 10^{7}) but comparable to humans. The daily load of enterococci from shrimp fecal mounds was even lower at 1.9 x 10^{4} (avg. 1.0 x 10^{4}). Given these values dogs contribute about 99.2% of the total enterococci load whereas humans (0.72%), birds (0.04%), and shrimp (<0.04%) contribute a relatively negligible amount. This indicates that dog feces have the largest potential impact on local water quality.

3.4.2 Recommendations. Given the results observed for this study, management practices for the beach should include a requirement that dog owners remove dog fecal matter from the beach. Ideally, this fecal matter should be disposed in water proof bags within trash bins that are covered. The trash bins should be frequently emptied, especially during weekends. Such a practice could reduce the direct enterococci input to the beach by as much as 99%, thereby potentially decreasing the number of beach advisories issued for this site.

Chapter 4: CONCLUSION

4.1 Conclusion

Enterococci, as an indicator microbe, are effective when there is a known point source of contamination to a water body. Hobie Cat Beach is a recreational beach which has no known point source of contamination, yet, there have been several beach advisories/warning due to exceedences of regulatory standards. These studies were conducted to better understand and evaluate the patterns of enterococci concentrations, as well as study potential sources of enterococci.

Chapter 2 identified the environmental factors that impact the enterococci concentrations and the location which contained elevated levels of enterococci. This was accomplished by several experiments which were efforts that focused on the analysis of enterococci from water samples and sand samples from various locations of the beach and under the water. Environmental factors which were targeted included antecedent rainfall, tides, sunlight impacts, and seasonal impacts. Rainfall was seen as a relatively important and influential environmental factor that has an effect on the enterococci concentrations. The tidal studies showed that when there was rain within six hours then the concentrations were increased to above detection limit (>500 CFU/100 ml), even the sample collected at 100 m from the shoreline was recorded at >173 CFU/10 ml. The hourly sampling showed for two rain events that the levels of enterococci that were well into 10^4 MPN/100 ml immediately following the rain events. The runoff events also showed high levels of enterococci from the water samples, supporting the fact that rain water impacts the enterococci in water samples and perhaps the source is the runoff. The next environmental factor indicated was the effects from tides. The tidal studies showed

higher levels of enterococci in from the knee samples collected during high tide than low tide. The hourly sampling supported the tidal influence by having higher concentrations during high tide and staying elevated at 1,700 CFU/100 ml for an hour, the levels then decreased during low tide. The 10-minute sampling showed variable results, where the first experiment had a good correlation to tidal height and enterococci concentrations, yet the second (replicated the first experiment) overall had low concentrations throughout the tidal event. This might be explained that the tidal signal is intermittent and dependent upon additional environmental factors. The impacts from sunlight may account for the decrease of concentrations in water samples. The hourly sampling showed a decrease of enterococci levels in the water samples during high daylight, intense solar radiation times, around noon. There was not enough sampling and evidence to support or deny any seasonal variations.

The geographic location identified by chapter 2, showed that the inter-tidal zone is the area for the source of enterococci within the study site. The tidal studies sampling showed consistently, even during antecedent wet conditions and high bather loads that the water samples collected within the inter-tidal zone contained the most elevated levels of enterococci, and that water sample concentrations decreased the further away from the shoreline. The spatially intensive sediment sampling also supported that the inter-tidal zone contained the high levels of enterococci in the sand samples, although the highest were observed just above the high tide line, or seaweed or wrack line. The enterococci contained in the pore waters of the sand were seen to be elevated within the swash or inter-tidal zone from the tidal studies and were observed to be higher when the sand was collected from the beach area, dry sand. The wetting and drying that occurs in the inter-

tidal zone is frequent, whereas, there is only brief wetting to the dry sand either from rain water runoff and/or wave action which simulates a wetting of the sand. When considering the sand results in terms of its pore water concentrations, the levels of indicator microbes in sand on a pore water basis were orders of magnitude higher than the levels observed in the water column. This was seen in the tidal studies and the hourly sampling. The extreme high concentrations observed in the sand pore waters suggests that the pore water is the main reservoir for enterococci, where it can then be released by washing-off via runoff events or through tidal action. The sand or sediments within the backshore zone, or dry beach sand area, may act as a reservoir for enterococci and grains cannot account for the elevated levels observed in the water column as the suspended solids concentrations are not high enough.

The ultimate source of the elevated levels of enterococci found in both the sand and water near the shoreline can possibly be attributed to the activities that impact this location of the beach, i.e. presence of human and animal activity. Chapter 3 addresses the sources which impact the inter-tidal zone and the area near the shoreline.

As mentioned earlier, humans, dogs, and birds are common at the beach and these may potential sources of enterococci to the water and sand. Results from animal feces showed that dogs were the highest at 7.4 x 10^6 CFU/g dry feces, then bird feces at 3.3 x 10^5 CFU/g feces, and then the lowest were found in shrimp fecal mounds at 2.0 CFU/g feces. The microbial load from dog feces, bird feces, and shrimp fecal mounds were measured at 3.2 x 10^9 CFU per dog event, 4.7 x 10^5 CFU per bird fecal event, and 10 CFU per shrimp fecal mound. This gives a result of one dog fecal event to have the same enterococci loading as 6,940 bird fecal events and 3.2 x 10^8 shrimp fecal mounds.

Overall, enumeration results indicated that there were high human counts during the weekdays at 276 (avg. 55) and during the non-holiday weekend day to be 351. Holiday weekends had the largest number of people (2,644 people maximum, with an average of 1,333). The overall weekend results were 1,088 people. Enumeration results for the dog counts showed that during weekdays there were 11 dogs, with the highest number at 89. During the one non-holiday weekend, 13 dogs were observed at the beach; during holiday weekends, the maximum number observed was 196, with an average of 83. Overall weekend results showed an average of 66 dogs. The average number of birds (combined species) documented was 150, with a maximum number of 587. Shrimp fecal mounds, as estimated from in-field counting surveys, showed an overall average of 984 mounds with a median value of 919.

Comparative contributions showed that one dog feces equated to 1,872 people, 6,940 bird feces, and 3.2×10^8 shrimp fecal mounds. The enterococci contribution from dog feces far surpasses the contribution from human shedding or from bird or shrimp feces. The contribution of enterococci from dog feces was significant due to the high concentration of enterococci within the feces and the quantity resulting in a large contribution per dog event.

4.2 Recommendations

Overall recommendations include the further analysis of rain water runoff and the impacts this may have to the inter-tidal zone and near the shoreline. In addition, the analysis of the release of enterococci from the pore waters in sand that are washed into the water should be evaluated. The possibility of a sunlight effects should be further

evaluated also. Although dogs, birds and shrimp have been evaluated, further research to determine the sources of enterococci within the inter-tidal zone should also be conducted. Perhaps management practices for the beach should be reassessed to require dog owners to remove dog fecal matter from the beach.

BIBLIOGRAPHY

Alderisio, K. A., and DeLuca, N., 1999. Seasonal enumeration of fecal coliform bacteria from the feces of ring-billed gulls (Larus delawarensis) and Canada geese (Branta canadensis). Applied and Environmental Microbiology 65:5628-5630.

Alm, E. W., Burke, J., and Hagan, E., 2006. Persistence and potential growth of the fecal indicator baceria, *Escherichia coli*, in shoreline sand at Lake Huron. International Association Great Lakes Research. 32:401-405.

Anderson, M. L., Whitlock, J. E., Harwood, V. J., 2005. Persistence and differential survival of fecal indicator bacteria in subtropical waters and sediments. Applied and Environmental Microbiology 71:3041–3048.

Anderson, S. A., Turner, S. J., and Lewis, G. D., 1997. Enterococci in the New Zealand environment: Implications for water quality monitoring. Water Science and Technology 35:325-331.

Bedard, J., Therriault, J. C., Berube, J., 1980. Assessment of the importance of nutrient recycling by searbirds in the St. Lawrence estuary. Canadian Journal of Fisheries and Aquatic Sciences 37:583-588.

Beversdorf, L. J., Bornstein-Forst, S. M., and McLellan, S. L., 2007. The potential for beach sand to serve as a reservoir for *Escherichia coli* and the physical influences on cell die-off. Journal of Applied Microbiology 102:1372-1381.

Boehm, A. B., and Weisberg, S. B., 2005. Tidal forcing of enterococci at marine recreational beaches at fortnightly and semidiurnal frequencies. Environmental Science and Technology. 39:5575-5583.

Boehm, A. B., Grant, S. B., Kim, J. H., Mowbray, S. L., McGee, C. D., Clark, C. D., Foley, D. M., and Wellman, D. E., 2002. Decadal and shorter period variability of surf zone water quality at Huntington Beach, California. Environmental Science and Technology 36:3885-3892.

Boehm, A. B., Keymer, D. P., Shellenbarger, G.G., 2005. An analytical model of enterococci inactivation, grazing, and transport in the surf zone of a marine beach. Water Research 39:3565-3578.

Bonilla, T. D., Nowosielski, Esiobu, N., McCorquodale, J. M., Rogerson, A., 2006. Species assemblages of enterococcus indicate potential sources of fecal bacteria at a south Florida recreational beach. Marine Pollution Bulletin 52:807-810.

Bonilla, T. D., Nowosielski, K. Cuvelier, M., Hartz, A., Green, M., Esiobu, N., McCorquodale, D. S., Fleisher, J. M., Rogerson, A., 2007. Prevalence and distribution of fecal indicator organisms in south Florida beach sand and

preliminary assessment of health effects associated with beach sand exposure. Marine Pollution Bulletin 54:1472-1482.

Brookes, J. D., Antenucci, J., Hipsey, M., Burch, M. D., Ashbolt, N. J., Ferguson, C., 2004. Fate and transport of pathogens in lakes and reservoirs. Environment International 30:741–759.

Cabelli, V. 1983. Health effects criteria for marine recreational waters. U.S. EPA Report EPA-600/1-80-031. Cincinnati, OH: U.S. Environmental Protection Agency.

Calci, K. R., Burkhardt III, W., Watkins, W. D., and Rippey, S. R., 1998 Occurrence of male-specific bacteriophage in feral and domestic animal wastes, human feces, and human-associated wastewaters. Applied and Environmental Microbiology 64:5027-5029.

Carrillo, M., Strada, E., and Haze, T. C., 1985. Survival and enumeration of the fecal indicators bifidobacterium adolescentis and *Escherichia coli* in a tropical rain forest watershed. Applied and Environmental Microbiology 50:468-476.

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

Cox, P., Griffith, M., Angles, M., Deere, D., and Ferguson, C., 2005. Concentrations of pathogens and indicators in animal feces in the Sydney watershed. Applied and Environmental Microbiology 74:5929-5934.

Davies, C. M., Long, J. A., Donald, M., and Ashbolt, N. J., 1995. Survival of fecal microorganisms in marine and freshwater sediments. Applied and Environmental Microbiology. 61 :1888-1896.

De Graef, E. M., Devrisde, L. A., Baele, M., Vancanneyt, M., Seings, J., and Haesebrouck, F., 2005. Identification of enterococcal, streptococcal and *Weissella* species in the faecal flora of individually owned dogs. Journal of Applied Microbiology 99:348-353.

Delgado, M., Neto, I., Correia, J. H., and Pomba, C., 2007. Antimicrobial resistance and evaluation of susceptibility testing amoung pathogenic enterococci isolated from dogs and cats. International Journal of Antimicrobial Agents 30 :98-100.

Desmarais, T. R., Solo-Gabriele, H. M., Palmer, C. J., 2002. Influence of soil on fecal indicator organisms in a tidally influenced subtropical environment. Applied and Environmental Microbiology 68:1165–1172.

Dunlap, B. G., Thies, M. L., 2002. *Giardia* in beaver (Castor canadensis) and nutria (Myocastor coypus) from east Texas. The Journal of Parasitology 88:1254-1258.

Durbin, M. E., Zaher, A. M., Heybeck, N. F., Solo-Gabriele, H. M., Elmir, S., Goodwin, K. D., and Sinigalliano, C., 2005. The source of enterococci to a subtropical recreational beach is the inter-tidal zone. Proceedings of the 105th General Meeting of the American Society for Microbiology. (Abstract).

Elmir, S. M., Wright, M. E., Abdelzaher, A., Solo-Gabriele, H. M., Fleming, L. E., Miller, G., Rybolowik, M., Shih, M. P., Pillai, S. P., Cooper, J. A., and Quaye, E. A., 2007. Quantitative evaluation of bacteria released by bathers in a marine water. Water Research 41:3-10.

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. Journal of Applied Microbiology 99:598-608.

"Florida Healthy Beaches Program." Florida Department of Health, 2007. My Florida.com. 20 Feb. 2007 http://esetappsdoh.doh.state.fl.us/irm00beachwater/default.aspx 2003.

Florida Department of Environmental Protection, 1996. Florida administrative code. Surface water quality standards. 62–302. Florida Department of Environmental Protection, Tallahassee.

Fogarty, L. R., Haack, S. K., Wolcott, M. J., and Whitman, R. L., 2003. Abundance and characteristics of the recreational water quality indicator bacteria *Escherichia coli* and enterococci in gull faeces. Journal of Applied Microbiology 94:865-878.

Fujioka, R. S., 1988. Naturally occurring fecal coliforms and fecal streptococci in Hawaii's freshwater streams. Toxic Assessment: An International Journal 3:613–630.

Fujioka, R. S., Hashimoto, H., Siwak, E. B., and Young, R., 1981. Effect of sunlight on survival of indicator bacteria in sewater. Applied and Environmental Microbiology 41:690-696.

Fujioka, R. S., Sian-Denton, C., Borja, M., Castro, J., Morphew, K., 1999. Soil: the environmental source of *Escherichia coli* and enterococci in Guam's stream. Journal of Applied Microbiology Symposium Supplement 85:83S–89S.

Gerba, C. P., 2000. Assessment of enteric pathogen shedding by bathers during recreational activity and its impact on water quality. Quantitative Microbiology 2:55-68.

Gould, D. J. and Fletcher, M. R., 1978. Gull droppings and their effects on water quality. Water Research 12:665-672.

Graczyk, T. K., Fayer, R., Trout, J. M., Lewis, E. J., Farley, C. A., Sulaiman, I., Lal, A. A., 1998. *Giardia* sp. cysts and infectious *Cryptosporidium parvum* oocysts in the feces of migratory Canada Geese (*Branta canadensis*). Applied and Environmental Microbiology 64:2736-2738.

Graczyk, T. K., Sunderland, D., Tamang, L., Shields, T. M., Lucy, F E., Breysse, P. N., 2007. Quantitative evaluation of the impact of bather density on levels of human-virulent microspordian spores in recreational waters. Applied and Environmental Microbiology 73:4095-4099.

Griffin, D. W., Donaldson, K. A., Paul, J. H., Rose, J. B., 2003. Pathogenic human viruses in coastal waters. Clinical Microbiology Reviews 16:129-143.

Haack, S. K., Fogarty, L. R., and Wright, C., 2003. *Escherichia coli* and enterococci at beaches in the Grand Traverse Bay, Lake Michigan: Sources, characteristics, and environmental pathways. Environmental Science and Technology 37:3275-3282.

Hanes, N. B., Fossa, A. J., 1970. A qualitative analysis of the effects of bathers in recreational water quality. Advanced Water Pollution Research 5:9/1–9/9.

Hardina, C. M. and Fujioka, R. S., 1991. Soil: the environmental source of *E. coli* and enterococci in Hawaii's streams. Environmental Toxicology and Water Quality. 6:185-195.

Harwood, V. J., Butler, J., Parrish, D., and Wagner, V., 1999. Isolation of fecal coliform bacteria from the Diamondback Terrapin (Malaclemys terrapin centrata). Applied and Environmental Microbiology 65:865-867.

Hatch, J. J. 1996. Threat to public health from gulls. International Journal of Environmental Health Research 6:5-16.

Haugland, R. A, Siefring, S. C., Wymer, L. J., Brenner, K. P., and Dufuor, A. P., 2005. Comparison of enterococcus measurements in freshwater at two recreational beaches by quantitative polymerase chain reaction and membrane filter culture analysis. Water Research 39:559-568.

Havelaar A. R., van Olphen, M., and Drost, Y. C., 1993. F-specific RNA bacteriophage are adequate model organisms for enteric viruses in fresh water. Applied and Environmental Microbiology 59:2956–2962.

Howell, J. M., Coyne, M. S., and Cornelius, P. L., 1996. Effect of sediment particle size and temperature on fecal bacteria mortality rates and the fecal coliform/fecal streptococci ratio. Journal of Environmental Quatlity 21:1216-1220.

Indest, K., 2003. Interim guidance on assessing the risk posed by pathogens associated with dredged material. (No. ERDC/TN EEDP-01-49).

Ishii, S., Hansen, D. L., Hicks, R. E., and Sadowsky, M. J., 2007. Beach sand and sediments are temporal sinks and sources of *Escherichia coli* at Lake Superior. Environmental Science and Technology 41:2203-2209.

Jiang, S. C., and Chu, W., 2004. PCR detection of pathogenic viruses in southern California urban rivers. Journal of Applied Microbiology 97:17–28.

Jones, F., Smith, P., and Watson, D. C., 1978. Pollution of water supply catchment by breeding gulls and the potential environmental health implications. Journal of the Institute of Water Engineers and Scientists 32:469-482.

Jones, K. and Obiri-Danso, K., 1999. Non-compliance of beaches with the EU directives of bathing water quality: evidence of non-point sources of pollution in Morecambe Bay. Journal of Applied Microbiology Symposium Supplement 85:101S-107S.

Kühn, I., Iversen, A., Burman, L. G., Olsson-Liljequist, B., Franklin, A., Finn, M., Aarestrup, F., Seyfarth, A. M., Blanch, A. R., Vilanova, X, Taylor, H., Cerrero, I. A., and Möllby, R., 2003. Comparison of enterococcal populations in animals, humans, and the environment – a European study. International Journal of Food Microbiology 88:133-145.

Kushlan, J. A., 1977. Population energetics of the White Ibis. American Ornithologists' Union. 94, 114-122.

Kushlan, J. A., 1979. Feeding ecology and prey selection in the White Ibis. Condor 81(4), 376-389.

Lévesque, B., Brousseau, P., Bernier, F., Dewailly, E., and Joly, J., 2000. Study of the bacterial content of ring-billed gull droppings in relation to recreational water quality. Water Research 34:1089-1096.

Lévesque, B., Brousseau, P., Simard, P., Dewailly, E., Meisels, M., and Joly, J., 1993. Impact of a ring-billed gull (Larus delawarensis) on the microbiology quality of recreational water. Applied and Environmental Microbiology 59:1228-1230.

Manning. R. B. and Kumpf, H. E., 1959. Preliminary investigations of the fecal pellets of certain invertebrates of the south Florida area. Bulletin of Marine Science of the Gulf and Carribean. 9:291-309.

Martin, A. and Gruber, S., 2005. Amplification of indicator bacteria in organic debris on southern California beaches. Weston Solutions, Technical Paper 0507.

Meals, D. W., and Braun, D. C., 2006. Demonstration of methods to reduce *E. coli* runoff from dairy manure application sites. Journal of Environmental Quality 35:1088-1100.

Messer, J. W. and A. P. Dufour., 2002. Method 1600: Enterococci in water by membrane filtration using membrane-enterococcus Indoxyl-β-D-Glucoside Agar (mEI). US EPA Office of Water, Washington, DC. EPA-821-R-02-022.

Mundt, J. O., 1963. Occurrence of enterococci in animals in a wild environment. Applied Microbiology 11:136-140.

Murphy, R. C. and Kremer, J. N., 1992. Benthic community metabolism and the role of deposit-feeding callianassid shrimp. Journal of Marine Research 50:321-340.

Murray, S. M., Patil, A. R., Fahey, Jr., G. C., Merchen, N. R., and Hughes, D. M., 1997. Raw and rendered animal by-products as ingredients in dogs diets. Journal of Animal Science 75:2497-2505.

National Climatic Data Center (NCDC), 2006. Average annual mean temperature (1970-2005). http://www.ncdc.noaa.gov/oa/climate/research/cag3/fl.html.

National Oceanic and Atmospheric Administration, 2007. National Data Buoy Center. Station FWYF1 - Fowey Rocks, FL, http://www.ndbc.noaa.gov/station_page.php?station=FWYF1.

National Oceanic and Atmospheric Administration, May 2004. Water level tidal predictions. Tides and Currents, http://tidesandcurrents.noaa.gov/tide_pred.html.

National Research Council. 1985. Nutrient requirements of dogs. National Academy of Sciences. Washington, D.C. 2-41.

Obiri-Danso, K. and Jones, J., 2000. Intertidal sediments as reservoirs for hippurate negative camplybacters, salmonellae and faecel indicators in three EU recongnized bathing waters in north west England. Water Research 34:519-527.

Oshiro, R. and Fujioka, R., 1995. Sand, soil, and pigeon droppings: sources of indicator bacteria in the waters of Hanauma Bay, Oahu, Hawaii. Water Science and Technology 31:251-254.

Papadakis, J. A., Mavridou, A., Richardson, S. C., Lampiri, M., and Marcelou, U., 1997. Bather-related microbial and yeast populations in sand and seawater. Water Research 31:799-804.

Rabinovici, S. J., Bernknopf, R. L., Wein, A. M., Coursey, D. L., and Whitman, R. L., 2004. Economic and health risk trade-offs of swim closures at a Lake Michigan beach. Environmental Science and Technology 38:2737-2745.

Rivera, S. C., Hazen, T. C., Toranzos, G. A., 1988. Isolation of fecal coliforms from pristine sSites in a tropical rain forest. Applied and Environmental Microbiology 54:513-517.

Robinton, E. D., Mood, E. W., 1966. A quantitative and qualitative appraisal of microbial pollution of water by swimmers a preliminary report. Journal of Hygiene (Cambridge) 64:489–499.

Rodriques, J., Poeta, P., Martins, A., and Costa, D., 2002. The importance of pets as reservoirs of resistant enterococcus strains, with special reference to vancomycin. Journal of Veterinary Medicine 49:278-280.

Rogerson, A., McCorquodale, D., Esiobu, N., 2003. Prevalence and survival of microorganisms in shoreline interstitial waters: a search for indicators of health risks. (EPA # 828830). Final Report—November 2003.

Roll, B. M., and Fujioka, R. S., 1997. Sources of faecal indicator bacterica in a brackish, tropical stream and their impact on recreational water quality. Water Science and Technology 35:179-186.

Shehane, S. D., Harwood, V. J., Whitlock, J. E., and Rose, J. B., 2005. The influence of rainfall on the incidence of microbial faecal indicators and the dominant sources of faecal pollution in a Florida river. Journal of Applied Microbiology 98:1127-1136.

Sherer, B. M., Miner, J. R., Moore, J. A., and Buckhouse, J. C., 1992. Indicator bacteria survival in stream sediments. Journal of Environmental Quality 21:591-596.

Shibita, T., Solo-Gabriele, H. M., Fleming, L. E., Elmir, S., 2004. Monitoring marine recreational water quality using multiple microbial indicators in an urban tropical environment. Water Research 38:3119-3131.

Smith, B. G., Dufour, A. P., 1993. Effects of the microbiological quality of recreational waters: a simulation study. American Society for Microbiology 93rd General Meeting, May 16–20, 1993, Atlanta, Georgia.

Solo-Gabriele, H. M., Wolfert, M. A., Desmarais, T. R., and Palmer, C. J., 2000. Sources of *Escherichia coli* in a coastal subtropical environment. Applied and Environmental Microbiology 66:230-237.

Spears, J. K., Grieshop, C. M., and Fahey, Jr., G. C., 2004. Evaluation of stabilized rice bran as an ingredient in dry extruded dog diets. Journal of Animal Science 82: 1122-1135.

Stetler R. E., 1984. Coliphages as indicators of enteroviruses. Applied and Environmental Microbiology 48: 668–670.

U.S. Environmental Protection Agency, 1976. Quality criteria for water. EPA-440976023. Washington D.C., U.S. Environmental Protection Agency

U.S. Environmental Protection Agency, 1986. Ambient water quality criteria for bacteria. EPA 440/5-84-002. Washington, DC: U.S. Environmental Protection Agency.

U.S. Environmental Protection Agency, 2001. Protocol for developing pathogen TMDLs First Edition. EPA 841-R-00-002. http://www.epa.gov/owow/tmdl/pathogen_all.pdf.

U.S. Geological Survey. 2006a. Microbiology and public beach safety: integrated science for the protection of public health. FS 2006-3045 ed.

U.S. Geological Survey. 2006b. Pathogen exposure through recreational water. Retrieved 10/18/2007 from http://health.usgs.gov/pathogens.

van Elsas, J. D., and Smalla, K., 1997. Methods for sampling soil microbes, p.383-390. *In* C. J. Hurst, G. R. Knudsen, M. J. McInernery, L. D. Stetzenbach, and M. V. Walter (ed.), Manual of Environmental Microbiology. ASM Press, Washington, D.C.

Whitman, R. L., and Nevers, M. B., 2003. Foreshore sands as a source of *Escherichia coli* in nearshore water of a Lake Michigan beach. Applied and Environmental Microbiology 69:5555-5562.

Whitman, R. L., Nevers, M. B., Korinek, G. C., and Byappanahhalli, M. N., 2004. Solar and temporal effects on *Escherichia coli* concentration at a Lake Michigan swimming beach. Applied and Environmental Microbiology 70:4276-4285.

Williams, A. P., Avery, L. M., Killham, K., and Jones, D. L., 2007. Persistence, dissipation, and activity of *Escherichia coli* O157:H7 within sand and seawater environments. FEMS Microbiology Ecology 60:24-32.

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. Environmental Science and Technology 41:4515-4521.

Ziebis, W., Forster, S., Huettel, M., and Jorgensen, B. B., 1996. Complex burrows of the mud shrimp *Callianassa truncata* and their geochemical impact in the sea bed. Nature 382:619-622.

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