UNIVERSITY OF MIAMI

DEVELOPMENT OF A WATER QUALITY MODEL WHICH INCORPORATES NON-POINT MICROBIAL SOURCES

By

Samir M. Elmir

A DISSERTATION

Submitted to the Faculty
of University of Miami
in partial fulfillment of the requirements for
the degree of Doctor of Philosophy

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DEVELOPMENT OF A WATER QUALITY MODEL WHICH INCORPORATES NON-POINT MICROBIAL SOURCES

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Traditionally monitoring the sanitation of recreational coastal waters has been regulated by measuring concentrations of fecal indicator bacteria (*E. coli*, fecal coliforms, and enterococci). The bacteria utilized are those typically found in human feces in high concentrations. Recently the use of fecal indicator bacteria to monitor and regulate the recreational use of coastal waters has come into question, particularly in the tropical and sub-tropical marine environment (e.g., Hawaii, Guam, Puerto Rico, and South Florida) where the non-point sources (i.e. beach sand and/or sediment, animals, run-off water, and bathers) are the dominant fecal bacteria input source. In addition, little work has been done in the area of recreational water quality modeling, especially water quality models that incorporate non-point sources of fecal bacteria indicators to predict the bacterial loading in the water column.

The primary objective of this dissertation was to characterize and quantify non-point sources of enterococci at a marine beach, Hobie Cat Beach, located in Miami-Dade County, Florida. This information will be incorporated into a water quality model to evaluate the relative importance of each of the non-point sources of enterococci. In order to achieve this objective, two main tasks were completed and discussed.

The first task focused on estimating the concentrations of enterococci and *Staphlococcus aureus* shed directly off the skin of bathers and the amount of beach sand and the corresponding concentration of enterococci that can be transported by bathers into the water column. Enterococci, a common fecal indicator, and *Staphylococcus aureus*, a common skin pathogen, can be shed by bathers affecting the quality of recreational waters and resulting in possible human health impacts. Two sets of field studies were conducted at Hobie Cat Beach. The first study, referred to as the "Large Pool" study, involved 10 volunteers who immersed their bodies in a 4700 liter inflatable plastic pool filled with off-shore marine water during four 15 minute cycles with exposure to beach sand in cycles 3 and 4. The second study, referred to as the "Small Pool" study involved 10 volunteers who were exposed to beach sand for 30 minutes before they individually entered a small tub. After each individual was rinsed with off-shore marine water, sand and rinse water were collected and analyzed for enterococci. Results from the "Large Pool" study showed that bathers shed concentrations of enterococci and *S. aureus* on the order of $6x10^5$ and $6x10^6$ colony forming units per person in the first 15 minute exposure period, respectively. Significant reductions in the bacteria shed per bather (50% reductions for *S. aureus* and 40% for enterococci) were observed in the subsequent bathing cycles. The "Small Pool" study results indicated that the enterococci contribution from sand adhered to skin was small (about 2% of the total) in comparison with the amount shed directly from the bodies of the volunteers.

The second task focused on developing the algorithms for simulating non-point sources of enteroccoci specific to the study site including sand, dogs, birds, water runoff,

and bathers, and the application of the developed algorithms to quantify the enterococci loads associated with each one of the sources. The five dominant non-point sources of enterococci were described and expressed as mathematical equations along with their variables. Estimates for all variables were defined and computed using the most recent literature, studies and direct field measurements values. The task showed that water run-off is the most significant non-point source contributing enterococci into the water column followed by dogs, sand, birds, and bathers respectively.

Overall this dissertation suggests that non-point sources of fecal bacteria indicators contribute significant amounts of enterococci into the water column and they should thus be considered when designing water quality models. Regulatory beach monitoring programs should include site specific predictive water quality models in order to assess the sanitation of coastal recreational water bodies.

I would like to dedicate my dissertation and all of my work to my mother, Fatima Abbass Hamoud (1927-2000), my wife, my daughter, and my coach and mentor, Dr. Helena M. Solo-Gabriele, for being a great inspiration in my life

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CHAPTER 1

DISSERTATION PROPOSAL

1.1 Introduction:

This chapter provides an overview of selected environmental and epidemiological studies aimed at establishing the relationship between traditional indicator microbes in human health and environmental sources, the most recent mathematical models to predict the sanitary water quality in recreational waters using historical meteorological and environmental data, regulatory criteria for monitoring recreational waters including state and local historical water quality data. Finally, the dissertation overall objective, tasks, and hypothesis including study site description are presented in this chapter.

1.2 Review of Environmental Studies:

This section reviews the significant literature published over the last 15 years in the area of microbial indicators for recreational waters in tropical and subtropical climates. This review introduces the names of some of the top scientists and technical experts who worked in this area. Sources of fecal bacteria in tropical environments and factors that influence their survival and growth in warm and humid environments and the search for the best alternative indicators to assess the recreational water quality in tropical climates were among the main areas studied on the subject. Recent evidence indicated that the significance of beach sands and other environmental sources is not necessarily limited to the sub/tropics. For example, sands have been implicated as a bacterial source

in the freshwater beaches of Lake Michigan (Whitman and Nevers, 2003) and Lake Huron (Alm et al., 2006), both in Michigan.

Studies conducted in Hawaii (Fujioka and Byappanahalli, 1996 & 1998; Fujioka, 1983; Fujioka and Shizumura, 1985; Fujioka et al., 1988 & 1999; Hardina and Fujioka, 1991), in Guam (Fujioka, 1989), and in Puerto Rico (Bermudez and Hazen, 1988; Hazen 1988; Toranzos, 1991), examined the validity and applicability of the USEPA recommended fecal indicators (fecal coliforms, *E. coli* and enterococci) in determining the hygienic water quality in subtropical/tropical regions of the world. Those studies have shown collectively that: 1- in the absence of any known sources of human/animal waste, fecal indicators are consistently present and recovered in high concentrations in the environment (fresh water streams, vegetation, soil/sediment and storm drains). This finding refutes the first main assumption the USEPA used in setting up the microbial water quality indicators/standards which is "there are no major environmental sources of these bacteria. Thus, environments in tropical islands are significant sources of fecal bacteria and the detection of such bacteria does not necessarily mean that the environment is contaminated with fecal matter. Therefore, the use of fecal indicators to measure the water quality in the tropics may not be applicable; 2- Obtained data from (Fujioka and Byappanahalli, 1998) reconfirmed earlier studies that fecal indicators are capable of multiplying under natural conditions. Study also showed that temperature, available nutrients, moisture, indigenous microbes of the soils play critical role in controlling the survival and regrowth of fecal indicators in Hawaii's soils. This finding contradicts one of the criteria used by the USEPA to establish the most suitable water quality indicator(s) which is "the microbial indicator(s) must not multiply outside the

human intestinal tract". Thus, using fecal indicators to predict fecal contamination in the waters of tropical islands may not be adequate; 3- *Clostridium perfringens* (an alternative indicator) can be used to establish the recreational water quality in Hawaii; *C. perfingens* were detected in the range of 56 to 2100 CFU/100ml in streams receiving wastewater effluent discharges and in streams upstream from wastewater discharges *C. perfringens* were detected in significantly low densities as compared with the traditional fecal indicators. Based on those studies, it was concluded that the use of the USEPA recommended fecal indicators to establish water quality standards in Hawaii and other Pacific Islands does not appear to be valid or appropriate.

Toranzos et al., 1987, conducted a study in a cloud rain forest watershed (a tropical climate) in Puerto Rico. The purpose of the study was to determine the distribution, activity and survival of *Klebsiella pnuemoniae* and *E. coli* in a tropical environment. In situ diffusion chamber studies were conducted at two sites that contained fecal bacteria with no known point pollution source. The study indicated that *K. pnuemoniae* and *E. coli* are naturally present in the pristine fresh waters and remain physiologically active thus; they can survive in the environment without a fecal source for a long period of time (approx. 5 days). Finally, the study concluded that the use of fecal coliform as indicators to measure the sanitary water quality in tropical waters like the waters of Puerto Rico might not be appropriate.

Solo-Gabriele et al., 2000, conducted a study in a section of the New River, a coastal waterway (brackish waters), in Ft. Lauderdale, Florida. The intent of the study was to identify and evaluate the sources of high *E. coli* concentrations in the river waters. Field studies and laboratory experiments were conducted for this project. It was found

that soils of the riverbanks contribute a significant amount of *E. coli* in the water column there was an instantaneous increase in *E. coli* densities during rainfall events. It was also found that the *E. coli* concentrations in the water column fluctuate with the tidal cycles; it increases with high tide and decreases during low tide. The laboratory soil analyses showed that the *E. coli* concentrations increased by several orders of magnitude when the soil samples were subjected to cyclical drying and wetting conditions (growth occurs during dry conditions) which suggests that soil water content plays an important role in regulating *E. coli* growth providing that all other favorable environmental conditions (warm temperature, limited sunlight and nutrients) are met. Based on those findings, the study questioned the suitability of using *E. coli* to test the microbial water quality in tidally influenced areas located in subtropical/tropical regions of the world.

Desmarais et al., 2002, studied the environmental factors that influence the survival and regrowth of *E. coli*, enterococci and *Clostridium perfringens* in the sediment and soil along the riverbanks of the New River in Ft. Lauderdale, Florida. Field sampling results indicated that *E. coli*, enterococci and *C. perfringens* are generally present in soil and sediment samples. *E. coli* and enterococci were detected in high numbers in the superficial samples 3 to 6 cm in depth, their values ranged from 75 to 600 MPN/g and from 25 to 100 MPN/g respectively. On the other hand, high concentrations of *C. perfringens* were found in the core soil samples 15 to 20 cm in depth and ranged from 250 to 550 MPN/g. While the concentrations of enterococci showed little variation as a function of distance from the edge of the water, the densities of *E. coli and C. perfringens* were the highest within 45 to 50 cm distance from the edge of the water where the water content was highest. Two different laboratory experiments were

conducted to evaluate the regrowth of the microbial indicators. The first one was designed to evaluate the effect of increasing nutrients or decreasing the number of indigenous microbes by adding sterile or unsterile sediment to the river water sample. The second experiment was designed to study the wetting and drying effects due to tidal cycles. The results from the laboratory experiments revealed that *E. coli* and enterococci were capable of multiplying when sterile sediments were added and under tide simulation whereas *C. perfringens* was not capable of multiplying in either experiment. The study concluded that the use of the traditional fecal indicators to assess the hygienic water quality in a subtropical/tropical environment is still doubtful. Thus, additional studies are necessary to further evaluate and characterize those indicators and their influencing factors in terms of survival and growth in such climates.

Shibata et al., 2004, conducted a pilot epidemiological and water quality study at two public beaches, Hobie and Crandon, located in southern part of Biscayne, Miami, Florida. The main objectives of the study were: evaluate the microbial water quality including soils at the selected beaches and the bay using the regulatory microbial indicators (total and fecal coliforms, *E. coli* and enterococci) and *Clostridium perfringens* (alternative microbial indicator recommended for tropical climate); conduct sanitary surveys to identify point and non point sources of fecal pollution; identify sources of microbial indicators; administer an epidemiological study to evaluate relationship between swimming related illnesses and microbial density. No dose-response relationship was found between density of microbes and health effects, the water quality at Crandon Beach was better than Hobie Beach regardless of the season (wet vs. dry), there was no fecal pollution point source identified in the sanitary survey, intensive spatial water

quality monitoring indicated the southern tip of the shoreline at Hobie Beach appears to be the source of microbes. This finding was supported by the soil sample results collected from this end of shoreline. The detection of those indicators in the soils/vegetation of the shoreline without a known point source fecal pollution again questions the suitability of those indicators for measuring the sanitary water quality in subtropical/tropical climates.

Rose et al., 1998, conducted a one-year water quality study in Charlotte Harbor, Florida. The purpose of the study was to determine: a) distribution and seasonal changes in microbial indicators and human pathogen levels in Charlotte Harbor shellfish and recreational waters, and b) factors that may influence the fate and transport of pathogens. Water and sediment samples were analyzed for fecal indicators (fecal coliform, enterococci, *Clostridium perfringens* and coliphage), enteric protozoa (*Cryptosporidium spp., Giardia spp.*) and enteroviruses. All sampling sites were marine waters. Fecal indicators were found in high concentrations in areas of low salinity and high densities of on site sewage disposal systems. Enterococci were shown to be highly correlated with the fresh water flows and proved to be a good indicator. Enteroviruses were detected at 75% of the sampling sites during El Nino related rain events between November 1997 and February 1998 (none were detected in other months). A significant increase in coliphage (virus indicators) was also indicated during the wet months. In this case the coliphage accurately predicted the presence of enteroviruses. *Cryptosporidium spp.*, and *Giardia spp.*, were detected infrequently and were not associated with seasonal changes.

Rose et al., 2000, published a water quality study, which was conducted along the Philippi Creek and coastal beaches in Sarasota County, Florida. The objectives of the study were to assess the water quality in the watersheds impacted by septic tank systems

and evaluate the occurrence of enteric viruses along the public beaches in the county. Fecal indicators (*Clostridium perfingens*, enterococci, coliphage, fecal coliform), enteroviruses and enteric protozoa (*Cryptosporidium spp.*, and *Giardia spp.*) were used to assess the water quality in the study areas. Enteric protozoa were infrequently detected; 4.5 % of the samples tested positive. Fecal indicators (ranged from 5 to 4000 cfu/100ml) were highly correlated with areas impacted with high densities of on site sewage and disposal systems. Enteroviruses were detected at low levels in approx. 83.3 % of the tested sites. These results indicate that the waters in Sarasota Bay are contaminated with human pathogens and the mechanism by which the contaminants are transported to the Bay is the subsurface flow generated from the watersheds with high densities of septic systems.

Griffin et al., 1999, conducted water quality studies in Florida Keys (Upper, Middle and Lower). The purpose of the study was to evaluate the impact of the domestic waste disposal practices (cesspools, septic systems and wastewater package plants) on the ambient water quality and to estimate the risk for human health. It was found that 95% of the 19 sites (canals, beaches and near shore waters) tested positive for at least one group of enteric viruses: enteroviruses, hepatitis A and B, or Norwalk viruses. This study suggested that recreational and navigational waters in the Keys were negatively impacted by sewage disposal practices and that traditional/regulatory microbial indicators may not be adequate to assess this impact.

The USEPA, May 2002 Draft, Implementation Guidance for Ambient Water Quality Criteria for Beaches, Section 4.3, stated its policy and provided recommendations to the states and authorized tribes regarding high levels of fecal indicators originating

from environmental sources in tropical climates. EPA continues the support of applying its recommended fecal bacteria indicators (enterococci for marine and fresh waters and *E. coli* for fresh water) in all states and authorized tribes in the U.S including those located in tropical climates. EPA does not believe that the scientific evidence presented on this issue at the 2001 expert workshop in Hawaii is sufficient to recommend the use of alternative indicator(s). However, EPA provided three options to the states and authorized tribes that wish to address the fecal indicators and their potential to exist and multiply in tropical climates; establishment of a new alternative indicator(s) using risk based methods; utilization of *Clostridium perfringens* or any other alternative indicator(s) that in addition to the EPA's recommended fecal indicators along with a sanitary survey; adoption of a subcategory of recreation use providing that the primary contact recreation is not an existing use and naturally occurring contaminants prevent the site from attaining the primary contact recreation use standards.

Nova Southeastern University 2001-2003 evaluated indicator bacteria and selected pathogens at Hobie Cat beach, Hollywood and Fort Lauderdale beaches, South Florida. The main objectives of the study were: 1-document the numbers of *E coli*, enterococci and fecal coliforms in beach sand and determine if they are attached or free in interstitial water, and 2- compare the survival of indicator organisms in water versus sand. Study found that concentrations of bacteria indicators were higher in dry sand, followed by wet sand (swash zone) and followed by seawater, and majority of indicators were attached to sand grains i.e. they were metabolically active. The study suggested that swash zone receives significant bacterial inputs from the beach, and sediment re-suspension plays significant role impacting bacterial loading in the water column.

The above environmental studies are summarized by author, study location(s), objectives, findings, and conclusions in table format, Table 1.1. These studies indicate that: fecal indicators are naturally present in tropical environments (soils, vegetation and waters) and are able to survive and multiply providing certain environmental conditions (nutrients and predation, moisture, temperature and rainfalls) are met. Thus, the detection of those indicators in recreational waters of the tropics may not be predicative of fecal contamination. Therefore, fecal bacteria indicators (enterococci and *E. coli*) as recommended by the USEPA, 1986 may not be appropriate to be applied in tropical and subtropical environments. Thus it is necessary to look for alternative indicator(s) such as *Clostridium perfringens* that best suit tropical and subtropical climates.

Limitations:

Despite the significant scientific evidence presented by many studies about the presence and recovery of traditional microbial indicators in high numbers from the environment (i.e. beach sand, river bank sediments, and plants), small efforts and resources have been spent on understanding re-growth, sources (source tracking), alternative microbial indicators and health risks.

1.3 Predictive Recreational Water Quality Models:

Limited review of the literature revealed that only a few recreational water quality-modeling efforts have been taken place in the US. The main objectives behind the development of such models are: a) provide real-time assessment of the recreational hygienic water quality in relationship with natural and man-made environmental changes

i.e., seasonal changes (rainfall events, temperature, winds and water currents) and sewage spills and bypasses; b) provide public health officials with the scientific tools necessary to make timely and accurate decisions on beach closures and openings, thus protect the health and safety of the public with a minimum economical impact; and c) using hydrodynamic modeling to predict bacterial loading in the water column due to non-point sources (e.g. runoff, bird feces and sediment resuspension).

Two models were completed and have been utilized and integrated in recreational water monitoring programs: the first one is in New Orleans, Louisiana. The model developed for New Orleans was designed to predict the densities of fecal bacteria indicators along the south shore of Lake Pontchartrain. The water quality of the lake is negatively impacted by the storm water runoffs pumped into it as a means to control flooding in New Orleans.

The second model was developed by the New York City Department of Environmental Protection. This model was designed as a regional tool to characterize and predict fecal pollution (fecal indicators densities vs. time and plume movement, size and locations) due to sewage spills and bypasses.

In addition, the two modeling projects in Florida were not materialized due to lack of funding and/or valid data. The Pinellas County Health Department and the College of Marine Sciences, University of South Florida proposed one to the Florida Legislation, 2000. The second model was sponsored by the USEPA and FDOH, 1999. The modeling effort was put on hold because the statewide water quality and environmental data collected (from 300 sampling sites in 34 coastal counties) in the one-year USEPA and

FDOH Beach Monitoring Study lacked valid statistical relationships which are necessary to design such a model.

The FDA has a statistical water quality model for shellfish harvesting waters. They have been using this model since 1985. This statistical model is used to predict the fecal coliforms concentration using current and historical water quality (microbial and physical-chemical), hydrological (river stage data) and weather data. Other states (TX, LA, MS, AL, NC, SC, VA, and others) have adopted similar models for predicting microbial contamination in shellfish harvesting waters to issue temporary closure advisories for theses sites. According with FDA, Bureau of Aquaculture Environmental Services, "Users did not understand and accept predictive temporary closures at first, but eventually did when explained that the alternative was not to allow shellfish harvesting. The economic impact was that shellfish harvesting could continue (when safe)". The following is the web site for this program www.floridaaquaculture.com.

Sanders et al., 2004, (note: at the time of writing this proposal, study was not published) conducted hydrodynamic modeling in Huntington Beach, California to predict the loading of enteric bacteria to surface waters of an inter-tidal wetland by urban runoff, bird feces and re-suspension in sediments. Results of this study suggest that re-suspension of bacteria in sediments is the main factor in influencing the bacteria concentrations in the water column.

Limitations:

In light of this review, it is evident that little work has been done in this area and additional research and studies may be necessary. A well designed predictive model that

is designed based on a deterministic approach as oppose to a probabilistic one using site specific historic and real-time environmental data can be an invaluable tool to protect public health as well as the economy in South Florida.

1.4 Review of Human Health Data:

This section provides literature review of the most significant epidemiological studies conducted in this field over the last 20 years worldwide. Those studies evaluated the relationships among swimming related illnesses (i.e. gastrointestinal and respiratory diseases, ear and eye infections and skin rashes) and traditional and non-traditional recreational microbial water quality indicators (i.e. fecal and total coliforms, enterococci, *Clostridum perfringens*, *E. coli,* fecal streptococci, hetrotrophic bacteria, *Pseudomonas aeruginosa* and total staphylococci, and etc.) In general, while there is a significant association between swimming associated illnesses and exposure to contaminated marine waters, there is no significant consistent association between adverse health outcomes with any particular microbial indicator.

Seyfried et al., 1985, conducted an epidemiological study in Canada. The main objective of the study was to evaluate swimming related illnesses associated with densities of microbes in fresh waters. Fecal coliforms, fecal streptococci, hetrotrophic bacteria, *Pseudomonas aeruginosa* and total staphylococci were used to assess the microbial water quality. In this study, total staphylococci correlated best with gastrointestinal illnesses as compared with the rest of the indicators. This finding however did not coincide with many other studies that used this indicator.

Fattal et al., 1987, reported a swimming related illnesses study conducted at three beaches (marine waters) with different water qualities in Tel-Aviv, Israel. *E. coli*, fecal coliforms and enterococci were used to evaluate the microbial water quality. The study design was modeled after the microbiological-epidemiological studies conducted by EPA. The two important findings of this study were: 1) at high densities of indicators (>24 cfu/100ml for E.coli and enterococci and >50cfu/100ml for fecal coliforms), there was a significant difference in gastroenteritis (GI) reported symptoms among swimmers and none-swimmers, 2) out of the three indicator- microbes tested, enterococci was the best indicator to predict GI illnesses among swimmers, this finding agreed with the EPA epidemiological studies conducted by Cabelli et al., 1986 in marine waters.

Cheung et al., 1990, reported on a study conducted at nine of the polluted (human waste discharge) beaches (marine waters) in Hong Kong. 19,000 individuals participated in the study. More than 65% of those individuals met the " swimmer" definition (complete exposure of the head to the water). Nine microbial indicators were used to evaluate the water quality; fecal coliforms, *E. coli, Klebsiella spp.*, fecal streptococci, enterococci, staphylococci, *Pseudomonas aeroginosa*, *Candida albicans*, and total fungi. At the study beaches, the *E. coli* to enterococci ratio ranged from 2.2 to 6.9 (the range for enterococci was from 31 to 248 cfu/100ml). Major findings were that a) the incidence rate of GI symptoms was significantly higher among swimmers as oppose to non-swimmers especially among the younger (<than 10 years old) population, and b) the strongest correlation between swimming related health effects and indicator density was between *E. coli* and highly credible gastrointestinal (HCGI) symptoms.

Balharajan et al., 1991, reported on a study that described the health risks related with exposure (wading, swimming, surfing and diving) to marine waters in the United Kingdom. 1,883 individuals participated in the study of which 839 were not exposed to the waters. Information was not provided as to the parameters/ indicator microbes used to evaluate the water quality at the study site. In this study, it was found that the rate of enteric disease symptoms was significantly greater among bathers than non-bathers. It was also found that the health risk for surfers/divers was approximately 1.4 times greater than swimmers and 1.5 times than waders. The increase or decrease in health risk was concluded to be a function of type and degree of exposure.

Von Schirnding et al., 1992, reported on a relatively small epidemiological and microbiological study conducted in marine waters at two beaches off the Atlantic coast of South Africa. One of the beaches was relatively clean the other was considered to be moderately polluted due to failing septic tank systems and storm water run–off. Only 733 individuals participated in the the study. In this study, enterococci, fecal coliforms, coliphages and staphylococci were among the indicator microbes tested. It was reported that there was a considerable increase in GI illness rates among swimmers than non-swimmers at the moderately polluted beach as oppose to the relatively clean beach.

Corbett et, al., 1993, conducted a study to assess the swimming related illnesses at the beaches (marine waters) in Sydney, Australia. Only fecal coliforms and fecal streptococci were used to measure the microbial quality of the waters. Out of 2,869 individuals that participated in the study, 924 of whom did not swim. Individuals younger than 15 years old were excluded from the study. Water samples were collected while people were swimming, 2 samples were collected from each sampling site. While the

study did not show a dose response relationship between swimming related illnesses and density of indicator microbes, it did show that the health risk significantly increased with an increase in exposure time (For individuals who swam for more than 30 minutes, their risk of reporting GI symptoms increased by a factor 4.6 times over those who swam less than 30 minutes). This study showed similar results with the EPA beach water studies in that increasing GI illness rates were not associated with increasing fecal coliform densities.

Kay et al., 1994, conducted a study to evaluate swimming related illnesses and water quality at the beaches in the United Kingdom. The study was a randomized controlled epidemiological study in that participants were recruited and randomly assigned to swimming or non-swimming groups. The microbial water quality was tested using total and fecal coliforms, fecal streptococci, total staphylococci and *Pseudomonas aeruginosa.* Water samples were collected every 30 minutes from the sites allocated for swimmers. 1,112 individuals participated in the study of which 512 were assigned to the swimmers group. Results of the study indicated that GI illness rates among swimmers were appreciably greater than non-swimmers. Out of the 4 indicator microbes, fecal streptococci was the best predictive for GI illness symptoms.

Pruss, 1998, reviewed all significant existing epidemiological studies on the health effects from exposure to recreational water. She found that most studies reported a dose related increase of health risk in swimmers with an increase in the indicator bacteria count in recreational water. The relative risks for reported symptoms (either gastrointestinal symptoms or highly credible gastro-enteritis) ranged from 1<relative risk

(RR)<3 in these studies. The indicator organisms that correlated best with the health outcomes were enterococci/fecal streptococci for marine and freshwater, and *E. coli* for freshwater. In both marine and freshwater, the increased risk of gastro-intestinal symptoms was associated with water quality values ranging from only a few indicator counts/100 ml to about 30 indicator counts/100 ml. These values are low compared to water qualities frequently encountered in coastal recreational waters. Of note, the majority of these studies were conducted in the US and UK, with few studies evaluated in tropical marine recreational waters.

Fleisher et al., 1998, conducted a study in 4 separate United Kingdom beaches during the summers of 1989 to 1992. This particular study focused on how domestic sewage contamination pollutes marine waters and affects public health. The results showed that the rates of illness (gastroenteritis, acute febrile respiratory illness, and eye and ear infections) among bathers were statistically significantly higher in relation to non-bathers. The average duration of illness was 4 to 8 days with 4 to 22% of participants seeking medical treatment, and 7 to 26% losing at least 1 day of normal activity, depending on the illness. Among the bathers cohort, 34.4% to 65.8% of the adverse health conditions reported were considered a direct result of bathing in sewage contaminated marine waters. Interestingly enough, at the time of the study, those waters met both USEPA and European "safe water" standards.

Kueh et al., 1995, analyzed bacteriological concentrations and examined how physico-chemical parameters such as air and water temperature and turbidity may contribute to changes in microbial count and therefore bathing related illness in Hong

Kong. Swimmers were in general two to three times more likely to develop illnesses than non-swimmers (swimmers only included those who wet their faces). The study was conducted in two Hong-Kong popular beaches, where one was considered more polluted than the other. Samples were analyzed for three bacterial indicators (*E.coli*, fecal coliforms, and staphylococci) and seven pathogenic bacteria (*Aeromonas spp., Clostridium perfringens, Vibrio cholerae, V. parahaemolyticus, V. vulnificus, Salmonella spp.*, and *Shigella spp.*). Interestingly, in this study *Clostridium perfringens* and *Aeromonas spp.* showed a significant correlation with GI and HCGI symptoms, while *V. cholerae and V. parahaemolyticus* were best associated with GI but not HCGI symptoms. *E. coli* and fecal coliforms levels were not associated with any adverse health symptoms evaluated in the study. However, in the analysis of physical-chemical water parameters, the study showed a strong correlation between water turbidity and GI and HCGI symptoms.

Fujioka et al., 1994, conducted a pilot study in Hawaii to clarify which microbiological indicator or other environmental parameter better associates with health outcomes therefore analyzing multiple indicators. Individuals participating were classified in three distinct groups: non-swimmers, swimmers who did not swallow water, and swimmers that did swallow water. The incidents of gastrointestinal illnesses in swimmers were more than twice as high as in non-swimmers. However, no association between swallowing water and GI adverse symptoms were found. Of note, the risk of an adverse condition was reported lower in individuals who swallowed water. There was no relationship between either swimming or swallowing water and frequency of the other symptoms studied. Furthermore, the study did not find any associations between the five

microbial indicators (fecal coliform, *E.coli*, enterococci, bacillus spores, and *Clostridium perfringens*) analyzed and human health effects.

Haile et al., 1999, evaluated the risk of reported gastrointestinal symptoms, "highly credible gastro-enteritis" and other symptoms with respect to reported distance from storm drains with untreated run off in the County of Los Angeles. Over 22,000 persons were interviewed 9 days after their facial immersion exposure to recreational beach waters concerning their symptoms. From the 22,085 subjects interviewed, 17,253 fulfilled the eligibility criteria, 15,492 agreed to participate, and from those 13,278 were contacted during follow-up. An increased risk of adverse health outcomes associated with swimming in ocean water contaminated by untreated urban runoff was found with a significant dose response relationship.

Prieto et al., 2001, established a cohort of 2,774 persons on 4 beaches in the north of Spain with follow up of 1,858 persons after 7 days from exposure for symptoms. Among those followed up, 135 (7.5%) experienced symptoms; visitors experienced symptoms more than residents, and symptoms were higher among bathers although not significantly. Gastrointestinal and skin symptoms correlated with total coliforms; an increased risk was observed with exposure to 2,500 to 9,999 total coliforms per 100 ml. This coliform count was below the European Union mandatory limit, although over a new proposed standard.

Fleming et al., 2002, conducted a prospective cohort epidemiological pilot study at 2 beaches (Hobie and Crandon) in South Florida, using multiple bacteria indicators (enterococci, total and fecal coliforms, *E. coli* and *C. perfringens*). The study was coducted one month each during wet and dry seasons. Final study population consisted of

63 families with 208 individuals. An epidemiological questionnaire was used to evaluate swimming related symptoms (GI and upper respiratory illnesses) and exposure. Daily monitoring of water quality using multiple bacteria indicators was conducted. There was no significant association between the number and the type of reported symptoms and the different sampling months or beach sites. There was a negative correlation between the number of bacteria indicators and the frequency reported by beach goers. Results of the daily monitoring indicated that different indicators provided conflicting results concerning beach water quality. Larger epidemiological studies with individual exposure monitoring are recommended to further evaluate these potentially important associations in subtropical recreational waters.

Nova Southeastern University 2001-2003, distributed a questionnaire on three beaches in South Florida: Hobie Cat, Hollywood and Fort Lauderdale. Out of 10,000 surveys handed out only 892 experimental forms and 609 control forms were returned. Symptoms reported included GI, upper respiratory, dermatological and constitutional.The results from the beach questionnaire did not show clear signs of symptoms in the recreational population in comparison with the control population. Questionnaire return was low around 10%. A future and more comprehensive epidemiology study may be warranted.

University of California Berkeley School of Public Health 2005, conducted an epidemiology study to evaluate the relationships between traditional indicators (enterococci, fecal coliforms, and total coliforms) and health risk. This study is one of the few studies that examined this relationship at beaches where no-point sources are the dominant fecal input source. One of the significant findings of this study was the risk of

swimming-related illnesses (gastrointestinal, respiratory, and dermal illnesses) were uncorrelated with levels of traditional indicators. In particular, it was found that the state water quality standards were not predictive of those illnesses. The study suggests that those findings are specific to Mission Bay beaches and can not be extrapolated into other sites. Note Study sites have been subjected to thorough cleanup activities that source tracking studies confirm leave human fecal sources a only a minor contributor. Finally, the study suggests the need for further evaluation of traditional indicators in conditions where non-point sources are the dominant fecal contributor.

The above epidemiological studies are summarized by author, study location(s), objectives, findings, and conclusions in table format, Table 1.2.

Limitations:

Based on the studies available, there is a significant general association between swimming associated illnesses and exposure to contaminated marine waters. However, there is no significant consistent association between adverse health outcomes with any particular microbial indicator. This association was derived from studies conducted at sites almost all the time impacted by a known point source of human and/or animal waste pollution and in cold regions of the world and not in the tropics. Therefore, researchers and beach regulators including public health officials have limited understanding of health risks associated with exposure to recreational waters impacted by non-point sources of fecal indicators especially in the tropics. A number of specific limitations to the existing studies should be mentioned, including small sample sizes, selection of target population, exposure definitions, and the use of particular indicator organisms.

1.5 Regulatory Criteria and Monitoring for Bacteria Indicators:

Under the 2002 guidelines fecal coliforms and enterococci are the indicator bacteria used by the Miami-Dade County Department of Health (MD-DOH) for testing beach water quality. Fecal coliform is the traditional indicator used since this microbe has been monitored since 1996 in Miami-Dade County, and it is also the official indicator of the Florida Department of Environmental Protection (FDEP) to test Class III Recreational Surface Brackish, Fresh and Marine waters for Swimming and other Recreational Water Activities (*Florida Administrative Code*, 62-302). Using this indicator a "health warning" is issued if confirmed fecal coliform levels exceed 400

CFU/100ml. Confirmation implies that the initial and resampling results exceed this standard. The second indicator is enterococci, which is the EPA recommended surface marine water indicator. Using this standard a "health advisory" is issued if the confirmed sample exceeds 104 CFU/100 ml. The MD-DOH lifts these "health advisories or warnings" after 2 consecutive acceptable results are obtained. The primary difference between a health advisory and a warning is the indicator microbe used to establish them.

Other applicable regulatory criteria include the total coliform standard (USEPA 1976), which is still implemented by the FDEP to test Class III waters in Florida. The monthly average total coliform concentration should not exceed 1,000/100 ml over a period of a month, nor exceed 2,400/100 ml for any single day sample. *E. coli* is recommended by the USEPA 1986 guidelines for freshwater but not for marine waters. For freshwater, the *E. coli* guideline is no more than 126/100 ml for a monthly average and no more than 235 for any single day sample. As mentioned, the use of *C. perfringens* has been recommended for the State of Hawaii (Fujioka and Shizumura 1985). The recommended open ocean standard for this microbe is 5/100 ml. For interior waters, the recommended standard is 50/100 ml.

A summary of the federal recommended guidelines and state regulatory standards are listed in Table 1.3.

1.6 General Description of the Study Site:

Miami-Dade County, Florida is the ideal site in which to study the issues of recreational water quality and possible water quality indicators in the tropical marine environment. There is approximately 25 miles of beach available for public recreational

use, with significant amounts of historic water quality data collected regularly by the Miami-Dade County Health Department as well as other regulatory agencies. These monitoring data have shown variable water quality in recreational areas throughout Miami Dade. There had been anecdotal evidence indicating a range of reported symptoms from beach users. In particular, wind surfing groups have voiced complaints associated with Hobie Cat Beach which has been characterized by historically elevated levels of indicator microbes.

Hobie Cat Beach is located in the southern portion of Biscayne Bay just northwest of the Miami Seaquarium and approximately 5.5 Km southeast of the mouth of Miami River. It is about 1 mile long and runs on the south side of Rickenbacker Causeway (Figure 1.1). Hobie Cat Beach is also known by the general public as the "dog beach," because it is the only beach in the county where beach visitors can bring their pets. The beach is owned and maintained by Miami-Dade County Public Works Department. There is no charge for admission or parking at Hobie Beach. There are no lifeguards or posted rules (e.g. hours of operation, safety, and sanitary rules) in the park.

Hobie Cat Beach is relatively shallow with poor water circulation; its shoreline is covered with seaweed over a very silty, muddy floor. It is a very narrow beach: the average distance between the mean water line and the outer edge of the sand and gravel is about 4.5 m. Vehicles park on the outer portion of the narrow sand strip. A paved access road is located immediately adjacent to the beach.

As noted, this beach has a history of poor water quality. Suspected sources of bacteriological "hits" include runoff from heavy rain events and the uncontrolled use of the beach (multi purpose use such as wading, swimming, other recreational water

activities, horse-back riding, dog training, bar-b-que parties, etc.). This beach is also surrounded by very dense urban and industrial facilities (i.e. the Port of Miami, Miami Dade County Central District Waste Water Treatment Plant (CDWWTP), restaurants, marinas, shopping centers, Miami Seaquarium and high rise office and residential buildings). The water quality at this beach is also likely influenced by storm water drainage (including the Miami River, its tributaries and storm drain outfalls along the seawall from the Port of Miami, Virginia Key, and the highly urbanized Brickel area).

There are also several concessions located at Hobie Cat Beach; these concessions provide a variety of services from renting recreational water equipment to food and beverage service. The Beach has two bathroom facilities at each end. These bathrooms have been connected to the county's sewage collection system since December of 2002. During the Hobie Cat Beach Epidemiological and Water Quality Pilot Study those bathrooms were connected to septic tank systems that were pumped regularly by the Miami Dade county public Works. Since December 2002, those tanks have been disconnected and properly abandoned under a permit from the Miami Dade County Health Department.

This beach has always been a critical candidate for water quality monitoring and studies. Summaries of the most recent and relevant environmental and epidemiology studies including results and conclusions conducted at Hobie Cat Beach are presented in Appendix B and Chapter 4. In 1999 Hobie Cat Beach was selected as one of the 10 beach sites to be monitored biweekly under the "EPA/FDOH One Year Water Quality and Public Notification Study." One of the purposes of the study was to test the 1986 EPA identified bacterial indicator, enterococci. During the year 2000, Hobie Cat Beach

exceeded the EPA Poor Water Quality Guideline (PWQG) for enterococci 29.2% of the times whereas the rest of the beaches on average (excluding Hobie Beach) exceeded the PWQG only 3.8 % of the times. This was a significant finding concerning the water quality at Hobie Cat Beach, which was one of the factors that triggered this particular study.

1.7 Historical Recreational Water Monitoring Programs:

Miami-Dade County Beaches including Hobie have been monitored since 1996. Monitoring between 1996 and 1999 was on a voluntary basis and there was no systematic approach to monitor on a regular basis. Between July 1999 and June 2000, surface water quality at Hobie Cat Beach was monitored for one year under a joint EPA/FDOH Beach Monitoring Study. Although the Florida State Laws at this time required beach water quality monitoring, public notifications did not exist until May 2000. In August 2002, the beach water-sampling program began collecting water samples on a weekly basis as opposed to biweekly with additional funding from U.S. EPA. Currently, Hobie Cat Beach (commonly known as "Dog Beach") is designated a sampling site, which is part of the Florida Healthy Beaches Monitoring and Public Notification Program. This program was effective July 2000 through new state legislation. The Florida Legislature, in the year 2000 allocated $529,000 to implement this mandatory statewide program. To review the FDOH Beach Monitoring and Public Notification Program (including the results), visit the following web site: http://apps3.doh.state.fl.us/env/beach/webout/default.cfm

The review of historical water quality data focuses on a review of the data collected by the Miami Dade County Health Department and the Miami Dade County Department of Environmental Resources Management.

1.7.1 Miami-Dade County Health Department (MDCHD) Beach Monitoring Data:

These data are reviewed and evaluated for two different periods. The first period corresponded to July 1999 to June 2000 and the second from August 2000 to August 2006 . During the first period ten sampling sites or beaches were monitored biweekly and tested for total and fecal coliforms and enterococci. The 10 study sites included sampling points at Hobie Cat, Crandon, and Cape Florida Beaches, all of which are located in the southern part of Biscayne Bay (Figure 1.2). Seventy-two enterococci and 122 coliforms (total and fecal) samples were collected from the three beaches. All coliform samples met the FDEP Water Quality Standards for Class III Recreational Waters, which are 2,400 CFU/100ml for total coliforms and 800 CFU/100ml for fecal coliforms for a single day sample. Out of the 72 enterococci samples (24 from each beach), 9 samples were classified as corresponding to "poor" water quality because they exceeded the EPA guideline of 104 CFU/100ml and whereas an additional 8 exceeded the "moderate" water quality limit of 34 CFU/100ml. Seven out of the 9 "poor" water quality samples were collected from Hobie Cat Beach alone and the remaining two were one each from Cape Florida and Crandon Beaches. Out of the 8 "moderate" samples, 4 were from Hobie Cat Beach and the rest were from Crandon Beach. Note, those exceedances did not occur on the same days. During this monitoring period Hobie Cat Beach was out of compliance for good water quality 29.2% of the time and exceeded the

moderate water quality guidelines 16.7 % of the time. On the other hand, enterococci concentrations at Crandon Beach exceeded the good and moderate range only 4.2% and 16.7% of the times respectively. The out of compliance rate for exceeding the good range for the 10 sites (including Hobie Beach) was at 14.2% and for exceeding the moderate range was 6.7%.

The second sampling period (August 2000 to present, data is analyzed up to August 2006) was part of an on-going Florida Healthy Beaches Monitoring Program. Fifteen beaches were monitored biweekly until July 2002 and weekly from August 2002 to present. Enterococci and fecal coliforms have been the official bacterial indicators used to test the microbial water quality at those selected beaches. Total coliform was dropped as an indicator during this second period, although DERM and FDEP still use this indicator along with fecal colifrom to test the microbial water water quality for Class III recreational water bodies. Six of the 15 beaches were located in the southern part of Biscayne Bay: Hobie Cat, Virginia, Matheson Hammock, Key Biscayne, Crandon, and Cape Florida Beaches (Figure 1.2).

Within the second sampling period, A total of 8,508 enterococci (4,254) and fecal coliforms (4,254) samples were collected from all 15 beaches in Miami Dade County. Review of the data (Table 1.4) indicates that: a- Hobie Cat Beach and Crandon Beach exceeded the USEPA recommended recreational water standard, enterococci for a single day sample 6% of the times. They ranked # 3 after North Shore Ocean Terrace Beach ranked #1 at 10% exceeding standards followed by 53rd Street, Miami Beach, ranked #2 at 7%. The overall county average exceeded the same standard over the same sampling period 4% of the times, b- Hobie Cat Beach exceeded the FDOH regulatory recreational

water standard, fecal coliforms, 7% of the times. It ranked #1 followed by Crandon Beach and Matheson Hammock #2 at 5%. The overall county average exceeded the same standard over the same sampling period 3% of the times, c- During this sampling period, the Miami Dade County Health Department issued to Hobie Cat Beach the most number of beach advisories (7) associated with 35 beach advisory days respectively. It ranked #1 followed by Crandon Beach 6 beach advisories with 19 beach advisory days, and Sunny Isles and North Shore Ocean Terrace Beach each 5 beach advisories with 34 and 23 beach advisory days respectively, and d- while the means enterococci and fecal coliforms for the rainy season (39 cfu/100ml, 145 cfu/100ml) were approximately 1.4 and 1.5 times greater than the means for the dry season (28 cfu/100ml and 94 cfu/100ml) respectively, seasonal effect showed no significant difference for both indicators (Table 1.5). The statistical test (t-Test: Two-Sample Assuming Equal Variances Statistic; confidence level 95%, p less than 0.05) was run for this analysis.

Looking at the same set of data from the perspective of before and after the 2 septic tanks were disconnected from the bathroom facilities at Hobie Cat Beach (December, 2002), it was observed that the mean fecal coliforms and enterococci for a single day sample after the removal of the tanks were 154 cfu/100ml and 38 cfu/100ml respectively (Table 1.6), approximately 6 and 1.7 times greater than the mean fecal coliforms and enterococci respectively before the removal of the tanks. In addition, the statistical test (t-Test: Two-Sample Assuming Equal Variances Statistic; confidence level 95%, p less than 0.05) conducted on the 2 sets of data before and after the septic tanks were removed, indicated that both fecal coliforms and enterococci showed no significant statistical difference before and after the tanks were removed (Table 1.6). This finding

thus questions the validity of the use of these indicators (enterococci and fecal coliforms) to predicate whether or not the beach is contaminated with human waste and refutes the hypothesis which is the water quality at the beach should improve after the removal of the septic tanks, a source for human waste.

1.7.2 Miami–Dade County Department of Environmental resource Management (DERM) Surface Water Quality Data:

DERM's (Miami-Dade County Department of Environmental Resources Management) data were reviewed and evaluated from July 1999 to present. It is important to note that DERM does not target the beaches; it tests the waters in Biscayne Bay, Miami River, and its tributaries. DERM's sampling efforts are intended to identify contamination sources (microbial, nutrients and chemical contaminants) in the Biscayne Bay drainage basins. This review includes only DERM's sampling stations surrounding Hobie Beach (Figure 1.3). At the request of the Miami-Dade County Health Department, DERM amended its sampling sites to add 6 stations in the vicinity of Hobie Beach. Five are located around the Miami Seaquarium at known outfalls and one station is located close to Hobie Beach's shore (but not in the swimming area). During this period 247 samples were collected from the DERM stations and analyzed for total and fecal coliform. The Miami-Dade County standards for these indicators in marine waters and for a single day sample are 1000 CFU/100ml for total coliforms and 200 CFU/100ml for fecal coliforms. Out 247 samples only 13 exceeded the County's surface water quality standards for coliforms, 4 out of the 13 exceedances occurred in November of 1999 and were collected near to the Port of Miami. The remaining nine exceedances occurred in the first week of October 2000 and were collected near Hobie Beach and the Miami

Seaquarium. All 13 violations were linked to heavy rains and flooding conditions. The October 2000 sampling event was characterized by 12.1 inches of rainfall (as measured at the Miami International Airport station) during the 48 hours prior to sampling.

At the request of the Miami Dade County Health Department, DERM began testing the surface water for enterocooci that in addition to their regulatory indicator microbes fecal and total coliforms. This change to DERM monthly sampling program affected only the Hobie Cat Beach Swim Buoy Line Station (HBE). Review of DERM's enterococci results from 2000 to 2006 of the Hobie Cat Beach Swim Buoy Line Station (HBE), Table 1.7 indicates that the water quality at this station meets the FDOH enterocci standards most of the time, the standards was exceed only once on March 6, 2003. There was no apparent reason for this spike. Note 40 samples were collected and analyzed during this period. 39 of the samples all met the standards and consistently were at least 2 orders of magnitude lower than the regulatory standards for a single sample. This finding supports the assumption of the human shedding study reviewed in Chapter 2 that the source water quality is characterized as good water quality. DERM's results are in agreement with the source water results obtained during the human shedding studies.

Preliminary Review: Data from the Department of Health monitoring suggest that on a day-to-day basis the beaches meet standards for total and fecal coliforms. DERM's data suggest that exceedances for total and fecal coliform occur during extreme rainfall events, only. The beaches do not always meet EPA guidelines for enterococci, even during dry conditions.

1.8 Suspected Sources of Sewage Contamination at Hobie Cat Beach:

This section describes the main suspected sources of sewage contamination impacting the beach and located within its drainage basin including sewage spills.

1.8.1 Sewage Spills:

Records review of state and local regulatory agencies (FDOH, DERM, and Miami Dade Water Sewer Department, WASD) from year 2000 to 2006 (calendar year) indicated that there was one incident of sewage spill within the study site water shed basin. On March 16, 2001, the 72" sub-aqueous force main conveys raw sewage from Central Miami Dade (approximately 150 MGD) to the Central District Wastewater Treatment Plant (CDWWTP) ruptured and released millions of gallons of raw sewage into Biscayne Bay just east of the mouth of Miami River. According to the same record, WASD's Central Lift Station (on NW 4 st.) that pumps sewage into the affected force main surcharged into the Miami River. As a result, the MDCHD issued beach advisories to all beaches in Key Biscayne including Hobie Cat Beach. The advisory was lifted on March 18, 2001, after the line was repaired and DERM's and the MDCHD's ambient and beach water testing results were satisfactory. Only two ambient water stations exceeded standards Figure 1.5. Since 2002 the 72" force main has been out of service (only to be used during emergencies), it was replaced by the new 102" force main which is laid on the Bay's floor parallel to the decommissioned 72" force main Figure 1.4.

1.8.2 Miami River

According with DERM's data, while the water quality in the Miami River is still out of compliance with the County standards (Figure 1.3), it has significantly improved. This improvement has been documented over the past five years which is attributed to the ongoing capital improvement program for the County's sewage collection system which includes upgrades to the sewage lift stations, increasing the system's conveyance capacity, completing the 102" force main crossing the bay to the CDWWTP and replacing old and decaying sewer pipes and through DERM's efforts to identify potential contamination sources and eliminate and/or reduce them i.e. combined sewer overflows (CSOs). Also, the storm drain outfalls will continue to play a significant role in bringing contamination including microbiological contaminants into the bay. This is due to alleged illegal hookups between the sanitary system and sewer pipes; sewage overflows from manholes after heavy rain events or tropical storms, leaky sewer pipes, CSOs and others. The Biscayne Bay Partnership Initiative 2001 Final Report to the Florida Legislature indicated that Biscayne Bay still receives chemical and biological contaminants. This is through storm water runoff from agricultural and urban land uses, canal discharge and discharges from industrial facilities and vessels.

1.8.3 Miami Seaquarium

There are a total of 6 outfalls from the Miami Seaquarium that drain water from the exhibit tanks. These outfalls are labeled "A" through "F". Details concerning these outfalls are as follows:

Outfall "A": 36-inch pipe from the manatee lagoon. Discharge of 1500 gpm.

Outfall "B": 6-inch pipe from the manatee tank. Discharge of 200 gpm.

Outfall "C": 36-inch pipe from the reef fish tanks, the dolphin tanks, and the manatee Pools and the satellite pool. Discharge of 2100 gpm.

Outfall "D": 24-inch pipe from the fish, turtle, and bird area. Discharge of 1000 gpm.

Outfall "E": 12-inch pipe from the training tanks. Discharge of 1000 gpm.

Outfall "F": 8-inch pipe from the sea lion golden dome. Discharge of 200 gpm.

Water that is withdrawn from Biscayne Bay is immediately treated via sand filtration prior to use within the exhibit tanks in order to protect the sea life contained within the tanks. In some mammal tanks, the water after filtration is chlorinated prior to entering a particular tank. However, the effluent from these tanks does not receive treatment before discharge into the bay, so fecal waste from the wildlife contained in the tanks is discharged to the bay without treatment. DERM's data indicate good water quality leaving those outfalls; only after extreme heavy rain events at some outfalls was the County's water quality standard exceeded. Most of the "human" sanitary system for the facility appears to be connected to the County's sewer system. The primary exception is the concession building that according to plans appears to be connected to a septic tank. Mr. German Hernandez, a consultant for the Miami Seaquarium, indicates that this facility, however, is connected to sanitary sewer. Field-testing and inspection are necessary to confirm the method of sewage disposal from this building. Also according to Mr. German Hernandez, all storm water runoff generated onsite is drained

via a French Drain system. None of the tank outfalls are interconnected with the storm drainage system.

1.8.4 Miami-Dade County Central District Wastewater Treatment Plant (CDWWTP)

The permitted capacity of the CDWWTP is 143 mgd. Of this amount, roughly 100 mgd are generated from central Miami-Dade County and conveyed to the treatment plant via two sub-aqueous force mains, the old 72" and the new 102" pipes, which are routed across the bay (Figure 1.4). 35 to 40 mgd of raw sewage are transmitted daily to the treatment plant via the sub-aqueous 54" force main that also crosses the bay. This force main carries the daily sewage generated from five coastal municipalities including Fisher Island. On the Rickenbacker Causeway there is a 16" force main that transmits approximately 5 mgd of sewage generated from the Village of Key Biscayne and all the facilities located along the causeway. The CDWWTP treats the wastewater using primary and secondary treatment. The effluent is discharged through a 72" outfall located 2.5 miles into the Atlantic Ocean. The effluent is chlorinated immediately prior to discharge.

1.9 Dissertation Overall Objective and Tasks:

The primary objective of this study is to characterize and quantify non-point sources of enterococci to the study site, Hobie Cat Beach located in South Florida, a sub-tropical environment. This information will be included within a simple water quality model to evaluate the relative importance of each of the sources. Specifically two main tasks were completed as part of this study: 1-Designed and implemented two human shedding field studies to estimate the concentrations of enterococci and *Staphlococcus aureus* shed directly off the skin of bathers and the amount of beach sand and the corresponding concentration of enterococci that can be transported by bathers into the

water column, and 2- Developed and quantified the algorithms for simulating non-point sources of enteroccoci including sand, dogs, birds, water runoff, and bathers. This information was used to develop and calibrate a simple "box" water quality model to evaluate the relative importance of each of the sources under various loading scenarios. Data from the sanitary survey (Shibata et al., 2004) and environmental monitoring efforts (Wright et al., 2005) were utilized to quantify the non-point source functions.

1.9.1 Tasks and Hypotheses:

The two main tasks outlined in this section are considered necessary steps for achieving the dissertation overall objective.

Task 1: Designed and implemented two human shedding field studies to estimate the concentrations of enterococci and *Staphlococcus aureus* shed directly off the bodies of bathers and the amount of beach sand and the corresponding concentration of enterococci that can be transported by bathers into the water column

H1: Bathers shed directly from their skin significant concentrations of *Staphlococcus aureus* and enterococci into the water column

H2: The enterococci contribution from sand adhered to bathers' skin is relatively smaller than the amount shed directly from the skin

Task 2: Developed and quantified the algorithms for simulating non-point sources of enteroccoci including sand, dogs, birds, water runoff, and bathers. This information was used to develop and calibrate a simple "box" water quality model to evaluate the relative importance of each of the sources under various loading scenarios.

Data from the sanitary survey (Shibata et al., 2004) and environmental monitoring efforts (Wright et al., 2005) were utilized to quantify the non-point source functions

H1: The five non-point sources identified in Task 2 are the most dominant enterococci source at the study site

H2: The study site is not impacted by a point source that contributes enterococci

H3: The predicted values of enterococci concentrations obtained from the model runs for various loading scenarios calibrates well with those concentrations obtained from earlier environmental studies (Shibata et al., 2004, and Wright et al., 2005) conducted at the study site

H4: Dogs contributes the most amount of enterococci followed by water run-off, beach sand within the inter-tidal zone, bathers, and birds respectively

Table 1.1 Tabulated Summary of Environmental Studies

Author(s)	Study Location(s)	Objectives	Findings	Conclusions
Fujioka and Byappanahalli, 1996 & 1998; Fujioka, 1983; Fujioka and Shizumura, 1985; Fujioka et al., 1988 & 1999; Hardina and Fujioka, 1991	Hawaii	Examine the validity and applicability of the USEPA recommended fecal indicators (fecal coliforms, *E. coli* and enterococci) in determining the hygienic water quality in subtropical/ tropical regions of the world.	In the absence of any known sources of human/animal waste, fecal indicators are consistently present and recovered in high concentrations in the environment (fresh water streams, vegetation, soil/sediment and storm drains).	Use of the USEPA recommended fecal indicators to establish water quality standards in Hawaii and other Pacific Islands does not appear to be valid or appropriate.
Toranzos et al., 1987	Cloud rain forest in Puerto Rico	Determine the distribution, activity and survival of *Klebsiella pnuemoniae* and *E. coli* in a tropical environment	*K. pnuemoniae* and *E. coli* are naturally present in the pristine fresh waters and remain physiologically active thus; they can survive in the environment without a fecal source for a long period of time (approx. 5 days).	Use of fecal coliforms as indicators to measure the sanitation water quality in tropical waters like the waters of Puerto Rico might not be appropriate.
Rose et al., 1998	Charlotte Harbor, Florida	Determine distribution and seasonal changes in microbial indicators and human pathogens (fecal coliforms, enterococci, *Clostridium perfingens* and coliphage, enteric protozoa: *Cryptosporidium spp.*, *Giardia spp.* and enteroviruses) levels in Charlotte Harbor shellfish and recreational waters	Fecal indicators were found in high concentrations in areas of low salinity and high densities of on site sewage disposal systems Enterococci were shown to be highly correlated with the fresh water flows and proved to be a good indicator.	Coliphage accurately predicted the presence of enteroviruses. *Cryptosporidium spp.*, and *Giardia spp.*, were detected infrequently and was not associated with seasonal changes.
Griffin et al., 1999	Florida Keys (Upper, Middle and Lower).	Evaluate the impact of the domestic waste disposable practices (cesspools, septic systems and wastewater package plants) on the ambient water quality and to estimate the risk for human health	95% of the 19 sites (canals, beaches and near shore waters) tested positive for at least one group of enteric viruses: enteroviruses, hepatitis A and B, or Norwalk viruses.	Recreational and navigational waters in the Keys were negatively impacted by sewage disposal practices and that traditional/regulatory microbial indicators may not be adequate to assess this impact.
Rose et al., 2000	Philippi Creek and coastal beaches in Sarasota County, Florida.	Assess the water quality in the watersheds impacted by septic tank systems and evaluate the occurrence of enteric viruses along the public beaches in the county.	Fecal indicators (ranged from 5 to 4000 cfu/100ml) were highly correlated with areas impacted with high densities of on site sewage and disposal systems.	Waters in Sarasota Bay is contaminated with human pathogens and the mechanism by which the contaminants are transported to the Bay is the subsurface flow generated from the watersheds with high densities of septic systems.

Table 1.1 Tabulated Summary of Environmental Studies

Author(s)	Study Location(s)	Objectives	Findings	Conclusions
Solo-Gabriele et al., 2000	The New River, a coastal water way (brackish waters), in Ft. Lauderdale, Florida	Identify and evaluate the sources of high E. coil concentrations in the river waters	Soils of the riverbanks contribute a significant amount of E. coil in the water column, there was an instantaneous increase in E. coli densities during rainfall events. It was also found that the E. coli concentrations in the water column fluctuate with the tidal cycles; it increases with high tide and decreases during low tide.	Questioned the suitability of using E.coli to test the microbial water quality in tidally influenced areas located in subtropical/tropical region of the world.
Desmarais et al., 2002	The New River in Ft. Lauderdale, Florida	Studied the environmental factors that influence the survival and regrowth of E. coli, enterococci and Clostridium perfingens in the sediment and soil along the riverbanks	E. coli and enterococci were capable of multiplying when sterile sediments were added and under tide simulation whereas C. perfingens was not capable of multiplying in either experiment	Use of the traditional fecal indicators to assess the hygienic water quality in a subtropical/tropical environment is still doubtful
Shibata et al., 2004	Hobie and Crandon, located in southern part of Biscayne, Miami, Florida	Evaluate the microbial water quality including soils at the selected beaches and the bay using the regulatory microbial indicators (total and fecal coliforms, E. coli and enterococci) and Clostridium perfingens .	Intensive spatial water quality monitoring indicated the southern tip of the shoreline at Hobie Beach appears to be the source of microbes, this finding was supported by the soil sample results collected from this end of shoreline	The detection of those indicators in the soils/vegetation of the shoreline without a known point source fecal pollution again questions the suitability of those indicators for measuring the sanitation water quality in subtropical/tropical climates.
Nova Southeastern University 2001-2003	Hobie beach, Hollywood and Fort Lauderdale beaches, South Florida	Main objectives: 1-document the numbers of E coli, enterococci and fecal coliforms in beach sand and determine if they are attached or free in interstitial water, 2-compare the survival of indicator organisms in water versus sand.	Concentrations of bacteria indicators were higher in dry sand, followed by wet sand (swash zone) and followed by seawater. Majority of indicators were attached to sand grains i.e. they were metabolically active.	Swash zone receives significant bacterial inputs from the beach. Sediment re-suspension plays significant role impacting bacterial loading in the water column.

Table 1.2 Tabulated Summary of Human Health Data

Author(s)	Study Location	Study Design & Indicators	Findings	Conclusions
Cabelli et al. 1972-1979, sponsored by the USEPA.	conducted at three beaches in the USA: New York City, NY; Lake Pontchartrain, New Orleans, LA; and Boston Harbor, MA.	Prospective epidemiological studies, aproximately 26, 686 total usable responses from all beaches over the 3-year studies. Enterococci, *E.coli*, *Klebsiella*, Enterobact-Citrobacter, Total coliforms, *C. Perfingens, P. aeruginosa*, fecal coliforms, *A. hydrophila, V. parahaemolyticus* and Staphylococci were the indicators used for those studies.	Fecal coliforms, the indicator originally recommended in 1986 by the Federal Water Pollution Control Administration of the Department of Interior, showed less correlation to swimming – associated gastroenteritis than some other indicator organisms. *E. coli* showed strong correlation in fresh waters where as Enterococci showed strong correlation both in fresh and marine waters.	The strong correlation may be a result of the survivability of the indicator organisms in the environment being similar to the survivability of the pathogens of concern. And, enterococci 's resistance to environmental factors, particularly saline environments, enhancing its ability as a suitable indicator for marine waters.
Fattal et al., 1987	at three beaches marine waters with different water qualities, Tel-Aviv Israel	*E. coli*, fecal coliforms and enterococci were used to evaluate the microbial water quality.	Out of the three indicator-microbes tested, enterococci were the best indicator to predict GI illnesses among swimmers.	This finding agreed with the EPA epidemiological studies conducted by Cabelli et al. in marine waters.
Cheung et al., 1990	at nine of the polluted (human waste discharge) beaches (marine waters), Hong Kong.	*19,000 individuals participated in the study. Nine microbial indicators were used to evaluate the water quality; fecal coliforms, E. coli, Klebsiella spp., fecal streptococci, enterococci, staphylococci, Pseudomonas aeroginosa, Candida albicans, and total fungi.*	The strongest correlation between swimming related health effects and an indicator density was between E. coli and highly credible gastrointestinal (HCGI) symptoms.	This finding does not agree with the EPA epidemiological studies conducted by Cabelli et al. in marine waters.
Balharajan et al., 1991	United Kingdom	*Study that described the health risks related with exposure (wading, swimming, surfing and diving) to marine waters. 1,883 individuals participated in the study. Information was not provided as to the parameters/ indicator microbes used to evaluate the water quality at the study site.*	The rate of enteric disease symptoms was significantly greater among bathers than non-bathers. The health risk for surfers/divers was approximately 1.4 times greater for swimmers and 1.5 times for waders.	The increase or decrease in health risk was concluded to be a function of type and degree of exposure.

Table 1.2 Tabulated Summary of Human Health Data

Author(s)	Study Location	Study Design & Indicators	Findings	Conclusions
Von Schirnding et al., 1992	at two beaches off the Atlantic coast of South Africa	*733 individuals participated in the study. One of the beaches was relatively clean the other was considered to be moderately polluted due to failing septic tank systems and water run – off. Enterococci, fecal coliforms, coliphages and staphylococci were among the indicator microbes tested.*	It was reported that there was a considerable increase in GI illness rates among swimmers than non swimmers at the moderately polluted beach as oppose to the relatively clean beach.	It was concluded that there is increase in health risks among individuals exposed to polluted waters in comparison with individuals exposed to moderately polluted waters.
Corbett et, al., 1993	at the beaches (marine waters) in Sydney, Australia	*Conducted a study to assess the swimming related illnesses, 2,869 individuals participated in the study. Only fecal coliforms and fecal streptococci were used to measure the microbial quality of the waters.*	It was found that individuals who swam for more than 30 minutes, their risk of reporting GI symptoms increased by 4.6 times than those who swam less than 30 minutes	This study showed similar results with the EPA beach water studies in that increasing GI illness rates were not associated with increasing fecal coliforms densities.
Kay et al., 1994	Beaches in the United Kingdom.	*1,112 individuals participated in the study of which 512 were assigned to the swimmers group. The study was a randomized controlled epidemiological study. The microbial water quality was tested using total and fecal coliforms, fecal streptococci, total staphylococci and Pseudomonas aeruginosa*	Results of the study indicated that GI illness rates among swimmers were appreciably greater than non-swimmers. Out of the 4 indicator microbes, fecal streptococci were the best predictor for GI illness symptoms.	This finding agreed with the EPA epidemiological studies conducted by Cabelli et al. in marine waters.
Fujioka et al., 1994	Hawaii	Individuals participating were classified in three distinct groups: non-swimmers, swimmers who did not swallow water, and swimmers that did swallow water.	Study did not find any associations between the five microbial indicators (fecal coliforms, *E.coli*, enterococci, bacillus spores, and *Clostridium perfringens*) analyzed and human health effects.	This finding does not agree with the EPA epidemiological studies conducted by Cabelli et al. in marine waters

Table 1.2 Tabulated Summary of Human Health Data

Author(s)	Study Location	Study Design & Indicators	Findings	Conclusions
Kueh et al., 1995	In Hong Kong	Analyzed the bacteriological aspect of water quality and examined how physico-chemical parameters such as air and water temperature and turbidity may contribute to changes in microbial count and therefore bathing related illness. Samples were analyzed for three bacterial indicators (*E.coli*, fecal coliforms, and staphylococci) and seven pathogenic bacteria (*Aeromonas spp., Clostridium perfringens, Vibrio cholerae, V. parahaemolyticus, V. vulnificus, Salmonella spp.*, and *Shigella spp.*).	In this study *Clostridium perfringens* and *Aeromonas spp.* showed a significant correlation with GI and HCGI symptoms, while *V. cholerae and V. parahaemolyticus* were best associated with GI but not HCGI symptoms.	Swimmers were in general two to three times more likely to develop illnesses than non-swimmers (swimmers only included those who wet their faces). The study also showed a strong correlation between water turbidity and GI and HCGI symptoms.
Pruss, 1998	The majority of these studies were conducted in the US and UK, with few studies evaluated in tropical marine recreational waters.	reviewed all significant existing epidemiological studies on the health effects from exposure to recreational water.	The indicator organisms that correlated best with the health outcomes were enterococci/fecal streptococci for marine and freshwater, and *E. coli* for freshwater.	Review found that most studies reported a dose related increase of health risk in swimmers with an increase in the indicator bacteria count in recreational water.
Fleisher et al., 1998	In 4 separate United Kingdom beaches during the summers of 1989 to 1992.	*This particular study focused on how domestic sewage contamination pollutes marine waters and affects public health.*	The results showed that the rates of illness (gastroenteritis, acute febrile respiratory illness, and eye and ear infections) among bathers were statistically significantly higher in relation to non-bathers.	The study showed a dose- response relationship between exposure and contaminated waters (among the bathers cohort, 34.4% to 65.8% of the adverse health conditions reported were considered a direct result of bathing in sewage contaminated marine waters)
Haile et al., 1999	Santa Monica Bay, County of Los Angeles.	Over 22,000 persons were interviewed 9 days after their facial immersion exposure to recreational beach waters concerning their symptoms. From the 22,085 subject interviewed, 17,253 fulfilled the eligibility criteria, 15,492 agreed to participate, and from those 13,278 were contacted during follow-up.	An increased risk of adverse health outcomes associated with swimming in ocean water contaminated by untreated urban runoff was found with a significant dose response relationship.	An increased risk of adverse health outcomes associated with swimming in ocean water contaminated by untreated urban runoff was found with a significant dose response relationship.

Table 1.2 (continued): Tabulated Summary of Human Health Data

Author(s)	Study Location	Study Design & Indicators	Findings	Conclusions
Prieto et al., 2001	North of Spain	Established a cohort of 2,774 persons on 4 beaches in the north of Spain with follow up of 1,858 persons after 7 days from exposure for symptoms. Among those followed up, 135 (7.5%) experienced symptoms; visitors experienced symptoms more than residents, and symptoms were higher among bathers although not significantly	Gastrointestinal and skin symptoms correlated with total coliforms; an increased risk was observed with exposure to 2,500 to 9,999 total coliforms per 100 ml. This coliforms count was below the European Union mandatory limit, although over a new proposed standard.	This finding does not agree with the EPA epidemiological studies conducted by Cabelli et al. in marine waters.
Fleming et al., 2002	In South Florida, (Hobie and Crandon Beaches)	Conducted a prospective cohort epidemiological pilot study at 2 beaches. using multiple bacteria indicators (enterococci, total and fecal coliforms, *E. Coli and C. perfingens*).Final study population consisted of 63 families with 208 individuals. An epidemiological questionnaire was used to evaluate illness vs. exposure.	No significant association between the number and the type of reported symptoms and the different sampling months or beach sites. There was a negative correlation between the number of bacteria indicators and the frequency reported by beach goers. Results of the daily monitoring indicated that different indicators provided conflicting results concerning beach water quality.	Larger epidemiological studies with individual exposure monitoring are recommended to further evaluate these potentially important associations in subtropical recreational waters.
Nova Southeastern University 2001-2003	In South Florida: Hobie, Hollywood and Fort Lauderdale.	A voluntary beach questionnaire on three beaches was administered. Out of 10,000 surveys handed out only 892 experimental forms and 609 control forms were returned. Symptoms to be reported GI, upper respiratory, dermatological and constitutional.	Beach questionnaire didn't show clear signs of symptoms in the recreational population in comparison w/the control population. Questionnaire return was low around 10%.	A future and more comprehensive epidemiological study may be warranted.
University of California Berkeley School of Public Health and Southern California Coastal Water Research Project 2005	California: Six popular public beaches in Mission Bay	A cohort epidemiology study to evaluate the relationships between traditional indicators (enterococci, fecal coliforms, and total coliforms) and swimming- related illnesses. Nearly 8,800 participants were recruited for the study.	Only skin rash and diarrhea were consistently significantly elevated in swimmers compared to non-swimmers, especially among children 5 to 10 years old. The risk of illness was uncorrelated with levels of traditional water quality indicators. And the state water quality standards were not predictive of swimming – related illnesses	Traditional fecal indicators were ineffective predictors of health effects and there is a need for further evaluation of traditional indicators at beaches where non-point sources are the dominant fecal contributors

Table 1.3 Guidelines for Recreational Marine Waters

Indicator Microbes	Guidelines	Guideline or Criteria Developed By:
E. coli	Not recommended for marine waters. For freshwater a geometric mean of 126/100 ml and 235/100 ml on a single day.	USEPA (1986)
Enterococci	A geometric mean of 35/100 ml and 104/100 ml on a single day	USEPA (1986) FDOH(present)
Fecal coliform Bacteria	400/100 ml for a single day sample. Monthly average or geometric measure doesn't apply to this indicator.	FDOH(present)
Fecal coliform Bacteria	Fresh and Marine Class III Waters: MPN and MF counts shall not exceed a monthly average of 200, nor exceed 400 in 10% of the samples, nor exceed 800 on any one day. Monthly averages shall be expressed as geometric means based on a minimum of 10 samples taken over a 30 day period.	USEPA (1976) FDEP
Total coliform Bacteria	Fresh and Marine Class III Waters: <or= 1,000 as a monthly average; nor exceed 1,000 in more than 20% of the samples examined during any month;<or=2,400 at any time. Monthly averages shall be expressed as geometric means based on a minimum of 10 samples taken over a 30 day period, using either the MPN or MF counts.	USEPA (1976) FDEP
C. perfringens	A geometric mean of 5/100 ml (open ocean) A geometric mean of 50/100 ml (Interior waters)	Suggested in Hawaii

Table: 1.4 Data analysis for the Miami-Dade County Beach Monitoring Program for all beaches from August 2000 to August2006

Beach Sampling Location	Number of Samples	Number of Enterococci Poors	Number of Fecal Coliforms Poors	Enterococci% Poor	Fecal Coliforms % Poor	Number of Advisories	Number of Advisory Beach Days
N. Shore Ocean Terr. Beach	299	30	11	10%	4%	5	23
53rd St.-Miami Beach	292	19	13	7%	4%	3	11
Hobie Cat Beach	296	19	21	6%	7%	7	35
Crandon Beach	293	17	16	6%	5%	6	19
Sunny Isles Beach	293	16	12	5%	4%	5	34
S.Beach-1st St.-Miami Beach	280	13	6	5%	2%	3	6
Haulover Beach	283	13	8	5%	3%	3	20
Surfside Beach	278	9	3	3%	1%	3	12
Key Biscayne Beach	279	9	7	3%	3%	1	5
Oleta State Park	272	6	3	2%	1%	1	7
Golden Beach	276	6	2	2%	1%	0	0
Matheson Hammock	283	5	14	2%	5%	3	9
Cape Florida Beach	272	4	3	1%	1%	0	0
Virginia Key Beach	272	4	3	1%	1%	2	6
21st St.-Miami Beach	286	1	2	0%	1%	0	0
Avg. Miami Dade Beaches	284	11	8	4%	3%	3	12

Table: 1.5 Statistical analysis for Hobie Cat Beach water quality monitoring data from August 2000 to August 2006. Evaluating the seasonal effect on enterococci and fecal coliforms

	Rainy Season	Dry Season		Rainy Season	Dry Season
	Enterococci, cfu/100ml	Enterococci, cfu/100ml		Fecal Coliforms, cfu/100ml	Fecal Coliforms, cfu/100ml
	Variable 1	*Variable 2*		*Variable 1*	*Variable 2*
Mean	39	28	Mean	145	94
Variance	9578	8456	Variance	130900	96617
Observations	151	139	Observations	153	140
Pooled Variance	9040		Pooled Variance	114525	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
Df	288		df	291	
t Stat	1		t Stat	1	
P(T<=t) one-tail	0		P(T<=t) one-tail	0	
t Critical one-tail	2		t Critical one-tail	2	
P(T<=t) two-tail	0		P(T<=t) two-tail	0	
t Critical two-tail	2		t Critical two-tail	2	
t-Test: Two-Sample Assuming Equal Variances:			$t_{Critical}$ greater than t_{Stat}		

Table: 1.6 Statistical analysis for Hobie Cat Beach water quality monitoring data from August 2000 to August 2006. Evaluating the effect of removal the septic tank systems on enterococci and fecal coliforms

	Before Dec.02	**After Dec.02**		**Before Dec.02**	**After Dec.02**
	Enterococci, cfu/100ml	**Enterococci, cfu/100ml**		**Fecal Coliforms, cfu/100ml**	**Fecal Coliforms, cfu/100ml**
	Variable 1	***Variable 2***		***Variable 1***	***Variable 2***
Mean	23	38	Mean	25	154
Variance	3208	11152	Variance	5223	148997
Observations	78	212	Observations	76	217
Pooled Variance	9028		Pooled Variance	111942	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
Df	288		df	291	
t Stat	-1		t Stat	-3	
P(T<=t) one-tail	0		P(T<=t) one-tail	0	
t Critical one-tail	2		t Critical one-tail	2	
P(T<=t) two-tail	0		P(T<=t) two-tail	0	
t Critical two-tail	2		t Critical two-tail	2	
t-Test: Two-Sample Assuming Equal Variances:			$t_{Critical}$ greater than t_{Stat}		

Table 1.7 DERM's Surface Water Quality Results at Hobie Cat Beach Swim Buoy Line Station (HBE)

Date	Enterococci CFU/100ml	Date	Enterococci CFU/100ml
10/10/02	2	9/9/04	2
12/5/02	5	10/7/04	2
2/6/03	1	11/4/04	2
3/6/03	150	12/9/04	4
5/8/03	2	2/10/05	2
6/5/03	2	3/10/05	10
7/10/03	2	4/7/05	10
8/7/03	2	5/5/05	10
9/11/03	2	7/14/05	10
10/9/03	2	8/4/05	10
11/6/03	2	9/15/05	2
12/4/03	2	10/6/05	10
1/8/04	2	11/14/05	10
2/5/04	2	12/8/05	10
3/4/04	2	1/12/06	10
4/8/04	2	2/9/06	10
5/6/04	2	3/9/06	20
6/10/04	2	4/6/06	10
7/8/04	2	5/4/06	10
8/5/04	2	6/8/06	10
Avg.	10		8

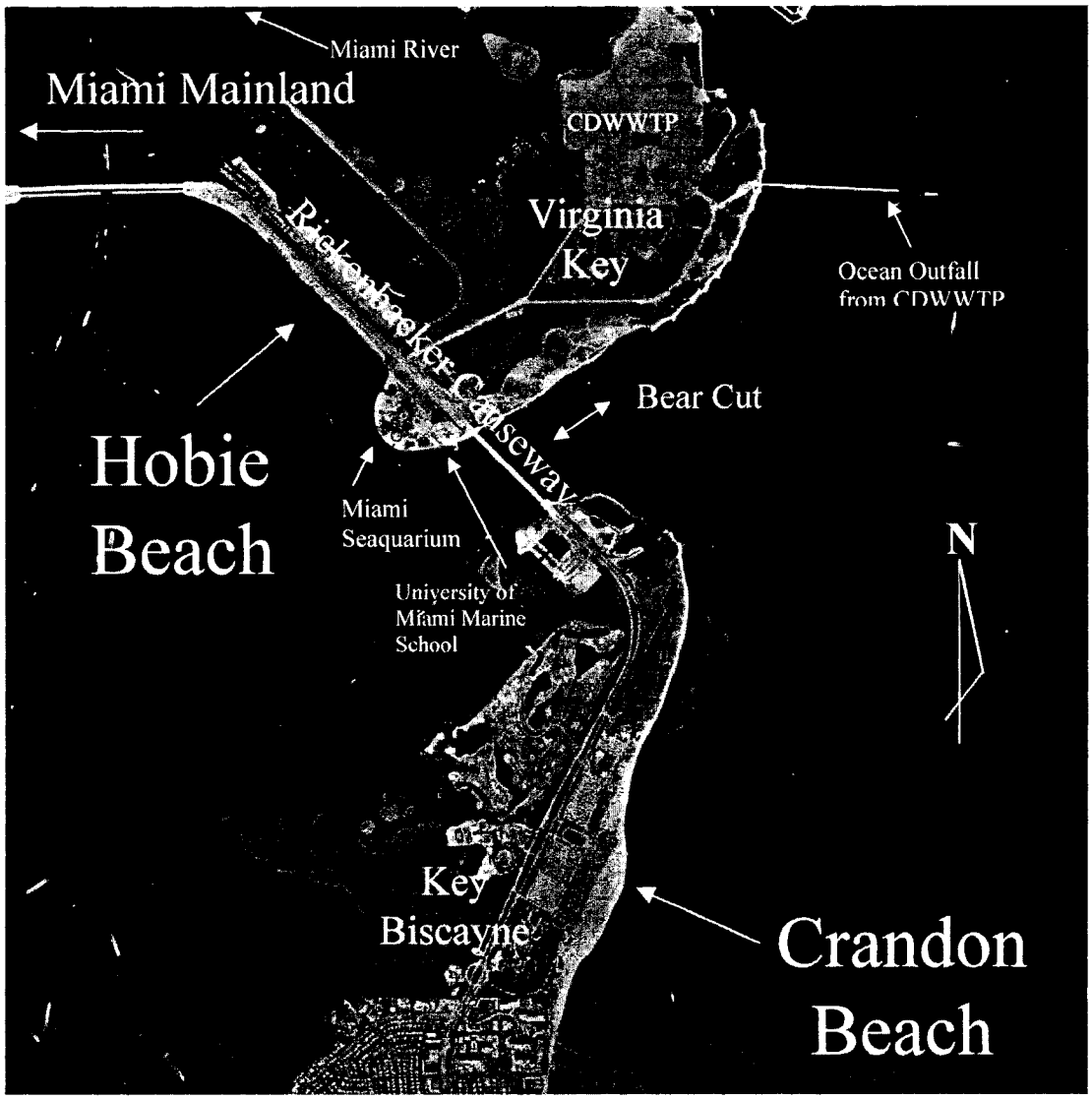

Figure 1.1 Location of Hobie Cat and Crandon Beaches

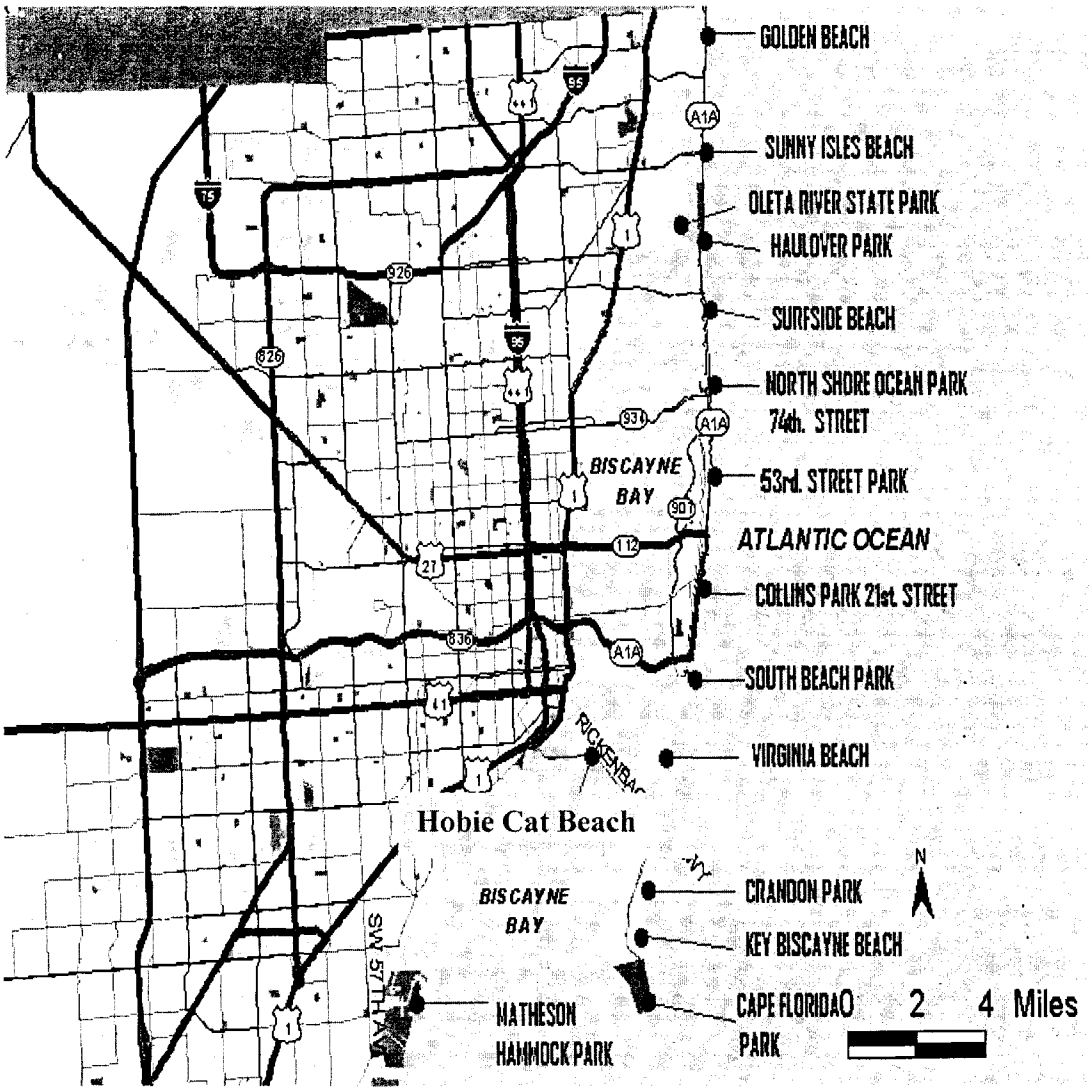

Figure 1.2 Miami Dade County Health Department beach sampling sites

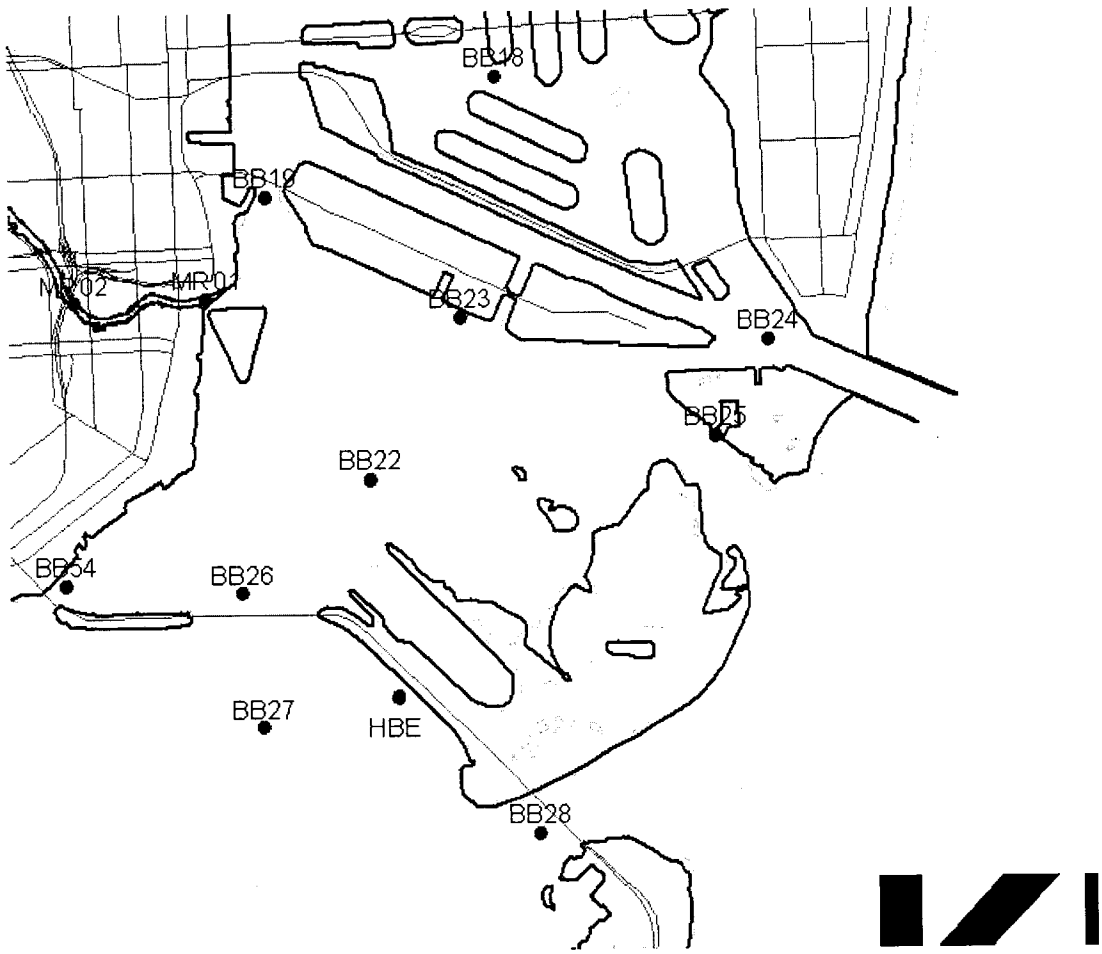

Figure 1.3 DERM's surface water sampling stations in Southern Biscayne Bay

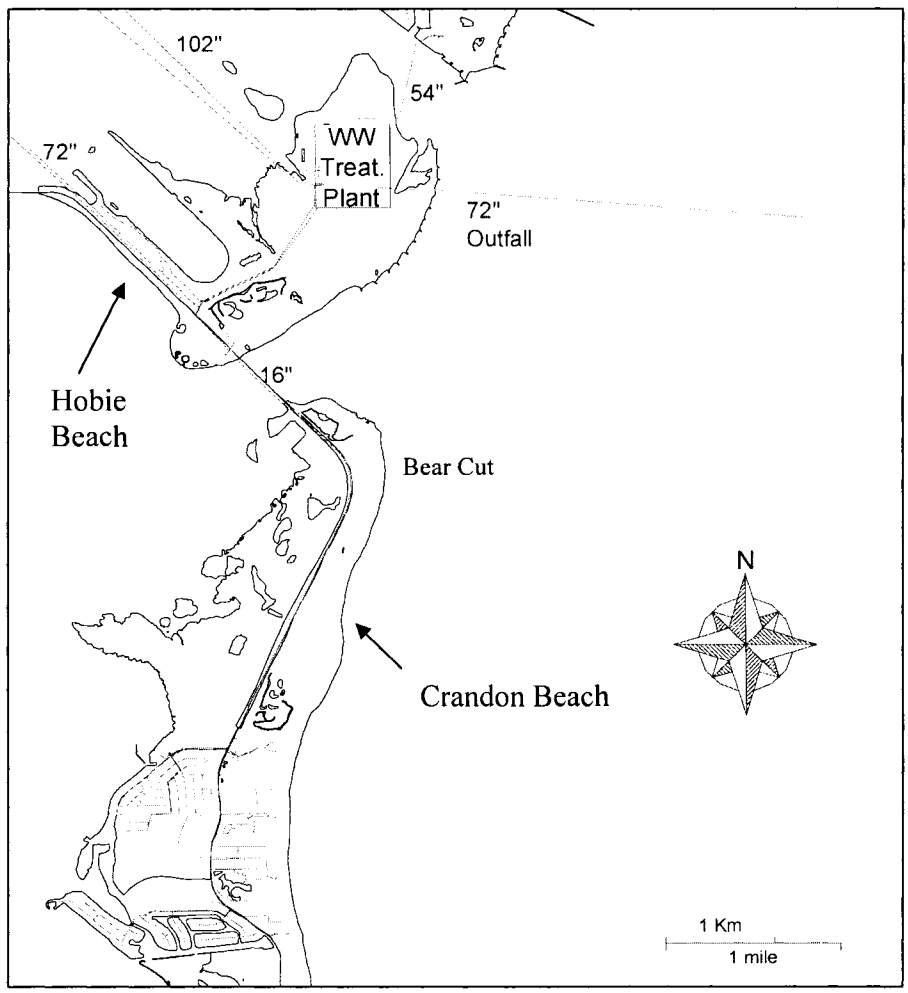

Figure 1.4 Sewer mains that serve the Miami-Dade Central District Wastewater Treatment Plant (CDWWTP)

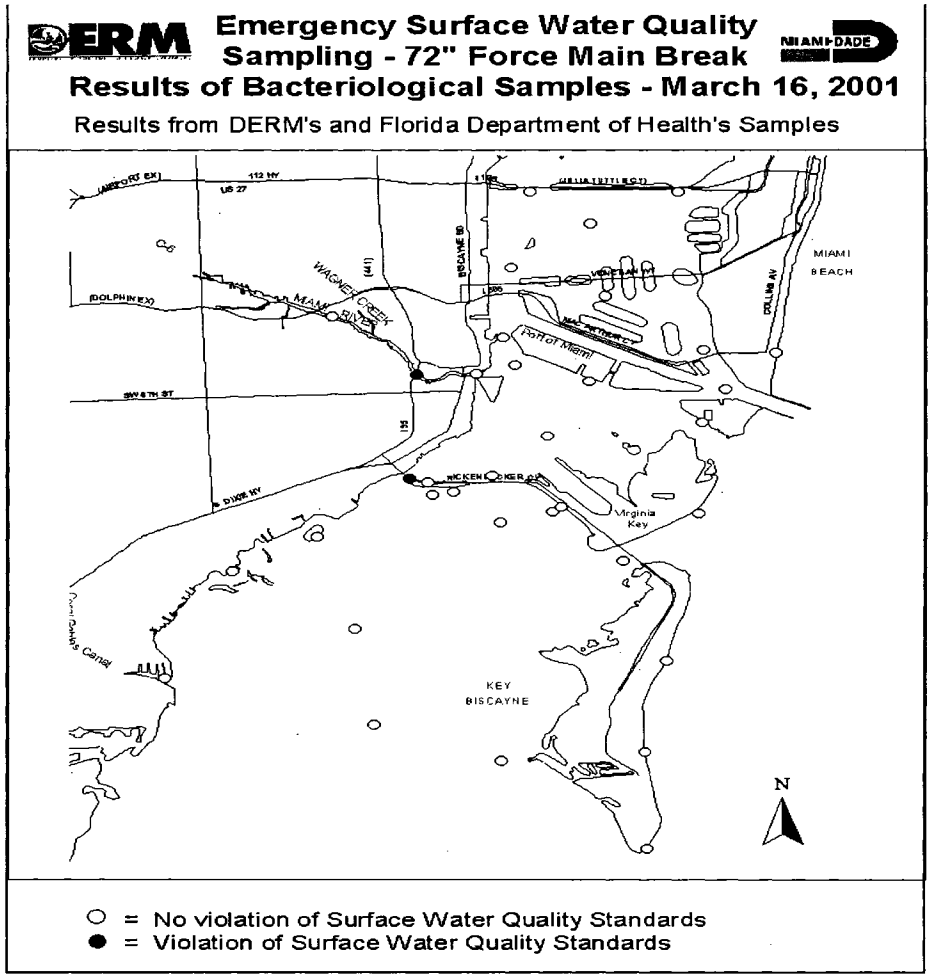

Figure 1.5 Results of DERM's and MDCHD's ambient and beach water sampling in response to the ruptured 72" sub-aqueous force main crossing Biscayne Bay on March 16, 2001

CHAPTER 2

HUMAN SHEDDING STUDIES

2.1 Introduction

Review of the design and implementation of the two human shedding field studies "Large Pool" study and "Small Pool" study are provided in this chapter. This includes background data, materials and methods, results and discussions, conclusions and recommendations.

2.2 Background

Beaches serve an important role in the U.S. economy. Coastal recreation is estimated to contribute approximately 85% of all U.S. tourist revenues (NRDC, 2005). However, this revenue depends upon the availability of coastal areas that are safe for recreational purposes. According to the latest surveillance of the U.S. Centers for Disease Control (CDC) (Yoder et al., 2004), the largest number of recreational water-associated outbreaks (65 outbreaks causing illness among an estimated 2,536 persons) occurred between 2001 and 2002. The National Resources Defense Council (NRDC, 2005) indicates that during 2004, U.S. beaches had 24,853 beach closing and advisory days, the highest in 15 years since the NRDC started reporting this data, a 9% increase from 2003. In 2004, 85% of the total closings and advisories were issued because water quality exceeded the recommended bacterial indicator standards for which the sources of contamination were not identified. The inability to identify sources, in particular when point sources of pollution are not obvious and/or not present, has made it difficult to remediate and prevent the impacts to beaches.

Bathers are considered a potential non-point source of contamination impacting recreational waters. Studies have found that bathers shed appreciable amount of microbes via their skin into the water column, and swimming related illnesses appear to be associated with the microbial water quality, even in the absence of point sources of fecal contamination. Mallman (1962) and Favero et al. (1964), suggested that large numbers of cocci are washed off the skin of bathers into freshwater swimming pools, and thus concluded that cocci are a valid indicator to measure the recreational quality. Calderon et al. (1991) found that gastrointestinal illnesses observed in swimmers were associated with higher numbers of bathers per day and high densities of *S. aureus*. Robinton and Mood (1966), Hanes and Fossa (1970), and Smith and Dufour (1993) concluded that high bacterial densities were shed by bathers into the water column, especially *S. aureus*. Finally, Gerba (2000) and Stewart et al. (2002) found that bathers shed pathogenic organisms via body contact and fecal accidents in drinking water reservoirs, and thus bathers increased the risk of water borne illnesses among drinking water consumers. Of note is that all of these studies were conducted in fresh waters and evaluated the effects of single washing events. Studies are lacking in marine waters and no studies to the authors' knowledge evaluated the effects from sequential bathing events.

Enterococci and *S. aureus* were the bacteria chosen for the current study. Enterococci are commonly found in the feces of humans and other warm-blooded animals. Although some Enterococcus species are also found naturally in the environment, the U.S. Environmental Protection Agency recommends the use of enterococci to measure potential fecal contamination in marine waters (US EPA, 1983; 1984; 2002). *S. aureus* is a Gram-positive coccus that commonly inhabits the anterior

nares of humans and *S. aureus* is considered one of the common causes of skin infections in the U.S. *S. aureus* can survive outside human hosts, and studies have shown correlations between *S. aureus* skin infections and swimming. Charoenca and Fujioka (1995), and Gabutti (2000) suggested that recreational waters characterized by high *S. aureus* densities may increase the risk of contracting skin, eye, and ear infections among bathers.

For over four decades, beach sands and sediments in tropical and subtropical environments have been documented to contain high concentrations of the bacterial indicators, *E. coli* and enterococci, and sand is one of the non-point sources of those indicators. Studies conducted in Hawaii and Guam (Fujioka, 1988; Fujioka and Roll, 1997; Fujioka et al., 1999), and in Puerto Rico (Toranzos and Marcos, 2000) have shown that in the absence of any known sources of human/animal waste, enterococci and *E. coli* are consistently present and recovered in high concentrations in the subtropical environment. Specifically in South Florida, river bank soils and beach sands have been implicated as the source of indicator microbes to the water column (Desmarais et al., 2002; Rogerson et al., 2003; Shibata et al., 2004). Recent evidence indicated that the significance of beach sands and other environmental sources is not necessarily limited to the sub/tropics. For example, sands have been implicated as a bacterial source in the freshwater beaches of Lake Michigan (Whitman and Nevers, 2003) and Lake Huron (Alm et al., 2006), both in Michigan. Given the high concentrations of indicator bacteria found in beach sands and sediments, bathers can contribute microbes to the water column by carrying sand on their bodies and washing it into the water column as they bath. Note, bacteria densities released from sand adhered to the bodies of bathers represent the total

indigenous bacteria attached to sand particulates and those from body contact. No studies have evaluated the contribution of sand carried by bathers as part of the bacterial load to a recreational water body.

In order to fill some of the gaps in human shedding studies, the current study focused on evaluating bacterial shedding in a marine water. Specifically, enterococci and *Staphylococcus aureus* were quantified by measuring the amount of bacteria shed by bathers directly off their skin and indirectly via sand adhered to skin. Experiments were conducted under controlled conditions where bathers were either washed or immersed in marine waters characterized by low indicator levels. Mass balance considerations were used to calculate the average colony forming units (CFU) of enterococci and *Staphylococcus aureus* per bather or per bather group.

2.3 Materials and methods:

This study was separated into 2 major efforts which are termed here as the "Large Pool" study and the "Small Pool" study. In the large pool study, microbial shedding from 10 volunteers as a group was evaluated. In the small pool study, microbial releases from individuals were evaluated, with a particular emphasis on measuring contributions from sand adhered to skin. Photographs illustrating the experimental set up are provided within the on-line supplemental information.

Work with the volunteers was approved by both the Miami Dade Department of Health Internal Review Board (IRB 1491) and by the University of Miami Internal Review Board (IRB 20057223). Consistent with IRB approval, consent forms were

signed by each volunteer and volunteer identity was kept confidential. All volunteers were from either Miami Dade County Health Department (MDCHD) staff or from the University of Miami (UM). Statistical differences between groups of data were computed using the t-Tests (Two-Sample Assuming Equal Variances at 95% confidence level, p=0.05).

2.4 Large pool field study:

This experiment was designed to estimate the amount of enterococci and *S. aureus* released from the bodies of bathers into the water column. Demographic characteristics of the 10 participants (7 males and 3 females) included age ranging from 18 to 50 years, and weight ranging from 52 to 91 kilograms. The experiment took place a few feet from the water line at the subtropical marine study beach, Hobie Cat Beach, located on Virginia Key, Florida. An inflatable pool (4700 liters) was first sanitized by wiping the pool with alcohol then the pool was filled with off-shore water using a gas powered pump from a point where water quality was consistently characterized by low concentrations of *S. aureus* and enterococci. Before each cycle, the complete water volume of the pool was emptied, the pool was resanitized, and then refilled with off-shore water. Volunteers wearing bathing suits went into the pool for four 15 minute cycles, and immersed themselves in the water up to their chest by sitting in the water. During each 15 minute cycle, the volunteers were asked to immerse their heads 3 times. During the first 2 cycles, volunteers were not exposed to beach sand. Volunteers used beach shoes prior to entering the pool and were asked to remain on a paved area between cycles 1 and 2 to prevent sand from touching their bodies. Volunteers entered the pool via a plastic

walkway between the paved area and the pool at which time they removed their shoes before entering the pool. In the last 2 cycles, volunteers were exposed to beach sand for 15 minutes before they entered the pool. Showers were not available at the beach site and so volunteers did not shower immediately before entering the pool or between cycles. During each cycle, pH, temperature, and salinity were measured using a field portable meter (YSI 600R series sonde,YSI Inc., Yellow Springs, OH) and water depth was measured using a ruler.

Before each cycle started, six 100mL water samples (3 for enterococci and 3 for *S. aureus* analysis) were collected from the pool after it was filled with off-shore water, and another two water samples (one for enterococci and one *S. aureus* analysis) were collected off-shore near the pumping location which served as a representative sample of source water quality. At the end of each cycle, the water in the pool was mixed by the volunteers walking around the inside of the pool and then six 100 mL water samples were collected (3 for enterococci and 3 for *S. aureus* analysis). Sample volumes used during analysis for enterococci and *S. aureus* were 50 mL for the samples collected before volunteers entered the pool and 10 mL for the samples collected after the volunteers exited the pool.

2.5 Small pool field study:

This experiment was designed to estimate the amount of sand transported on the bodies of bathers into the water column, and to estimate the enterococci concentration found in the transported sand. Two groups (Group I and Group II) of volunteers participated in this experiment which took place at the beach site. Each group consisted

of five volunteers for a total of ten volunteers. The field study for Group I and II was conducted during two different dates in July and August of 2005, respectively.

During this "Small Pool" study, each volunteer wearing a bathing suit spent 15 to 30 minutes on the beach sand (i.e. sitting, lying, playing, walking, etc). Thereafter, each volunteer was individually asked to enter a 190 liter tub that had been sanitized by wiping with alcohol followed by air drying. Each volunteer was rinsed with off-shore marine water using 3 pre-sanitized plant water containers (approximately 3 liters each). Initial water samples were collected prior to rinsing each subject.

After rinsing, water was collected for subsequent enterococci analysis and the total volume of water collected was measured. During water collection, the sand in the pool was directed towards one edge, and this sand was then placed via sterilized spoon into a pre-weighed and pre-sterilized WhirlPak bag. This procedure was repeated until all volunteers were rinsed. In the laboratory, the total weight, water content, and the enterococci concentrations of the sand were measured. Sample volumes of 25 mL, 50 mL, and 75 mL were used for water analysis and five pre-determined volumes of liquid extract (2 mL, 6 mL, 12 mL, 20 mL, and 50 mL) were used for sediment analysis.

2.6 Laboratory analysis:

Analyses of all samples were conducted the same day of delivery, no longer than 6 hours after collection.

2.6.1 Water analysis:

All water samples from the large pool experiment were analyzed for enterococci and *S. aureus* by the Florida Department of Health Bureau of Laboratories Miami Branch, and all sediment and water samples collected from the small pool experiment were analyzed for enterococci only at the UM laboratories. Use of two laboratories was necessary to address limitations in laboratory capacity.

A standard membrane filtration (MF) method was used for the analysis of enterococci (Method 1600, US EPA 1997) and *S. aureus* (Fowler et al., 2004). Enterococci were analyzed by placing the filter membrane on a selective medium (mEI agar, Becton Dickinson, Sparks, MD) and incubating the filters at 41°C for 24 hours. All colonies that were blue or characterized by a blue halo were recorded as enterococci colonies. *S. aureus* was analyzed using a chrome agar method. The membrane filter containing bacteria was placed on a selective medium (BD BBL^{tm} CHROMagar^{tm}), and the agar plates were incubated aerobically at 35 ± 2 °C for a minimum of 24 hours (the incubator limited the amount of light that the agar was exposed to in order to preserve the chromogens as recommended by BD BBL^{tm}). After incubation, the plates were read against a white background with the aid of a magnifying glass. All mauve colonies were counted as positive for *S. aureus*. Additional tests were performed to confirm that the mauve colonies were *S. aureus*. A representative few were sub cultured onto sheep blood agar and incubated for 24 hours. The isolates were pure, cream colored, beta hemolytic, and coagulase positive. A Gram stain of the isolates showed Gram positive cocci in clusters.

2.6.2 Sediment analysis:

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 (Mettler, AG245) difference of sand before and after drying (110 ^{o}C for 24 h) approximately 18 g of sample on pre-weighed weighing dishes.

The second step was to extract the organisms from the sand particles to a predefined volume of sterile water. 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 filtered using pre-sterilized 30μm 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 were also filtered through the same 30μm pore size nylon net filters. The final volume of filtrate was recorded, and this filtrate was analyzed for enterococci using the mEI agar method (Method 1600, US EPA 1997).

2.7 Results and discussion:

2.7.1 Large pool study:

The water depth ranged from 17 to 26 cm for all four cycles. Water temperature ranged from 30.3 °C to 31.3 °C and showed a consistent increase after each cycle. Water pH readings ranged from 7.95 to 6.82 and consistently decreased after each cycle.

The mean concentrations for source and initial water for enterococci were 170 CFU/100mL and 9 CFU/100mL, and for *S. aureus* were 17 CFU/100mL and 10 CFU/100mL, respectively (Table 2.1). Relatively high concentrations of bacteria were measured in the source water just before the first cycle, with a significant decrease in source water bacteria in subsequent cycles. The higher bacteria levels in the source water measured before cycle 1 was likely due to a rain event that occurred immediately before the first sampling event. Although no point sources of bacteria have been found at the beach, the increase in bacteria levels immediately after a rain event could be due to the wash-in of non-point sources of bacteria from the shoreline carried by water runoff. The first source water sample was collected before the initial pool water sample, and so the effect of the rainfall event had diminished by the time the first initial pool sample was collected.

The concentrations of enterococci and *S. aureus* in the initial pool samples for all four cycles were relatively low and the enterococci concentrations were well below the recommended guidelines for marine recreational waters (US EPA, 2002). This finding is important in that future human shedding studies can use marine water as source water as oppose to treated freshwater. All previous human shedding studies used filtered freshwater as source water (Table 2.2).

After immersion of the 10 individuals in the large pool, the bacteria concentrations in the pool increased by 1 to 2 orders-of-magnitude (Table 2.1). The range for mean *S. aureus* and enterococci for all four cycles were from 520 to 4,200 CFU/100 mL and from 80 to 400 CFU/100 mL, respectively. Between cycles, the bacteria detected in the water column decreased after each subsequent cycle. The decrease was faster for *S. aureus* (50%) relative to enterococci (42%), on average (Figure 2.1). This observation may be due to a washing effect leaving less bacteria on the body for shedding in the subsequent cycle.

Significant difference analysis was conducted to compare the means of six possible combinations of any two different cycles (1-2, 1-3, 1-4, 2-3, 2-4, 3-4). The concentrations of bacteria were significantly different for all combinations, except for cycle 2 to 3 for *S. aureus* and cycle 2 to 3 and 2 to 4 for enterococci. The lack of significant difference during these cycles may be due to the combined effects of variations in the replicate analyses observed in cycle 2, and the exposure to sand during cycles 3 and 4. It is possible that the introduction of sand to the body during cycle 3 may have served as an added source of enterococci and *S. aureus,* and this added source resulted in the decrease in the washing effect with additional cycles.

From mass balance computations which take into account the volume of water in the pool, the results indicated that *S. aureus* levels shed from the bathers (CFU/person) during the first three cycles were consistently greater by one order of magnitude than the enterococci numbers shed (Figure 2.2). During the first three cycles, *S. aureus* densities ranged from 6.1 x 10^6 to 1.3 x10^6 CFU per person, and enterococci densities ranged from 550 000 CFU to 165 000 CFU per person. In cycle 4, the total average *S. aureus* shed per

bather was approximately 6 times greater than enterococci, 670 000 CFU versus 110 000 CFU. This observation may be due to the effects of washing which may reduce *S. aureus* by a greater factor than enterococci. Between cycles 3 and 4, bathers were exposed to beach sand thus increasing available enterococci levels from sand for shedding. Enterococci are found in the sand at Hobie Cat Beach at typical concentrations of 380 CFU/g-dry sand, on average, immediately above mean high tide (Durbin et al., 2005).

The mean concentration of enterococci shed per subject in this study ($3x10^5$ CFU) was consistent, within an order of magnitude, with the amount typically released from feces during bathing ($2.3x10^5$ CFU; Gerba, 2000), as determined by comparing ratios of fecal indicator bacteria in sewage and feces (Rose et al., 1991). However, fecal releases cannot explain the *S. aureus* shed per subject as the mean concentration observed in this study (average for all 4 cycles, $3x10^6$ CFU) was 4 orders of magnitude higher than would be expected from a fecal release ($4.1x10^2$ CFU). These findings support that the main source of entrococci is from the release of fecal matter, while *S. aureus* is from non-fecal sources predominantly shed from the skin and possibly anterior nares of bathers.

The results from the Large Pool Study are consistent with the results from 3 other bather shedding studies (Table 2.2), even though the design of the studies was different. They differed in terms of the number of subjects, demographics, targeted bacteria indicators, type of water (marine versus fresh) and exposure (individual exposure as opposed to group exposure). Hanes and Fossa (1970) used a mix of 64 subjects including children with group exposures. Smith and Dufour (1993) used 8 demographically mixed adult subjects with individual exposures. Robinton and Mood (1966) used 5 adult females with individual exposures. Taken together, the results suggest that most bacteria shedding

occurs within the first 15 minutes of swimming activities or exposure. Despite the differences in design, this study, Smith and Dufour (1993), and Robinton, and Mood (1966) reported that *S. aureus* shed in significant densities per bather at $6.1x10^6$, $7.5x10^6$, and $1.3x10^6$, respectively. The type of water apparently did not impact the degree of shedding as the results from the current study using a marine water were consistent with prior studies which exclusively used freshwater.

The results suggest that *S. aureus* can serve as an indicator of bathing load. Studies that evaluated the risk of swimming related illnesses associated with exposure to waters contaminated with non-point sources, indicated that gastrointestinal illnesses observed in swimmers were correlated with high numbers of bathers and high densities of *S. aureus* (Calderon et al., 1991; Charoenca and Fujioka 1995). Since *S. aureus* is isolated from human waste in relatively low numbers, on the order of 10^3 CFU/100 mL (Gerba, 2000), it can be used as an indicator to predict human bather impacts which would include the combined effects of bather density, mixing, and dilution.

2.7.2 Small pool study:

The amount of sand released per subject ranged from 24 to 70 g/subject (mean=51) for Group I and from 7 to 65 g/subject (mean=28) for Group II (Figure 2.3 A). The average enterococci density released from sand adhered to the bodies of the subjects ranged from 210 to 870 CFU/ g-dry sand (mean=390) for Group I and from 4 to 55 CFU/g-dry sand (mean=24) for Group II (Figure 2.3 B). These concentrations may be due to enterococci naturally present in the sand or from contact of sand with the skin of the volunteers. The total numbers of enterococci shed per subject ranged from 5020 to 44

500 CFU/subject (mean=20 300) and from 42 to 2150 CFU/subject (mean=840) for Group I and II respectively (Figure 3b). The data generated from Group I and II showed considerable variation. Differences in weather conditions and differences in participant behavior within each group may be part of the reason for this variation. The field study that included Group I participants was conducted shortly after a rain event whereas no rain occurred during the day of the Group II field study. The rain event may have affected the microbial quality of the sand and may have increased the degree to which the wet sand may have adhered to the participants. Furthermore, all of the Group I participants actively assisted in the set-up of the experiment whereas the majority of the Group II participants did not assist.

The total average enterococci density released from sand adhered to the bodies of bathers per subject for Group I and II combined was 10 600 CFU, assuming no desorption of the enterococci from the sand. This value represents approximately 16 %, 1.9%, and 1.8% of the total average enterococci shed per bather per 15 minute exposure estimated from Smith and Dufour (1993) ($6.6x10^4$ CFU), the current "Large Pool" study ($5.5x10^5$ CFU), and Hanes and Fossa (1970) ($6x10^5$ CFU), respectively. The relatively small contribution from sand in this study may be impacted by the characteristics of the beach sand which was relatively coarse (mean size of 620 μm with less than 2% finer than 30 μm).

2.8 Conclusions and Recommendations:

This study demonstrated that bathers shed significant concentrations of enterococci and *S. aureus* into the water column and that *S. aureus* was shed at

concentrations at least one order of magnitude greater than enterococci. This study also showed that total enterococci and *S. aureus* released by bathers decreased significantly between bathing episodes, in particular after the first wash cycle. This conclusion agrees with the long standing universal requirement that bathers should shower before entering recreational waters to reduce the microbial load in particular at swimming pools since the water volume is limited. It is concluded from this study that the enterococci contribution from sand adhered to skin, was small relative to the amount shed directly from the skin and represented less than 5% of the total enterococci shed by bathers.

Future studies should be designed to evaluate the potential use of *S. aureus* as a measure of possible health effects from bather to bather transmission of illness, as *S. aureus* is shed in quantities one order of magnitude higher than enterococci. This study recommends additional targeted studies to confirm the results of this effort and to estimate how much *S. aureus* bathers carry into the water column via sand. Furthermore, given the significance of bathing load, water quality models of recreational beach waters impacted by non-point sources of microbes should include bathing load as one of the significant pollution sources. The contribution from sand adhered to skin can be potentially ignored in models which simulate non-point sources of enterococci as the quantities from sand on skin is small, on average, in comparison to the total body burden.

Table 2.1 Concentrations of enterococci and *S. aureus* in the source water and in the pool water before and after immersion by 10 volunteers

Cycle #	Enterococci CFU/100 mL			*S. aureus* CFU/100 mL		
	Source[a]	Pool Initial[b]	Pool Final[c]	Source[a]	Pool Initial[b]	Pool Final[c]
1	672	21 (8)	400 (44)	64	13 (7)	4187 (439)
2	8	3 (1)	153 (64)	2	7 (3)	2080 (859)
3	<1	7 (2)	140 (10)	<1	11 (6)	1027 (189)
4	<1	3 (2)	87 (25)	<1	9 (2)	523 (81)
Average	170	9	195	17	10	1954

[a] Single ocean water sample collected at the pump intake

[b] Average of three pool initial water samples before bathers entered pool. Value in parenthesis corresponds to the standard deviation of the 3 measurements.

[c] Average of three pool final water samples after bathers exited pool. Value in parenthesis corresponds to the standard deviation of the 3 measurements.

Table 2.2 Densities of enterococci and *S. aureus* shed per bather for the current study and comparisons with three additional studies

Study	Enterococci, CFU	*S. aureus*, CFU	Summary of Study Design				
			No. Subjects	Gender & Ages	Exposure Period	Individual or Group	Water Type
Current Study[a]	$5.5x10^5$	$6.1x10^6$	10	Males and Females. Ages 18-50	15 min.	Group	Marine
Smith and Dufour 1993	$6.6x10^4$	$7.5x10^6$	8	Males and Females. Ages 4-59, mostly adults.	15 min.	Individual	Fresh
Hanes and Fossa 1970	$6x10^5$	Not Measured	64	Males and Females. Ages 6-38.	10-30 min.	Group	Fresh
Robinton and Mood 1966	Not Measured	$1.3x10^6$	5	All Females. Ages 25-45.	15 min.	Individual	Fresh

[a] Average of 4, 15-minute exposure periods was 3×10^5 for enterococci and 3×10^6 for *S. aureus*

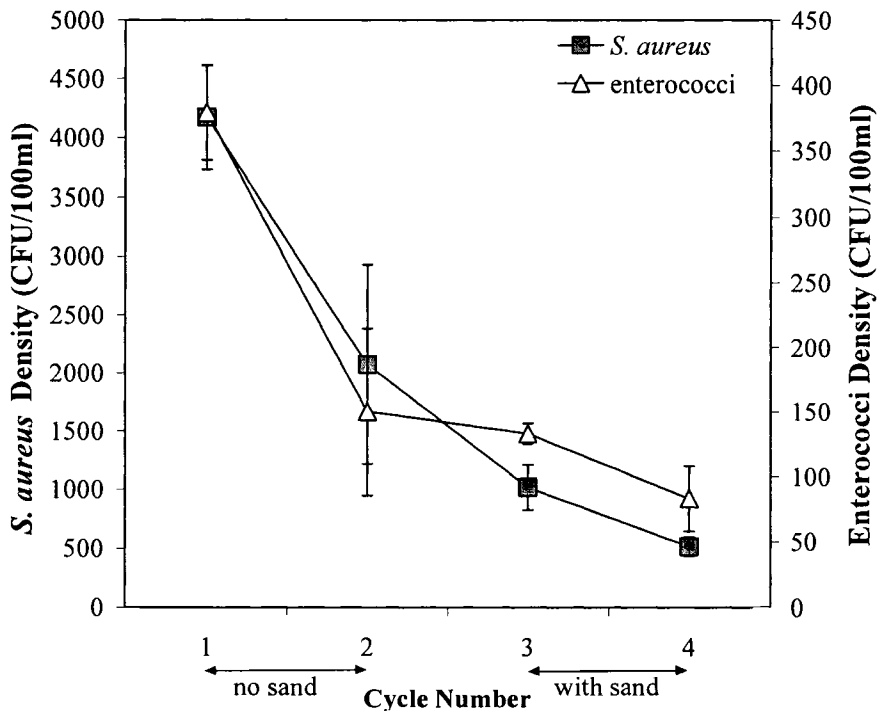

Figure 2.1 Total average enterococci and *S. aureus* densities (CFU/100mL) in the water column per cycle as observed during the large pool experiment. Error bars correspond to the standard deviation of replicate analyses

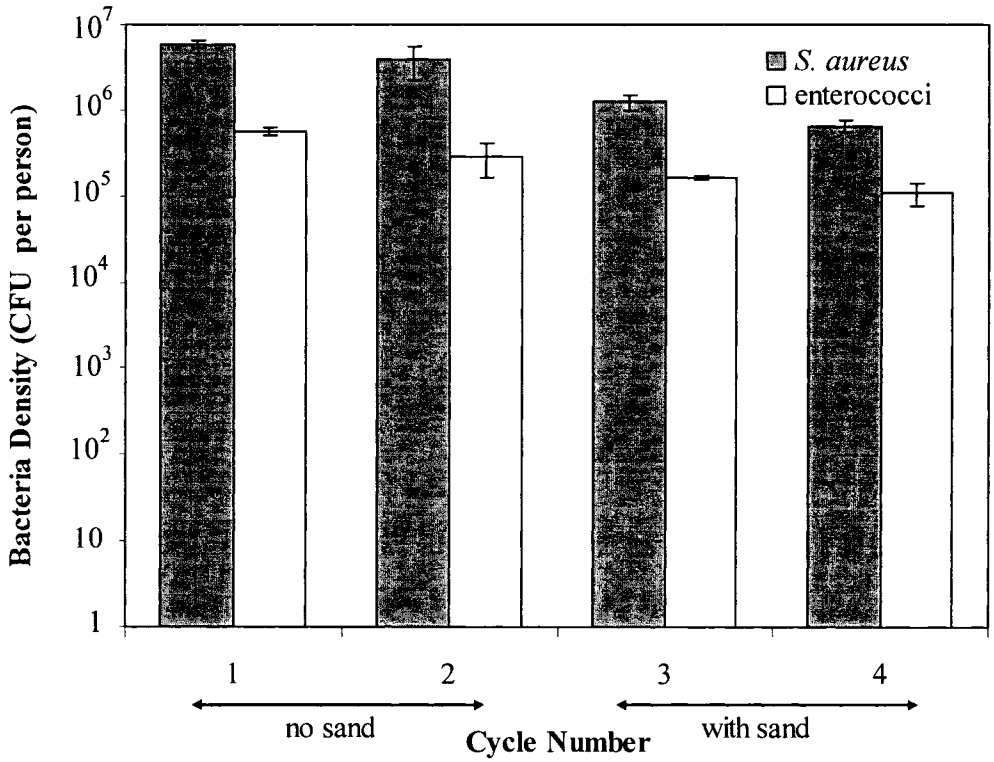

Figure 2.2 Mean total bacteria shed per person per 15 minute exposure. Error bars correspond to the standard deviation of three replicate samples collected per cycle. Considering all 4 cycles, the overall average shedding of microbes shed per bather were $3x10^6$ CFU for *S. aureus* and $3x10^5$ CFU for enterococci.

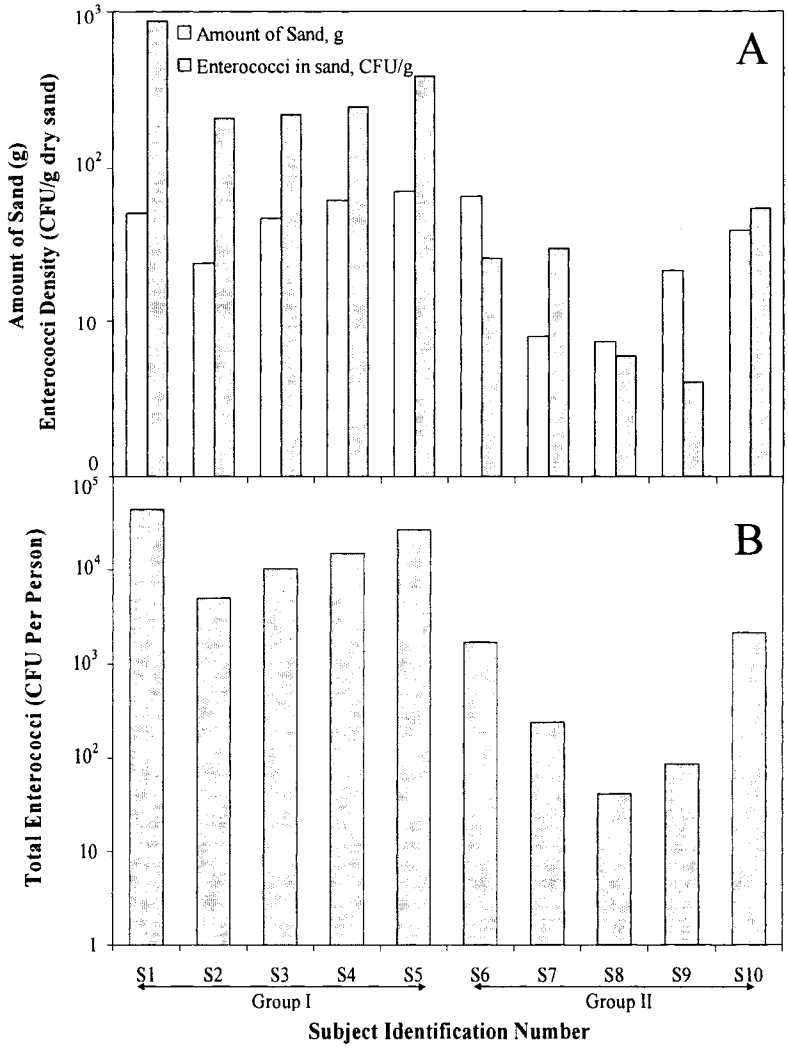

Figure 2.3 Results for group I and group II for the "Small Pool" experiment.
(A)Average amount of sand released per subject and, average enterococci density in sand.
(B) Average total enterococci, CFU per subject

CHAPTER 3

DEVELOPMENT OF THE WATER QUALITY MODEL AND THE ALGORITHMS FOR SIMULATING NON-POINT SOURCES FOR ENTEROCOCCI

3.1 Introduction:

This chapter describes the conceptual hydrodynamic water quality model in schematic format, including the basic mass conservation equations that were used to develop the conceptual model, the model assumptions, and the mathematical expressions of all non-point microbial input source functions.

This chapter also describes the conceptual hydrodynamic/water quality model in chematic format (Figure 3.1). The basic mass conservation equations are defined along with the individual terms in the equation. One mass balance equation is provided for water and another for enterococci, equations (3-1) and (3-13) respectively. In this chapter, all significant non-point sources of enterococci are described and listed. Individual sources are then expressed as mathematical equations (3-20) through (3-26) along with their variables and summed up to calculate and express in a mathematical form the total microbial input and output loads (L_{input}, L_{output}). The impact of enterococci decay (K_bC_tV) is also defined.

3.2 Conceptual Water Quality Model and Assumptions:

The following assumptions are made in the mass balance conservation equation developed for this study:

1. Water and bacteria are fully mixed within the control volume
2. Evaporation of water within the control volume is negligible
3. Flow into the control volume comes from four vectors;
    a. water runoff due to rainfall from shore
    b. tidal flows which cross the offshore face of the tidal prism,
    c. inflow of water parallel to the shoreline
    d. outflow of water parallel to the shoreline
4. Enterococci concentrations in offshore water is equal to zero
5. All bacteria loads come from shore due to non-point sources
6. Enterococci concentration in the parallel flow entering the control volume at time t is assumed to equal zero in most scenarios. If not, the value would be explicitly stated.
7. The die-off of bacteria within the control volume is governed by a first-order differential equation (r_c=-kC), where r_c represents the decrease in concentration per time.
8. Settling of enterococci attached to sand grains out of the water column is not considered in this model
9. Flows parallel to the shoreline, into and out of the control volume, are equal
10. Enterococci concentration in the parallel flow leaving the control volume at time t is assumed to equal the enterococci concentration within the control volume
11. The system is at steady-state at any given time, t.

3.3 Assumption of Complete Mixing:

The tidal prism will be modeled as a completely mixed system. Dispersion within the tidal prism will not be considered. Tidal dispersion is a function of tidal velocity, lateral and vertical gradients in velocity, and water density differences (Thomann and Muller 1987). In developing the model, we will not consider the mixing effect of lateral and vertical gradients in velocity and density differences. These assumptions are based on the following:

a. Water depth within the control volume (inter-tidal zone) is very shallow. The maximum depth is approximately 2.0 feet. Therefore we can assume vertical velocity equal to zero.

b. Water temperature is roughly constant with time therefore water density will remain constant. Water density is inversely proportional to water temperature.

c. As a result of the complete mixing assumption at time t, C_t is set equal to the enterococci concentration in the control volume. This concentration is equal to the enterococci concentration found in the parallel flow leaving the control volume and in the outgoing tidal flow.

3.4 Water Balance:

This section describes graphically the control volume and provides more details concerning the mathematical expression for water balance within the control volume. The control volume is bounded from the South by the center line between transects K and J (KJ), from the North by the center line between transects K and L (KL), from the East by

the interface of water and sand, and from the west by the buoy line. The width of the control volume B is constant, the length L and height H (tidal prism height) are variable with time. B, L, and H were measured in the field (Figure 3.2 and 3.3). Equation (3-1) describes the water balance mathematically.

3.4.1 Water Balance Mathematical Equation:

The fundamental expression for water balance states that the rate of change of volume per unit time (dV/dt) is given as the difference between the amount of water entering the control volume, Q_{in}, and the water exiting the control volume, Q_{out}, as shown in equation (3-1).

$$\frac{dV}{dt} = Q_{in} - Q_{out}, \text{ m}^3/\text{day} \qquad (3\text{-}1)$$

Where: $\frac{dV}{dt}$ = is the water volume change within the control volume per unit time (per one hour).

Q_{in} and Q_{out} are the total water flows that enter and exit the control volume at time t (Figure 3.4). In accordance with Figure 3.4 Q_{out} has only one water flow component which is $Q_{parallelout}$. Therefore:

$Q_{out} = Q_{parallelout}$ = is the parallel flow exiting the control volume at time t

$$Q_{out} = Q_{parallelout} = A \times v, \text{ m}^3/\text{day} \qquad (3\text{-}2)$$

Where:

A= is the cross sectional area of the tidal prism Figure 3.2 at time t

$$A = 0.5 \times H_t \times L_t, \text{ m}^2 \qquad (3\text{-}3)$$

Where:

H_t, m and L_t, m are the tidal prism height and length at time t, (Figure 3.2). Actual field measurements were conducted to estimate the values of H_t and L_t

v = is the lateral water flow velocity entering and exiting the control volume, m/day. Values of v are estimated using historic monitoring data collected by NOAA, the National Oceanic Atmospheric Agency.

Substituting equation (3-3) into equation (3-2), gives us equation (3-4) which is the mathematical expression of $Q_{parallelout}$ in terms of tidal prism height and length and lateral flow velocity.

$$Q_{parallelout} = 0.5 \times H_t \times L_t \times v, \ m^3/day, \quad (3\text{-}4)$$

The total water flows (Q_{in}) entering the control volume at time (t) equals the sum of tidal flow, parallel flow and water runoff, (Figure 3.4). Equation (3-5) below expresses this statement into a mathematical form:

$$Q_{in} = Q_{tidal} + Q_{runoff} + Q_{parallelin} \quad (3\text{-}5)$$

Equation (3-6) below is developed by substituting equations (3-2) and (3-5) into equation (3-1), where the term dV/dt (the water volume change within the control volume in unit of time) is expressed in terms of all individual water flow components entering (Q_{in}) and exiting (Q_{out}) the control volume:

$$\frac{dV}{dt} = (Q_{tidal} + Q_{runoff} + Q_{parallel\ in}) - (Q_{parallel\ out}) \quad (3\text{-}6)$$

Assumption number 9 in section 3.1 states that flows parallel to the shoreline, into and out of the control volume, are equal.

Therefore:

$$Q_{parallelin} = Q_{parallelout}, \quad (3\text{-}7)$$

Substituting equation (3-7) into equation (3-6) leads to equation (3-8) which states that the water volume change (dV/dt) within the control volume is the sum of two water flow vectors: Q_{tidal} (tidal flows which cross the offshore face of the tidal prism), and Q_{ruoff} (water runoff due to rainfall from shore). Q_{tidal} and Q_{runoff} are input water flow vectors.

$$\frac{dV}{dt} = Q_{tidal} + Q_{runoff} \tag{3-8}$$

3.4.2 Computation of $(\frac{dV}{dt})$:

The computation of (dV/dt) is based on the assumption of a tidal prism (Figure 3.2). The tidal prism volume is controlled by the tides regardless of the amount of water that enters the system; the surface elevation of the tidal prism will be controlled by the tidal height. Thus the boundary condition is tidal height which is a function of time.

Using geometry (Figure 3.2) then the term (dV/dt) can be calculated as follows:

$$(\frac{dV}{dt}) = 0.5 \text{ x } B \text{ x } (H_t \text{ x } L_t) - 0.5 \text{ x } B \text{ x } (H_{t-1} \text{x} L_{t-1})$$

$$= 0.5 \text{ x } B \ (H_t \text{ x } L_t - H_{t-1} \text{ x } L_{t-1}) \tag{3-9}$$

$(\frac{dV}{dt})$, will have the same sign as $Q_{tidal,}$ it will be positive for incoming tide (e.g. from low to high tide) and it will be negative for outgoing tide (e.g. from high tide to low tide).

Where:

B=Width of the tidal prism, it is constant with time, m

H_{t-1}=Tidal prism height at time (t-1), m

L_{t-1}= Tidal Prism Length at time (t-1), m.

Note, L_t is a function of H_t as the bottom geometry of the beach area or control volume is considered constant (i.e. the slope for the ocean floor within the control volume is constant with time).

3.4.3 Computation of Q_{runoff}:

Q_{runoff} is estimated using the rational formula, $Q_{runoff} = DIA$, (Lundeberg 1992) where I is the rainfall rate (L/T), A is the area over which the runoff will occur (L^2), and D is the runoff coefficient (Figure 3.5). This value can be estimated from the literature based on land use, population, and degree of imperviousness, and ranges from 0.1 to 0.3 for population of about 1 person/acre (rural) to 0.7 to 0.9 for heavy industrial and commercial areas with densities greater than 50 persons/acre. (Thomann, and Muller, 1987). As there are different land types within the area contributing runoff, a different runoff coefficient is assigned to each contributing area such that:

$$Q_{runoff} = I(\sum D_{c,i}A_{d,i}) \quad (3\text{-}10)$$

Where:

$A_{d,i}$= Drainage area of contributing area i, m^2

I = Rainfall intensity, m/day

$D_{c,i}$ = Drainage Coefficient of contributing area i, value varies from 0 to 1 based on the land use and cover

3.4.4 Computation of Q_{tidal}:

Q_{tidal} can be derived algebraically from equation (3-8) which states that: when there is no rainfall event (e.g. $Q_{runoff}=0$), Q_{tidal} equals (dV/dt) and if rainfall occurs, Q_{tidal} will be less than dV/dt by a the flow equivalent to the amount of water that enters the control volume as runoff (Q_{runoff}).

Therefore:

From equation (3-8):

$$\frac{dV}{dt} = Q_{runoff} + Q_{tidal}$$

Or:

$$Q_{tidal} = \frac{dV}{dt} - Q_{runoff} \qquad (3\text{-}11)$$

Substituting in equation (3-11) all parameters associated with the terms (dV/dt) and Q_{runoff}:

$$Q_{tidal} = 0.5 \times B(H_t \times L_t - H_{(t-1)} \times L_{(t-1)}) - I(\sum D_{c,i} A_{d,i}) \qquad (3\text{-}12)$$

Note, (Q_{tidal}) is negative during outgoing tide (e.g. from high tide to low tide) and is positive during incoming tide (e.g. from low tide to high tide). The sign computed for Q_{tidal} should be checked to validate the water balance calculations.

3.5 Bacteria (Enterococci) Balance in the Water column:

A second mass-balance equation was developed, as a simple way to define the behavior of enterococci within the control volume as a function of time. It is assumed that enterococci and water within the control volume behaves as a completely-mixed reactor. The boundary conditions of the control volume are schematically shown in Figure 3.1.

Section (3.1) of this chapter lists the assumed boundary conditions for the model. The mass balance conservation equation in general word statement can be described as follows:

Rate of accumulation of enterococci within the system boundary = [(Rate of flow of enterococci into the system boundary – Rate of flow of enterococci out of the system boundary) - Rate of decay of enterococci within the system boundary + Inputs internal to the boundary].

Equation (3-13) converts the mass balance conservation from a general word statement to a general mathematical form.

$$\frac{d(CV)}{dt} = \sum Q_{input} C_{input} - \sum Q_{output} C_{output} - K_b CV + \sum L \qquad (3\text{-}13)$$

where:

$\frac{d(CV)}{dt}$	Rate of increase in enterococci numbers in the control volume, (M/T)
$\Sigma Q_{input} C_{input}$	Rate of enterococci numbers entering the control volume, (M/T)
$\Sigma Q_{output} C_{output}$	Rate of enterococci numbers leaving the control volume, (M/T)
Q	Volumetric flow rate of water entering the control volume (L^3/T)
C	Enterococci concentration, (M/L^3)
V	Control Volume, (L^3)
K_b	Overall net decay rate for enterococci in the water column, (1/T)
ΣL	Sum of the sources internal to the boundary, (M/T)
t	Time, T

$\Sigma Q_{input}C_{input}$ and $\Sigma Q_{output}C_{output}$ equal the rate of flows of enterococci into and out of the control volume as a function of time respectively. The mathematical expression of $\Sigma Q_{input}C_{input}$ and $\Sigma Q_{output}C_{output}$ are described in equations (3-14) and (3-15).

$$\sum Q_{input}C_{input} = Q_{tidal}C_{tidal} + Q_{runoff}C_{runoff} + Q_{parallelin}C_{in} \qquad (3\text{-}14)$$

$$\sum Q_{output}C_{output} = Q_{parallelout}C_{out} \qquad (3\text{-}15)$$

Where:

C_{tidal} = the concentration of enterococci associated with the tidal flow Q_{tidal}, CFU/m^3

C_{in} = the concentration of enterococci entering the control volume with $Q_{parallelin}$, CFU/m^3

C_{out} = the concentration of enterococci leaving the control volume with $Q_{parallel\ out}$, CFU/m^3

C_{runoff}= the concentration of enterococci entering the control volume with Q_{runoff}, CFU/m^3

Of note, during incoming tide (e.g. from low tide to high tide), it is assumed that offshore water is clean therefore C_{tidal} = 0. Whereas C_{tidal} will equal C which is the concentration of enterococci within the control volume, during outgoing tide (e.g. from high tide to low tide). The value of C_{in} can be assumed based on site conditions and historical beach monitoring including surface water quality data .C_{out} is assumed to equal C within the control volume. Substituting equations (3-14) and (3-15) into equation (3-13):

$$\frac{d(CV)}{dt} = Q_{tidal}C_{tidal} + Q_{runoff}C_{runoff} + Q_{parallelin}C_{in} - Q_{parallelout}C_{out} - K_bCV + \sum L$$

(3-16)

Incoming Tide:

Simplifying equation (3-16) by applying the above boundary conditions and substituting for $\mathbf{C_{tidal}=0}$, results in the following equation:

$$\frac{d(CV)}{dt} = Q_{runoff}C_{runoff} + Q_{parallelin}C_{in} - Q_{parallelout}C - K_bCV + \sum L \qquad (3\text{-}17)$$

Outgoing Tide:

Simplify equation (3-16) by applying the above boundary conditions:

$$\frac{d(CV)}{dt} = Q_{tidal}C + Q_{runoff}C_{runoff} + Q_{parallelin}C_{in} - Q_{parallelout}C - K_bCV + \sum L \qquad (3\text{-}18)$$

3.6 Sources and Sinks for enterococci:

The conceptual model developed for this study assumes that there are no point sources of enterococci. The following non-point sources are considered:

3.6.1 Sources:

a. Sand from inter-tidal zone: Sediment from the inter-tidal zone contains high enterococci concentrations (Durbin et al. 2005). Re-suspension of sediments due to tidal, rainfall, wind storms, and bathers' activities release enterocci into the water column.

b. Bird and dog feces: Bird and dog droppings are transported into the water column via runoff and tidal activities. In some cases these feces may be deposited directly into the water column.

c. Bathers: Bacteria are transported via sand grains adhered to bather's skin and directly off skin of bathers into the water column.

3.6.2 Sinks:

Die-off of enterococci: Die-off rate (K_b) of enterococci in the water column may be due to many factors including salinity, nutrient deficiencies, predation, sunlight, temperature, and toxic substances. The K_b value used in this study is a lumped factor that was measured experimentally and incorporates all of the factors that may promote die-off.

3.7 Computation of Enterococci Non-Point Input Sources:

Based on the sanitary survey conducted at the Hobie Cat Beach, all input bacteria sources are non-point sources including beach sand, bathers, birds, and dogs. There are no fecal point sources impacting the water quality and the beach area. Below is the mathematical expression (3-19) of all non-point sources identified in the sanitary survey.

$$\sum L = L_{s(t)} + L_p + L_b + L_d \qquad (3\text{-}19)$$

Where:

$L_{s(t)}$= Bacterial load from sand (dry and intertidal zone), (M/T)

L_b= Bacterial load from bird droppings, (M/T)

L_d= Bacterial load from dog droppings, (M/T)

L_p= Bacterial load from people entering the water column, (M/T)

3.7.1 Beach Sand Non-Point Input Source:

The mathematical expression for beach sand input source $L_{s(t)}$ is shown in equation (3-20). $L_{s(t)}$ is a function of the beach sand bulk density, surface area over which the transfer of bacteria from sand grains to the water column occurs (Figure 3.3), scouring depth of beach sand, average enterococci concentration in beach sand, and frequency of tides.

$$L_{s(t)} = \rho * A_{s(t)} * d_s * C_s * f, \text{ cfu/hour,} \qquad (3\text{-}20)$$

Where:

ρ = Bulk density of sediments in the swash zone, g/m^3 .

$A_{s(t)}$ = Surface area over which the transfer of bacteria from sand grains to the water column occurs, m^2.

d_s = Scouring depth or sub-tidal surficial sediments, m.

C_s= Average enterococci concentration, CFU/g of dry sand.

f= frequency of scouring (4/day) or $(1/6)hr^{-1}$

3.7.2 Birds Non-Point Input Source:

Birds have been documented via a digital camera and a sanitary survey. Birds are one of the important non-point enterococci sources impacting the microbial water quality at Hobie Cat Beach. The mathematical expression for this non-point source input is shown in equation (3-21). In general, it is a function of the bird population that congregates at the beach and the enterococci loading rate.

$$L_b = N_b x W_b x U_b \qquad \text{CFU/day} \qquad (3\text{-}21)$$

Multiply equation (3-21) by (1/24) to convert its units from CFu/day to CFU/hour:

$L_b = (N_b x W_b x U_b) x (1/24)$ CFU/hour (3-22)

Where:

N_b =Average bird population documented on the study site at any time (number of birds)

W_b=Average concentrations of enterococci, CFU per g of dry bird feces

U_b = Average weight of dry feces released per bird per day, g per bird day

3.7.3 Dogs Non-Point Input Source:

Dogs have been documented via a digital camera and a sanitary survey. Dogs are one of the important non-point enterococci sources impacting the microbial water quality at Hobie Cat Beach. The mathematical expression for this non-point source input is shown in equation (3-23). In general, it is a function of the dog population congregates at the beach and the enterococci loading rate.

$L_d = N_d x W_d x U_d$ CFU/day (3-23)

Multiply equation (3-23) by (1/24) to convert its units from CFU/day to CFU/hour:

$L_d = (N_d x W_d x U_d) x (1/24)$ CFU/hour (3-24)

Where:

N_d =Average dog population documented on the study site at any time (number of dogs)

W_d=Average concentrations of enterococci, CFU per g of dry dog feces

U_d= Average weight of dry feces released per dog per day, g per dog day

3.7.4 Bathers Non-Point Input Source:

Average number of bathers found at Hobie Cat Beach has been documented via a digital camera and a sanitary survey. The bathing load is one of the important non-point enterococci sources impacting the microbial water quality at Hobie Cat Beach. The

mathematical expression for this non-point source input is shown in equation (3-25). In general, it is a function of the bathing load and the enterococci loading rate i.e the average concentrations of enterococci shed per bather during 15- minutes exposure periods to marine waters.

$L_p=f_pxN_pxY_{tskin}$ CFU/day (3-25)

Multiply equation (3-25) by (1/24) to convert its units from CFU/day to CFU/hour:

$L_p=(f_pxN_pxY_{tskin})(1/24)$ CFU/hour (3-26)

Where:

N_p= Average number of bathers

f_p = Average number of 15-minute exposures per bather per day

Y_{tskin}= Enterococci loading rate, CFU/15 minutes bather exposure

3.7.5 Sinks Output Function:

Die-off of enterococci within the control volume (Figure 3.1) is the only sink factor included in the general mass-balance conservation equation (3-13). The die-off of enterococci in the water column is governed by a first-order differential equation (r_c=-kC), where r_c represents the decrease in concentration per time. The mathematical expression for the decay of enterococci within the control volume is shown in equation (3-27).

K_bCV CFU/day (3-27)

Multiply equation (3-27) by (1/24) to convert its units from CFU/day to CFU/hour

$(K_bCV)(1/24)$ CFU/hour (3-28)

Where:

K_b= Overall net decay rate for enterococci in the water column, day^{-1}. The K_b value used in this study is a lumped factor that was measured experimentally and incorporates all of the factors that may promote die-off.

C= Average enteroccocci concentration in the water column within the control volume CFU/m^3. This variable will be computed from the enterococci balance equation.

V= Control Volume, m^3

3.8 Mathematical Expression of the Model:

Equation (3-16) describes the mass balance equation for enterococci in the water column as a function of time. This equation states that the rate of accumulation of enterococci within the control volume as a function of time is equal to the sum of enterococci loading rates from all incoming flows (tidal, runoff, and parallel) and non-point sources (birds, dogs, sand, and bathers) minus the sum of enterococci loading rates from all outgoing flows (parallel flow only) and the loading rate resulting from the overall decay of enterococci in the water column.

$$\frac{d(CV)}{dt} = Q_{tidal}C_{tidal} + Q_{runoff}C_{runoff} + Q_{parallelin}C_{in} - Q_{parallelout}C_{out} - K_bCV + \sum L \quad (3\text{-}16)$$

At steady state (Assumption 11, Section 3.2) the rate of accumulation of enterococci within the control volume as a function of time is equal to zero.

Therefore:

$$\frac{d(CV)}{dt} = 0$$

Or:

$$Q_{tidal}C_{tidal}+Q_{runoff}C_{runoff}+Q_{parallelin}C_{in}-Q_{parallelout}C_{out}-K_bCV+\sum L=0 \qquad (3\text{-}29)$$

Simplify equation (3-29) and substituting C_{out} with C (Assumption 10, Section 3.1)

$$Q_{tidal}C_{tidal}+Q_{runoff}C_{runoff}+Q_{parallelin}C_{in}-Q_{parallelout}C-K_bCV+\sum L=0 \qquad (3\text{-}30)$$

Solving equation (3-30) for C, the concentration of enterococci within the control volume:

$$C = \frac{[(Q_{tidal}C_{tidal} + Q_{runoff}C_{runoff} + Q_{parallelin}C_{in}) + \sum L]}{(Q_{parallelout} + K_bV)} \qquad (3\text{-}31)$$

During incoming tide, $C_{tidal} = 0$.

Substituting $C_{tidal} = 0$ into equation (3-31)

$$C = \frac{[(Q_{runoff}C_{runoff} + Q_{parallelin}C_{in}) + \sum L]}{(Q_{parallelout} + K_bV)} \qquad (3\text{-}32)$$

During outgoing tide, $C_{tidal} = C$:

Substituting $C_{tidal} = C$ into equation (3-31)

$$C = \frac{[(Q_{runoff}C_{runoff} + Q_{parallelin}C_{in}) + \sum L]}{(Q_{parallelout} - Q_{tidal} + K_bV)} \qquad (3\text{-}33)$$

Using equation (3-11) to substitute for Q_{tidal}:

$$C = \frac{[(Q_{runoff}C_{runoff} + Q_{parallelin}C_{in}) + \sum L]}{[Q_{parallelout} - (\frac{dV}{dt} - Q_{runoff}) + K_b V]} \quad (3\text{-}34)$$

Using equations (3-19), (3-9), (3-4), and (3-10) to substitute for $\sum L$, $\frac{dV}{dt}$, $Q_{parallelout}$, and Q_{runoff} into equation (3-34) , the following expression can be derived for outgoing tide:

$$C = \frac{[(I(\sum D_{c,i}A_{d,i})C_{runoff} + (0.5H_tL_tv)C_{in}) + (L_s + L_p + L_d + L_b)]}{[I(\sum D_{c,i}A_d, i) - 0.5B(H_tL_t - H_{(t-1)}L_{(t-1)}) + 0.5H_tL_tv + K_bV]} \quad (3\text{-}35)$$

3.9 Definition of non-point source input functions Terms and Application Values:

In this section, all terms used to develop non-point source input functions are defined and computed. This section provides recommended estimates for all variables to be used in association with the application of the water quality model developed in this chapter. Most recent literature and direct field measurements values and estimates were presented in this section.

3.9.1 Sand Input Function:

$L_{s(t)}$= ρ* $A_{s(t)}$*d_s*C_s*f, cfu/hour, (3-20)

ρ, Bulk density for sand, dense and uniform = 109 lb/ft^3 (Lundeberg, 1992).

Convert the units into (kg/m^3):

ρ = 109 (lb/ft^3)x16.018(ft^3/lb)x(Kg/m^3) ≈ 1,746 kg/m^3

Convert the units into (g/m^3):

$$\rho = 1{,}746\ (kg/m^3) \times 10^3\ (g/kg) \approx 1.75 \times 10^6\ g/m^3$$

$A_{s(t)}$, Width of the control volume (B, m) multiplied by the ebbed or flooded horizontal distance (ΔX_t, m, will be determined from direct field measurements, Figure 3.3) within one hour time scale during one tidal cycle i.e. high tide to low tide and low tide to high tide 12 hours period.

Therefore:

B= the distance between the center lines of transects KL and KJ, approximately 400 feet

$$B = 0.3048\ (m/ft) \times 400\ (ft) = 122\ m$$

$$A_{s(t)} = B \times \Delta X_t,\ m^2$$

Definition of ΔX_t is provided in Figure 3.3

d_s, Value can be obtained from the literature, Sanders et al. (2005) reported values (10^{-3} to 10^{-2} m), or from the sand deposit/erosion field work conducted at the study site by Wright et al.(2006). In this field study direct measurements of sand deposit or erosion were collected each hour for a complete tidal cycle (i.e. high tide to low tide and low tide to high tide) along 3 transects perpendicular to the shore line within the inter-tidal zone; for outgoing tide, sand deposition occurred at 4×10^{-3}m on average and for incoming tide sand erosion occurred at 6×10^{-3} m on average.

For outgoing tide sand deposition occurs:

$d_s = 4x10^{-3}m$

For incoming tide sand erosion occurs:

$d_s = -6x10^{-3}m$

C_s, Average enterococci concentration, CFU/g of dry sand. This number is obtained from the comprehensive environmental beach sand analysis, Wright et al. (2005). The following average concentrations of enterococci CFU/g of dry sand were found in sand from the inter-tidal and dry sand zones.

Average enterococci concentration of sand from the inter-tidal zone:

C_s = **56** CFU/g of dry sand

Average enterococci concentration of sand from the dry sand zone:

C_s = **380** CFU/g of dry sand

f, Frequency of scouring (4 tidal cycles/day)

$\mathbf{f} = (1/6)\ hr^{-1}$

3.9.2 Runoff Input Function:

The mathematical expression for the runoff non-point source input function is described in equation (3-36) which equals the estimated water runoff flow (L^3/T) multiplied by the average concentration of enterococci (M/L^3) found in runoff water specific to the study site.

$$\mathbf{Q_{runoff}\,x\,C_{runoff}} \qquad (3\text{-}36)$$

Or:

$$\mathbf{I(\sum D_{c,i}A_{d,i})x\ C_{runoff}} \qquad (3\text{-}37)$$

Where:

$\mathbf{A_{d,i}}$= Drainage area of contributing area i, m^2

I = Rainfall intensity, m/day

$\mathbf{D_{c,i}}$ = Drainage Coefficient of contributing area i, value varies from 0 to 1 based on the land use and cover

$\mathbf{A_{d,i}}$, this is the drainage surface area impacting the control volume. From the field it is estimated to be the width of the control volume (B, m), multiplied by the distance between the edge of water and the center line of the paved road. This drainage area is

divided into three different areas based on the degree of imperviousness; from the edge of the inter-tidal zone at high tide to the water line (A_{d1}), the entire dry sand zone (A_{d2}), and from the center line of the paved road to the adjacent edge of the dry sand (A_{d3}), Figure 3.5.

Therefore:

$$\sum A_{d,i} = A_{d1} + A_{d2} + A_{d3}\ (m^2)$$

I, this value is estimated based on either direct measurements or the following assumptions: S. Biscayne Bay watershed receives between 60 to 80 inches of rain/year on average. This estimate was derived by reviewing the South Florida Water Management rainfall data from January 01, 2000 to June 30, 2006 Miami International Airport rainfall gauge. Most of the precipitation occurs during the Hurricane Season or Rainy season from June 1st to November 30th of each year, 6 months. If we take the value of 70 inches/6 months or 9.88×10^{-3} m/day.

Therefore:

I = 70 (inches/6 months) x (ft/12 inches) x (0.3048 m/ft) = 1.778 (m/6 months)

$$\mathbf{I} = 1.778\ (m)/\ (6\ months \times 30 days/month) = 9.9 \times 10^{-3}\ m/day$$

$\mathbf{C_{runoff}}$, This value was estimated from the field data collected at the study site during rainfall events by the MDCHD inspectors Elmir et. al 2004. Runoff samples were collected directly from runoff channels discharging into the inter-tidal zone. The average

levels of enterococci in runoff water were $1.5x10^4$ CFU/100 ml with a range from $2x10^3$ to $4.9x10^3$ CFU/100 ml.

Therefore:

$$C_{runoff} = 1.5x10^4 \text{ (CFU)} / (100\text{ml} x \text{m}^3/10^6\text{ml}) = 1.5x10^8 \text{ CFU/m}^3$$

$D_{c,i}$, This value can be estimated from the literature based on land use, population, and degree of imperviousness, and ranges from 0.1 to 0.3 for population of about 1 person/acre (rural) to 0.7 to 0.9 for heavy industrial and commercial areas with densities greater than 50 persons/acre (Thomann and Muller 1987). The study site is zoned commercial/industrial with low to mid population density. The paved area is 12.5 % of the total drainage area and the remaining area consists mainly of beach sand which drains very well. Thus D_c will have a value of 1, 0.7, and 0.5 for the paved, dry sand, and wet sand areas respectively.

3.9.3 Birds Input Function:

$L_b = (N_b x W_b x U_b) x (1/24)$ CFU/hour (3-22)

Where:

N_b, average bird population will be estimated from analyzing the digital photographs taken by the camera installed at Miami Seaquarium for two years.

W_b, average level of enterococci in bird feces was estimated by Wright et al. (2005) $3.8x10^5$ CFU/g of dry feces (23 x 10^6CFU/100 mL fecal water) with a range of 350 to 3.1 x 10^6CFU/g-dry feces.

$W_b = 3.8x10^5$ CFU/g of dry feces

U_b, average weight of dry feces released per bird per day, g/day/bird

Computation of U_b and Assumptions:

U_b, This parameter was estimated using the assumptions used by Kushlan (1977 and 1979), Table 3.1.

$$U_b = \left[\frac{Ax(1-B)}{BxC}\right], \text{ g/day/bird} \quad (3\text{-}38)$$

Where:

A = Average daily (kcal) an adult Ibis needs to meet existence, kcal/day/bird

B = % Assimilation efficiency in adults

C = Average fecal energy content, kcal/g of dry feces

U_b value is presented in Table 3.1:

U_b = 11.4 g of dry feces/day/bird

3.9.4 Dogs Input Function:

$$L_d = (N_d x W_d x U_d) x (1/24) \quad \text{CFU/hour} \quad (3\text{-}24)$$

Where:

N_d, average dog population documented on the study site per day. This data will be obtained from the analysis of pictures taking via a digital camera positioned at the site throughout the study.

W_d, Wright, et. al (2005). Estimated that the enterococci levels observed for dog feces were the highest and most variable, with an average concentration of 6.6 x 10^7 CFU/g of dry feces (6.4 x 10^9CFU/100 mL fecal water) and a range of 5.7x10^4 to 2.8x10^8CFU/g dry.

W_d = 6.6 x 10^7 CFU/g of dry feces

U_d, average weight of dry feces released per dog per day, g per dog day

Computation of U_d and Assumptions:

The values of U_d were estimated using the NRC, National Research Council (2005), formula.

$$U_d = \left[\left(\frac{F}{G}\right) x H x K\right], \text{ g of dry feces/day/dog} \quad (3\text{-}39)$$

Where:

F = The daily food consumption of dogs, calories

G = Average fecal energy content, calories/g of matter

H = % of the daily food intake will be released as waste

K = Portion of the dry matter in the waste

U_d values are presented in Table 3.2:

Using NRC (2005) method:

$U_d^{(2)}$ =15.7 g/day/dog

Using Wright, et. al (2005):

$U_d^{(3)}$ =29.7 g/day/dog

On average, U_d Value obtained fromWright et. al is approximately 1.9 times greater than the value calculated from the NRC method.

Note, Wright, et. al (2005), estimated via direct field observations that a 3.2 Kg and a 27.2 Kg dog on average (Average of 7 samples collected and weighted daily) release 7.6 g/dry feces /day and 51.8g/dry feces/day respectively Table (3.2).

3.9.5 Bathers Input Function:

$L_p=(f_p x N_p x Y_{tskin})(1/24)$ CFU/hour (3-26)

Where:

f_p, average number of 15 minutes exposure per bather per day. It is estimated that a bather's exposure to marine waters is four times per day, Elmir, et al. 2005.

$f_p = 4$ Fifteen minutes bather exposure/day/bather

N_p, average number of bathers documented on the study site. This data will be obtained from the analysis of pictures taking via a digital camera positioned for a couple of months at the site.

y_{tskin}, Enterococci loading rate, CFU/15 minutes bather exposure. This value was field determined by Elmir, et al. 2005. On average a bather sheds $3.15x10^5$ per 15 minute exposure to marine waters. A 1.05 multiplier is included to account for the numbers of organisms transported via sand particles adhered to bather skin.

$y_{tskin} = 1.05x3.15x10^5 = 3.31x10^5$ CFU/15 minutes bather exposure

3.9.6 Sinks Output Function:

$(K_bCV)(1/24)$ CFU/hour (3-28)

Where:

K_b= Overall net decay rate for enterococci in the water column, day^{-1}. The K_b value used in this study is a lumped factor that was measured experimentally and incorporates all of the factors that may promote die-off.

C= Average enteroccocci concentration in the water column within the control volume CFU/m^3. This variable will be computed from the enterococci balance equation.

V= Control Volume, m^3

K_b, Overall net decay rate for enterococci in the water column, day^{-1}. This rate includes the following factors: Sunlight, temperature, salinity, predation, nutrient deficiencies, toxic substances, and regrowth. Fujioka et al. (1981) reported values up to 55/day in seawater exposed to sunlight for *fecal streptococci* (enterococci are members of this group of organisms). Wright et al. (2006), reported an average value of 22/day. This average was estimated via a series of field experiments using water and sand collected from Hobie Beach. During those experiments enterococci die–off rates were tracked and plotted hourly.

$K_b = 0.92\ hr^{-1}$

Limitations:

Environmental and meteorological factors that are know to influence the enterococci levels in the water column were not included in the model including wind and ocean current speed and directions, temperature and turbidity levels. In addition re-suspension of bacteria from beach sediments into the water column as source function and settling of enterococci from the water column into beach sediment as a sink function were not modeled or incorporated into the model. Bulk density of sand used to estimate the input function was not determined from the field instead literature value was used. Overall net decay rate of enterococci (K) was used instead of using separate decay rates due to sunlight, salinity, predation, and toxic chemicals. Model was not using real-time data (i.e. bathing load, animal load, rainfall) monitored and collected specifically for the testing purpose. The model developed in the dissertation is a simple conceptual water quality model subject to substantial development to become a full scale hydrodynamic model.

Conclusions:

Model testing and calibrations including results and discussions are represented in the next chapter. Data from the comprehensive environmental monitoring efforts Wright et al.(2004, 2005) (water, soil sampling results and meteorological data), and from the two bather shedding field experiments Elmir et al.(2006) will be utilized to run the model and to estimate the non-point source microbial loads. Finally, the model will be run and calibrated using various combinations of non-point source of microbial loads. Results from the model will be analyzed and discussed to determine which of the non-point

microbial source(s) influence most the beach water quality and under what environmental and meteorological conditions.

Table: 3.1 Computation of U_b using values for A, B, and C Suggested by Kushlan (1977 and 1979)

A	B	C	U_b
114	80%	2.5	11.4

A: Average daily (kcal) an adult Ibis needs to meet existence, kcal/day/bird
B: % Assimilation efficiency in adults
C: Average fecal energy content, kcal/g of dry feces
U_b, average weight of dry feces released per bird per day, g/day/bird

Table 3.2 Computation of U_d values using the NRC(2005) Method and the direct field measurements by Wright et al (2005)

Name of Dog	**Dog Size**	**Dog weight, Kg**	**F(1)**	**G**	**H**	**K**	**U_d(2)**	**U_d(3)**
Ginger	Medium	27.2	155.3	4.0	0.2	0.333	26.2	51.8
Bingolina	Small	3.2	22.7				5.2	7.6
						Average	15.7	29.7

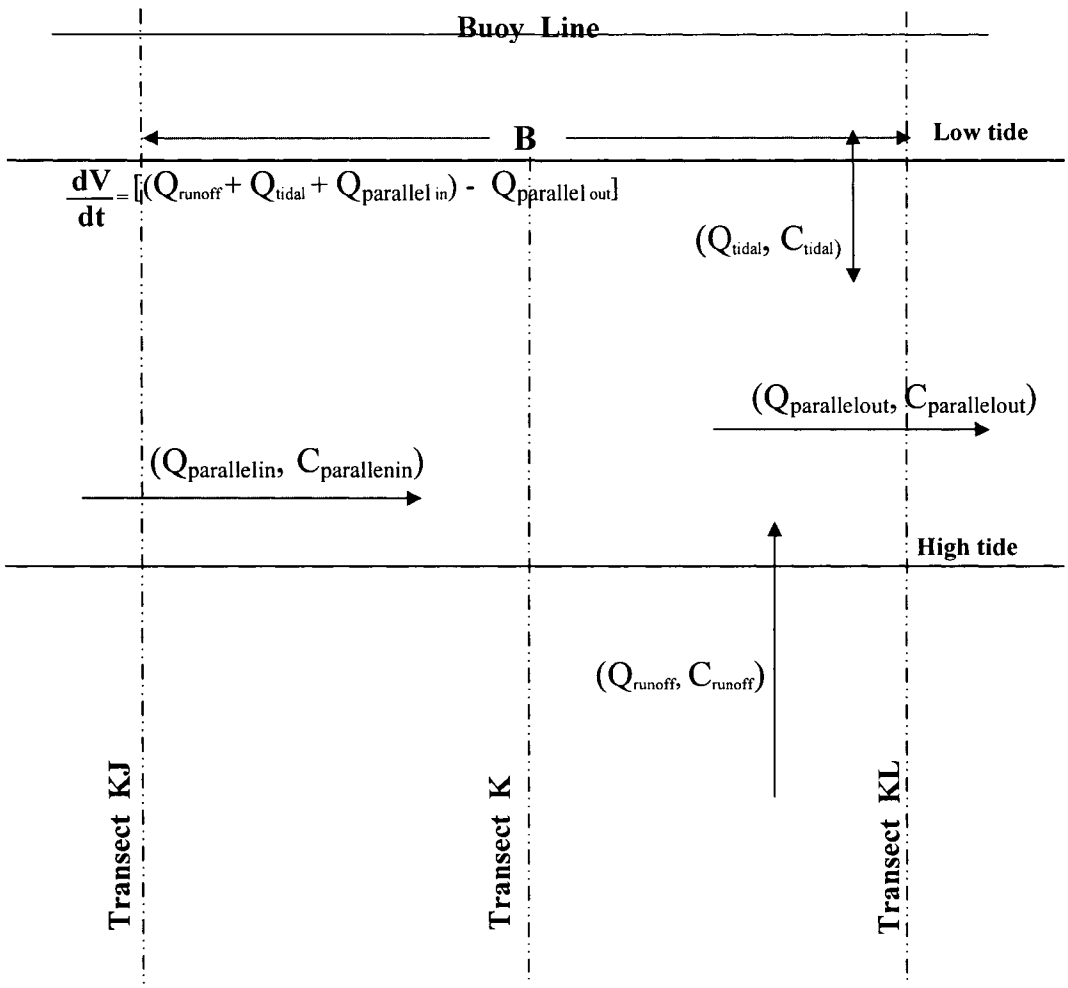

Figure 3.1 Conceptual water quality model within the inter-tidal zone

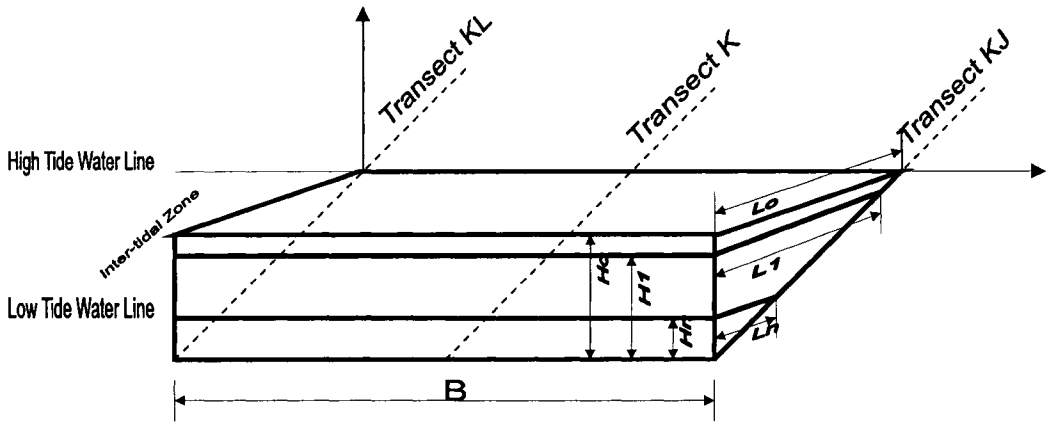

Figure 3.2 Three dimensional view of the tidal prism

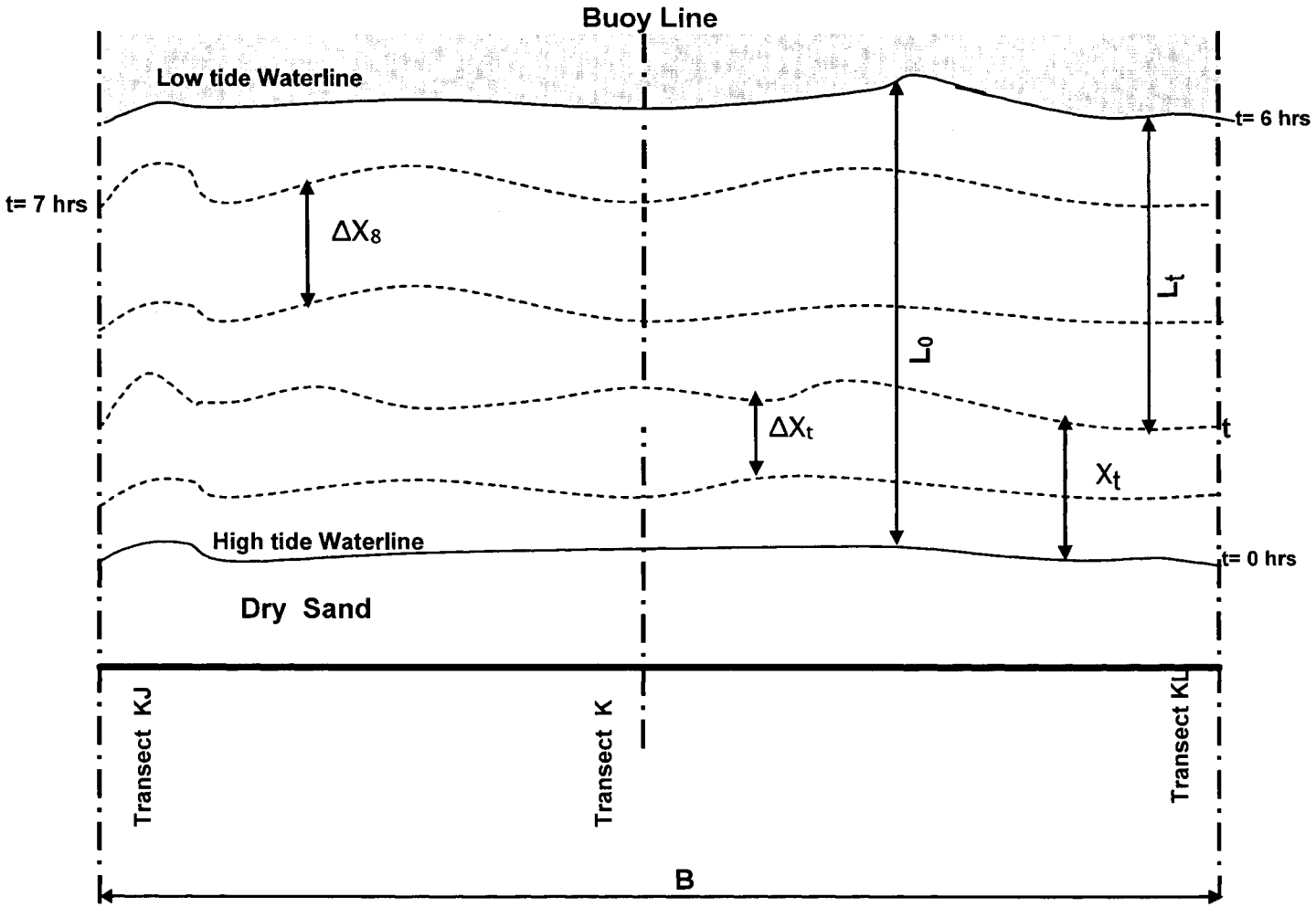

Figure 3.3 Plan view of the tidal prism within the control volume

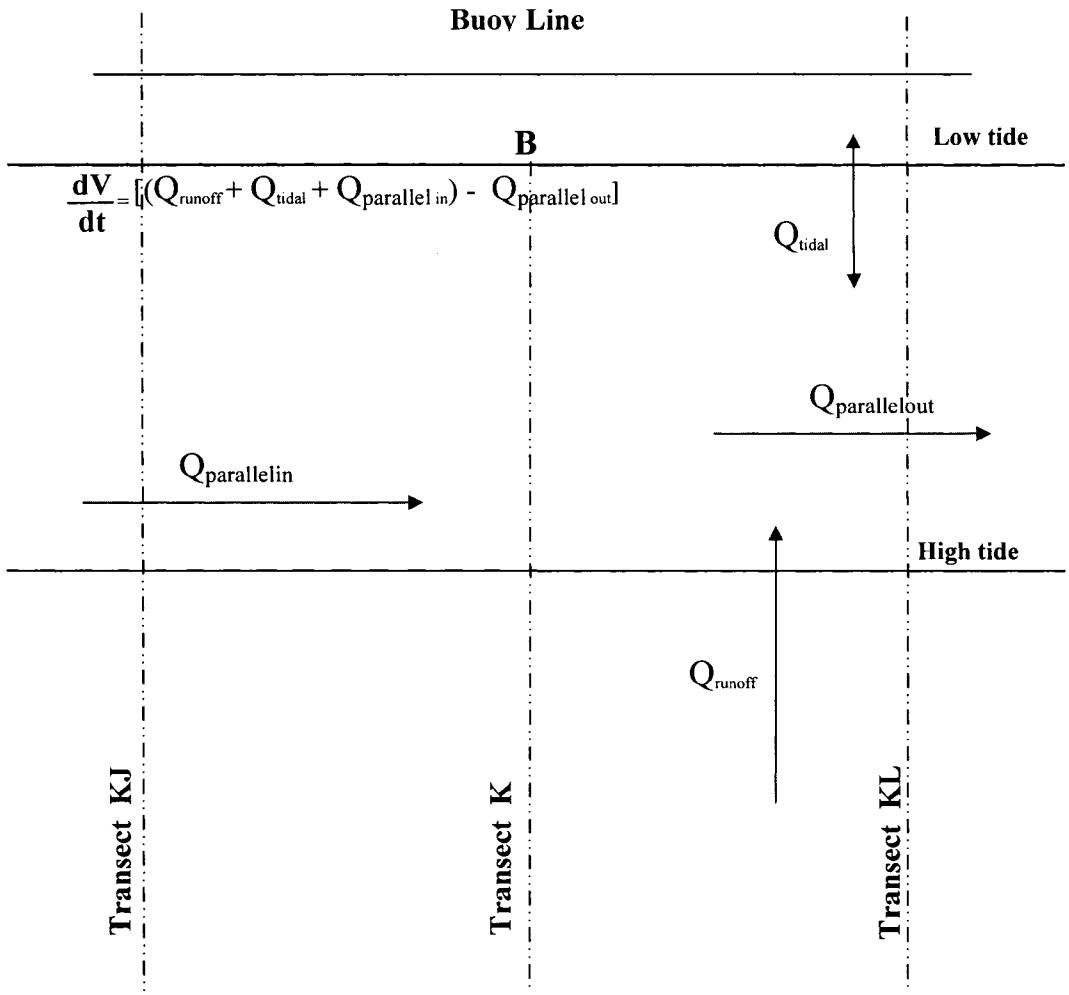

Figure 3.4 Schematic of the water balance within the control volume

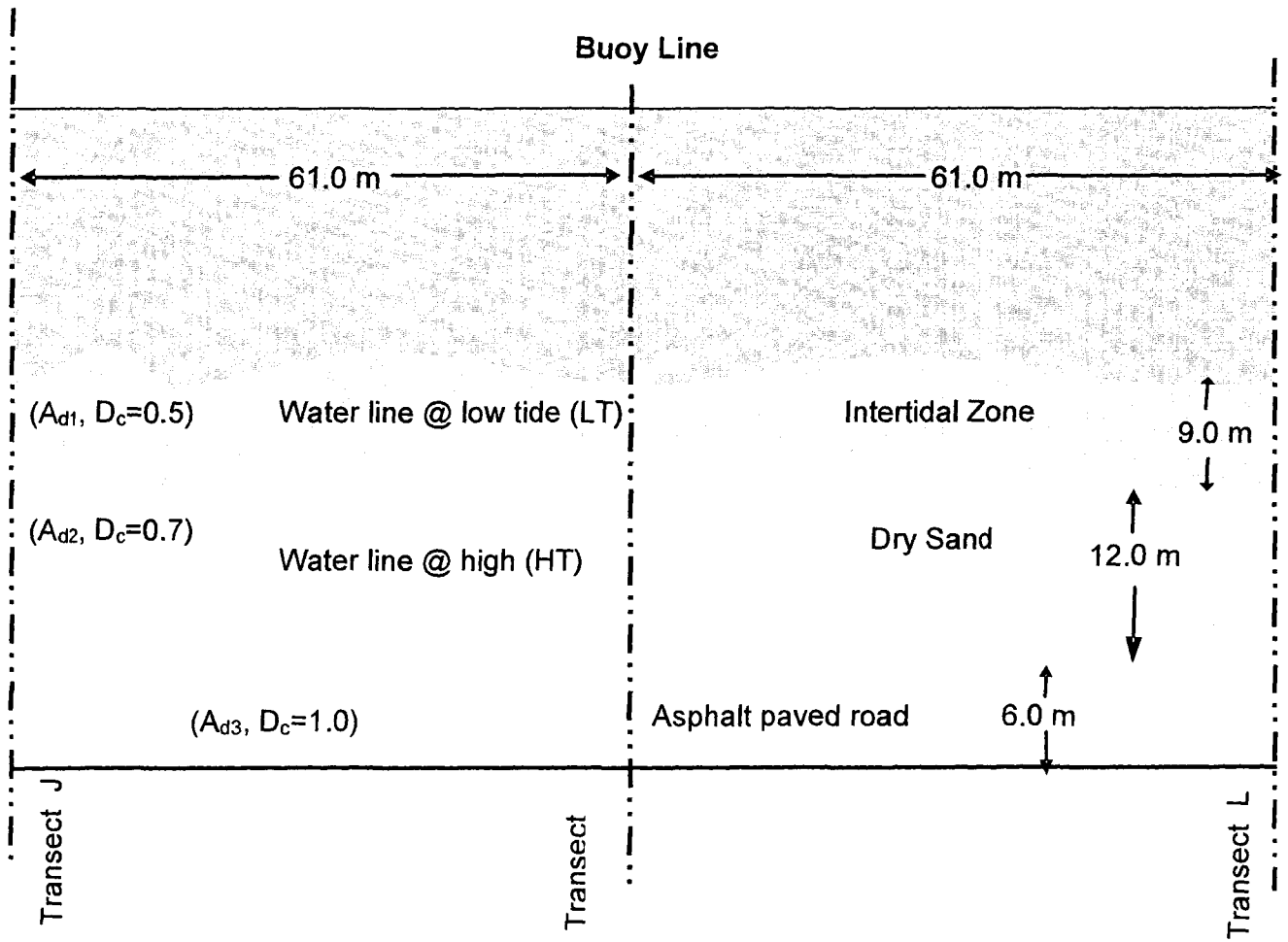

Figure 3.5 Schematic of drainage areas within the control volume

CHAPTER 4

DISSERTATION OVERALL CONCLUSION

4.1 Introduction

The study is aimed to develop a water quality model that estimates the concentrations of enterococci in marine waters at Hobie Cat Beach. Enterococci is the USEPA recommended microbial indicator. A literature review of the most recent and relevant environmental and epidemiology studies, regulatory monitoring data and standards concerning the use and applicability of the traditional fecal indicator microbes (*E.coli*, fecal coliforms,and enterococci) in particular as they apply to marine waters in tropical and subtropical environment are reviewed in great detail in chapter 1. The design, implementation, and results and discussions including conclusions of the two human shedding field experiments are presented in chapter 2. Chapter 3 presents step by step the process used to develop the water quality model specific to the study site. The model includes the development of the general equation for bacteria balance using the mass conservation principle and the mathematical expressions of all non-point microbe source functions (bathers, dogs, birds, water runoff, and sediments) as identified in the site sanitary survey. Data from the comprehensive environmental monitoring efforts (water, soil sampling results and meteorological data), and from the two bather shedding field experiments were utilized to run the model and to estimate the non-point source microbial loads. Finally, the model was run and calibrated using various combinations of non-point source of microbial loads. Results from the model were analyzed and discussed

in this chapter to determine which of the non-point microbial source(s) influence most the beach water quality and under what environmental and meteorological conditions.

4.2 Review of Microbial indicators:

Monitoring the sanitation of recreational coastal waters has been regulated by measuring concentrations of fecal indicator bacteria. The bacteria utilized are those typically found in human feces in high concentrations (*E. coli*, fecal coliforms, and enterococci). An elevated concentration of these indicator microbes within a water body would thus indicate that the water body has been contaminated by human waste and is unsafe for recreational use.

The U.S. Environmental Protection Agency recommends (USEPA 1986) that States utilize the indicator microbes enterococci and/or *Escherichia coli* to determine whether health advisories or closures should be issued for recreational coastal waters. *E. coli* is recommended for freshwaters and enterococci are recommended for both fresh and marine waters.

Recently the use of fecal indicator bacteria to monitor and regulate the recreational use of coastal waters has come into question, particularly in the tropical and sub-tropical marine environments. Specifically the *USEPA's Action Plan for Beaches and Recreational Water (EPA/600/R-98/079) in 1999* states that, "Currently recommended fecal indicators may not be suitable for assessing human health risks in the tropics. Studies have suggested that at tropical locales such as Puerto Rico, Hawaii, and Guam, *E. coli* and enterococci can be detected in waters where there is no apparent warm-blooded animal source of contamination. If this phenomenon is widespread under

tropical conditions, additional research should be conducted to modify approaches for monitoring, or to develop new tropics-specific indicators."

In 2001 as a follow up to the *USEPA's Action Plan for Beaches and Recreational Water*, the Hawaii State Department of Health conducted a workshop titled " Tropical Water Quality Indicators". A total of 18 national and international experts on the subject were selected to participate in the workshop. The following are the four workshop consensus statements issued: 1- Soil, sediments, water, and plants may be significant indigenous sources of indicator bacteria in tropical waters, 2- The inherent environmental characteristics of the tropics affect the relationship between indicators of fecal contamination (*E. coli*, fecal coliforms, enterococci) and health effects observed in bathers, which may compromise the efficacy of EPA guidelines, 3- Fecal indicator bacteria (*E. coli*, fecal coliforms, enterococci) can multiply and persist in soil, sediment, and water in some tropical/subtropical environments (Hawaii, Guam, Puerto Rico, south Florida), and 4- Recreational water quality guidelines for the tropics/subtropics should be supplemented with additional alternative indicators (*C. perfringens*, coliphages) for watershed assessment (or sanitary survey).

To make matters even more complicated, there have been documented cases where coastal waters monitored for both sets of fecal indicator bacteria (fecal coliforms and enterococci) have passed regulatory limits for enterococci and not for fecal coliforms, and vice versa Table 1.4. So a regulator is left with a perplexing situation where it is not clear which indicator microbe(s) should be utilized, and once the data are obtained, how these data should be interpreted.

4.3 Summary of Environmental and Epidemiology Studies at Hobie Cat Beach

Nova Southeastern University 2001-2003 evaluated indicator bacteria and selected pathogens at Hobie beach, Hollywood and Fort Lauderdale beaches, South Florida. The main objectives of the study were: 1-document the numbers of *E coli*, enterococci and fecal coliforms in beach sand and determine if they are attached or free in interstitial water, 2- compare the survival of indicator organisms in water versus sand, and 3- evaluate swimming related illnesses and exposure to beach waters via epidemiological questionnaire. The study found that concentrations of bacteria indicators were higher in dry sand, followed by wet sand (swash zone), and followed by seawater, and majority of indicators were attached to sand grains i.e. they were metabolically active. The study suggested that the swash zone receives significant bacterial inputs from the beach, and sediment re-suspension plays significant role impacting bacterial loading in the water column. The results from the beach questionnaire did not show clear signs of symptoms in the recreational population in comparison with the control population.

Shibata et al., 2004, conducted a pilot epidemiological and water quality study at two public beaches, Hobie and Crandon, located in southern part of Biscayne, Miami, Florida. The main objectives of the study were: 1- evaluate the microbial water quality including soils at the selected beaches and the bay using the regulatory microbial indicators (total and fecal coliforms, *E. coli* and enterococci) and *Clostridium perfringens* (alternative microbial indicator recommended for tropical climate), 2- conduct sanitary surveys to identify point and non point sources of fecal pollution; identify sources of microbial indicators, and 3- administer an epidemiological study to evaluate relationship between swimming related illnesses and microbial density. Intensive spatial water quality

monitoring indicated the southern tip of the shoreline at Hobie Cat Beach appeared to be the source of microbes (Figure B.2). This finding was supported by the soil sample results collected from this end of the shoreline. The detection of those indicators in the soils/vegetation of the shoreline without a known point source of fecal pollution again questioned the suitability of those indicators for measuring the sanitary water quality in subtropical/tropical climates. The sanitary survey indicated that there is no point source of microbe contamination impacting the beach. Pets mainly dogs and birds, urban runoff, natural sources such as sand and weeds and people were the principle non-point source of microbial contamination documented at the site. The epidemiological pilot study concluded that, "No dose-response relationship existed between density of microbes and health effects."

Wright et al., 2005, conducted a comprehensive environmental study at Hobie Cat Beach. The objectives of the study were: 1- determine sources of enterococci to the beach waters and environmental conditions that control enterococci levels, and 2- confirm the findings from the earlier study conducted at the site, Shibata et al.,(2004). Four monitoring efforts were designed and implemented: a- transect work which included high and low tide comparisons of water and sediment samples, b- spatially intensive water and sediment samples, c- hourly water and sediment sampling during a 48-hour period, and d- runoff water sampling. Results showed that enterococci levels in water increased as the shore was approached. The average level in knee deep water within a few feet of the shore (83 CFU/100 ml) was higher than the level in water 100 m from the shore (29 CFU/100 ml). On average, levels in knee deep water were 69 CFU/100 ml during high tide and 5 CFU/100 ml during low tide. Sediment samples collected under water from the

inter-tidal zone during high tide had lower numbers (5,400 CFU/100 ml pore water), while sediment samples collected during low tide from the same area but above water were higher (23,600 CFU/100 ml pore water). The highest levels of enterococci were measured in "dry" sediments above the high tide line but within a few meters of the inter-tidal zone (35,900 CFU/100 ml pore water). Microbe levels in sediments consistently decreased away from the inter-tidal zone. Hourly sampling showed that tides were a more important factor than sunlight effects. Runoff water was found to contribute water with high levels of enterococci (14,500 CFU/100 ml). Overall, levels of enterococci were higher in sediment samples than in water samples, and levels were found to be more concentrated closer to the shore. These results suggest that the wash-in of sediments and accompanying pore waters from the inter-tidal zone play a major role in controlling enterococci levels in recreational beach waters. Wash-in occurs through both tidal fluctuations and runoff. The sampling site and locations is shown in Figure B.3 and a summary of the results generated from the Four sampling efforts are presented in Figures B.4 through B.13.

4.4 Bathers Shedding Field Studies:

General results and conclusions from the two field experiments aimed to estimate the concentrations of enterococci and *Staph. aureus* shed by bathers are presented in this section.

Enterococci, a common fecal indicator, and *Staphylococcus aureus*, a common skin pathogen, can be shed by bathers affecting the quality of recreational waters and resulting in possible human health impacts. Due to limited information available

concerning human shedding of these microbes, this study focused on estimating the amounts of enterococci and *S. aureus* shed by bathers directly off their skin and indirectly via sand adhered to skin. Two sets of field experiments were conducted at Hobie Cat Beach, a marine beach, located in Miami-Dade County, Florida.

Results from this study demonstrated that bathers shed significant concentrations of enterococci and *S. aureus* into the water column. Bathers shed *S. aureus* and enterococci on the order of $3x10^6$ and $3x10^5$ CFU per bather per 15 minutes exposure period respectively Table 2.2. Comparison of the results from the current study with the results from prior studies indicates that the type of water apparently did not impact the degree of shedding as the results from the current study using marine water were consistent with prior studies which exclusively used freshwater. Between cycles, the bacteria detected in the water column decreased after each subsequent cycle.

All studies evaluated including the current study showed that *S. aureus* was shed at concentrations at least one order of magnitude greater than enterococci. Repeat washing or exposure of bathers has not been evaluated previously. The current study showed that total enterococci and *S. aureus* released by bathers decreases significantly between bathing episodes, in particular after the first wash cycle. The decrease was faster for *S. aureus* (50%) relative to enterococci (42%), on average (Figure 2.1). This observation may be due to a washing effect leaving less bacteria on the body for shedding in the subsequent cycle. This conclusion agrees with the long standing universal requirement that bathers should shower before entering recreational waters to reduce the microbial load in particular at swimming pools since the water volume is limited.

Studies that evaluated the risk of swimming related illnesses associated with exposure to waters contaminated with non-point sources indicated that gastrointestinal illnesses observed in swimmers were correlated with high numbers of bathers and high densities of *S. aureus* (Calderon et al. 1991; Charoenca and Fujioka 1995). These prior studies proposed the use of *S. aureus* as an indicator to predict and design appropriate bathing load. The current study supports such a recommendation as *S. aureus* is shed in quantities one order of magnitude greater than enterococci. Studies should thus be designed to evaluate the potential use of *S. aureus* as a measure of possible health effects from bather to bather transmission of illness.

There are no other data available to the authors' knowledge that estimates the total densities of bacteria transported via sand adhered to skin into the water column indirectly via bathers. The enterococci contribution from sand adhered to skin, was small relative to the amount shed directly from the skin and represented less than 5% of the total enterococci shed by bathers. Those numbers are site specific due to the many variables that can impact these values including physical and microbial quality of beach sand.

This study recommends additional targeted studies to confirm the results of this effort and to estimate how much *S. aureus* bathers carry into the water column via sand. Furthermore, given the significance of bathing load, water quality models of recreational beach waters impacted by non-point sources of microbes should include bathing load as one of the significant pollution sources. The contribution from sand adhered to skin can be potentially ignored in models which simulate non-point sources of enterococci as the quantities from sand on skin is small, on average, in comparison to the total body burden.

4.5 Water Quality Model Application Results and Discussion:

In this section the results from running the water quality model for various loading scenarios are presented and discussed. The model was developed in Chapter 3.

Various loading scenarios were tested using the developed water quality model. Table 4.1 shows all various scenarios and their corresponding results. Scenarios were designed to determine the degree of influence by each non-point sources (rainfall, dogs, birds, and people) independent from the other sources. Review of the results indicates that water runoff is the most significant non-point source impacting the levels of enterococci in the water column at Hobie Cat Beach. Dogs are the second most significant non-point source followed by people and birds, Figure 4.1.

In addition, the model was calibrated by inputting the estimated non-point loads using the variables (rainfall, number of dogs, birds, people, and densities of enterococci in sediment within the inter- tidal zone and the water column) documented at the site from the four field studies (Wright et al. 2004-2005) (Table 4.2). These studies included:1- transect intensive sediment and water sampling study during Summer 2004, 2- transect intensive sediment and water sampling study during Winter 2005 , 3- 48-hour sediment and water sampling study 2004, and 4- Labor Day weekend sediment and water sampling study 2005. Results from this calibration effort are presented in Figures 4.3 and 4.4. Sample calculations of all non-point source input functions including the complete model run corresponding to each study are presented in table format in Appendix E. Results indicate that there is a strong correlation between the density of enterococci in the water column obtained from Wright et al. for all four studies vs. the corresponding model runs. Except for the transect intensive water and sediment sampling

study winter 2005, the model runs yielded higher concentrations than the other studies but higher within the same order of magnitude. During the 48 hour study there was documented high rainfall recording which resulted on high concentrations of enteroccci in the water, the model tracked this concentration very well. This finding indicates that the model responds well to rainfall. This calibration effort was limited however due to the following facts: 1-number of birds was not documented, the data from the surveillance digital camera did not capture the days when the four studies were conducted, thus manual documentation of non-point sources was used instead. This method may be subject to human error. 2- Average concentrations of enterococci in the water column for the entire tidal cycle was used for comparison as oppose to hourly concentrations. Note, model is designed to calculate hourly concentrations, 3- meteorological factors such as wind direction, and speed, sediment re-suspension were not included in the water quality model. Despite these limitations the overall calibration results are significant thus use of such models can be a powerful tool to aid the regulatory beach monitoring program for assessing the microbial water quality thus making informed decisions to protect public health and the economy.

4.6 Dissertation Overall Recommendations:

This section lists the overall recommendations resulting from this study. These recommendations are intended to improve and expand the existing research data and the scientific understanding of this area, especially as they apply to the impact of subtropical climates and environmental factors on the use of indicator microbes for assessing the sanitary quality of recreational waters.

1. Conduct additional targeted human shedding studies to confirm the results of this study in particular the effect of repeat washings. Literature review indicates that data concerning the effect of repeat washing is limited only to this work.
2. Conduct a comprehensive epidemiology study to determine dose response relationships between health effects and exposures to marine at different levels of indicator microbes. This study will be the first one to be conducted in South Florida a sub-tropical environment at a site not impacted by direct point sources of human waste pollution.
3. Utilize the data from all previous environmental studies at Hobie Cat Beach and at other locations in South Florida (Fort Lauderdale) to evaluate and model the survival, growth and transport of fecal indicator microbes in sand, in particular within the inter-tidal zone.
4. Conduct a health assessment to determine the health risks associated with exposure to beach sand. It has been well documented that beach sand contains high levels of fecal indicator microbes several times above the recommended guidelines for recreational waters.
5. Conduct an environmental study to evaluate the relationships between fecal indicator microbes and with human pathogens in soil and water to accurately determine health risks.
6. Develop and use predictive mathematical models using site specific historic and real-time environmental and meteorological data derived from previous research studies and regulatory monitoring programs as a tool to aid regulators in the decision making process for protecting public health and the economy.

7. Conduct and constantly update beach sanitary surveys. Use Geographical Information Technology (GIS) and surveillance cameras similar to the one used in this project to document land application and use, population and animal density, and sewage spills and storm events. Sanitary surveys should always be used to supplement the regulatory beach monitoring programs.
8. Encourage public health departments to develop and implement policies aimed to eliminate and or minimize bacteria loads due to water runoff, bathers, animals, and sand. For example, dog owners should be required to clean after their dogs. Provide showers at the beach and request from bathers to shower before entering the swimming or wading area, put up permanent signs at conspicuous places at the beach that promote good bather and beach hygiene practices.

Table 4.1 Results of model runs for pre-designed loading scenarios

			Scenario No.			
			1	2	3	4
Tidal Direction	t hrs	t, time interval, hrs	Ct, CFU/100ml	Ct, CFU/100ml	Ct, CFU/100ml	Ct, CFU/100 ml
High to Low Tide Low to High Tide	0					
	1	0 to1	113	2	2	167
	2	1 to 2	162	4	4	222
	3	2 to 3	236	6	6	303
	4	3 to 4	355	8	9	431
	5	4 to 5	563	12	13	652
	6	5 to 6	1311	28	30	1512
	7	6 to 7	1746	1	4	1843
	8	7 to 8	765	1	2	856
	9	8 to 9	492	0	1	586
	10	9 to 10	322	0	1	409
	11	10 to 11	215	0	1	293
	12	11 to 12	147	0	0	217
		Avg.	**535**	**5**	**6**	**624**
Unit Number of Non-Point Source Equivalent to 0.1 inch/hr Rainfall Event			**1**	**1029**	**869**	**9**

Scenario #:

1=Rainfall equivalent to 0.1 inch/hour,no people,no dogs,no birds

2= 10 people, no birds, no dogs, and no rain

3= 10 birds, no dogs, no people, and no rain

4= 10 dogs, no birds, no people, and no rain

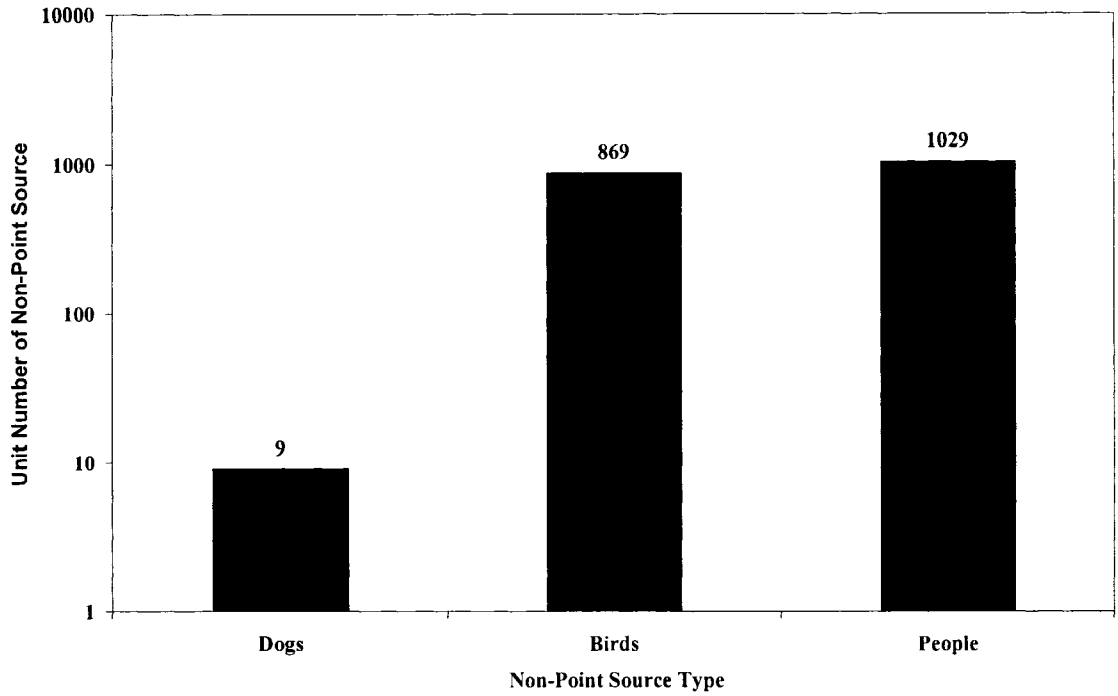

Figure 4.1 Quantity of non-point source equivalent to 0.1 inch/hr rainfall event

Table 4.2 Field studies Wright et al. (2005) documented variables

Study	Avg. daily Rainfall, m/hr	C_{sand}, CFU/g dry sand	No. birds	No.dogs	No. people	C^{a}_{water}, CFU/100ml	C^{b}_{water}, CFU/100ml
1	0.00013	39	Not Available	1	7	> 63	93
2	0.00002	13	Not Available	1	4	> 212	68
3	0.00302	56	Not Available	0	3	614	625
4	0.00031	19	Not Available	4	30	210	315

1: Transect intensive sampling study (Summer 2004)
Study date: June 22, 28, 29, 30, July 1, 6, 7, 12, 14
2: Transect intensive sampling study (Winter 2005)
Study date: February 3 and 10
3: 48 hour sampling study 2004
Study date: July 27 to July 29
4: Labor Day study 2005
Study date: May 31
[a]: Wright et al. (2005)
[b]: Water Waulity Model (Elmir et al. 2006)

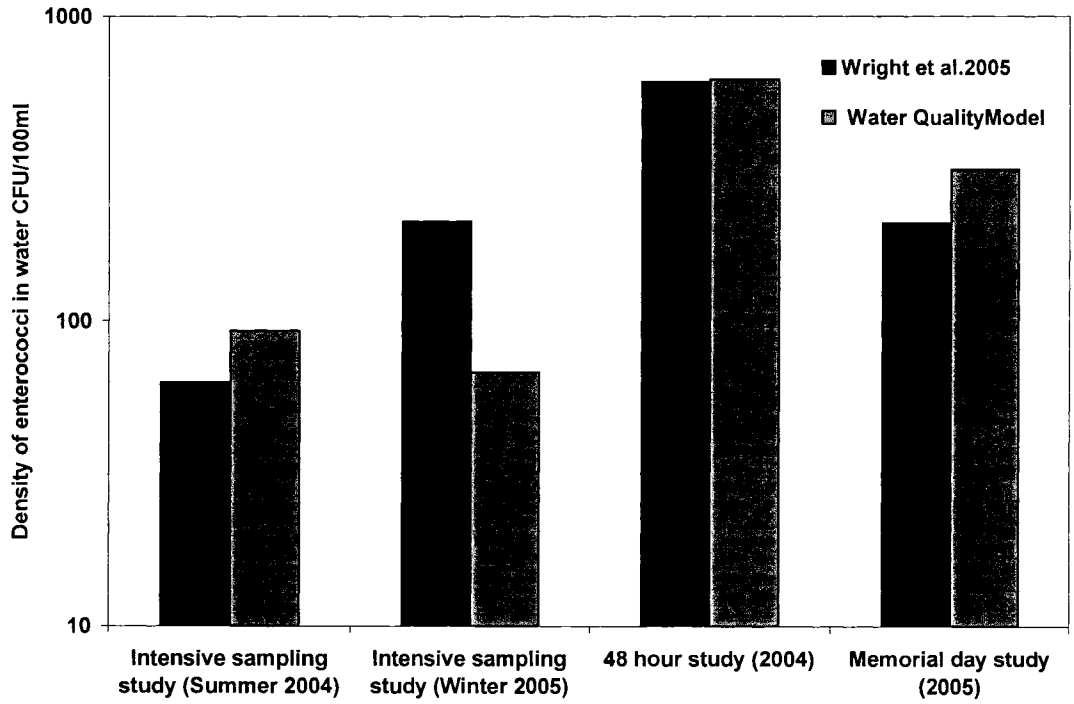

Figure 4.2 Comparisons of enterococci density in the water column between Wright et al. (2005) vs model for all four studies

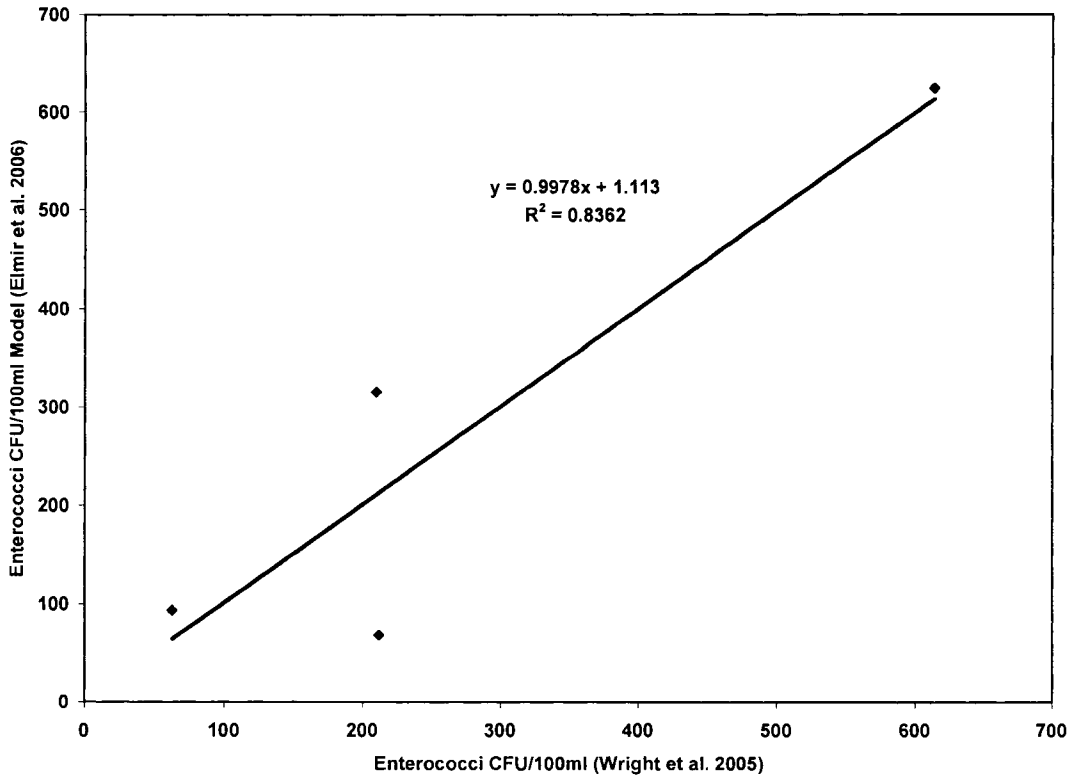

Figure 4.3 Correlation analysis for enterococci density in the water column between wright et al. (2005) vs. model for all four studies

APPENDICES

Appendix A

Laboratory Methods for Sediment and Water Microbial Analysis:

All samples were analyzed for total coliform, fecal coliform, *E.coli*, enterococci (IDEXX and membrane filter method), and *Clostridium perfringens* at the University of Miami, Environmental Engineering Laboratory, with the exception of fecal coliforms and enterococci (using the membrane filter method) for the spatially intensive water sampling effort, which were analyzed at the Florida Department of Health.

Sample Pre-processing for Microbe Analysis

Each sample was processed within six hours. Sand samples, however, were processed within 24 hours due to the more time consuming procedure (requires two filtration steps) and the large number of samples collected.

Two preliminary processing steps were performed for sand analysis. These steps included measurement of the water content of the sample and extraction of the microbes from the sand grains into a liquid. To measure water content, two scoops or approximately 10 g of the samples were collected from the sampling bags using a small spoon and were weighed (Mettler, AG245) on pre-weighed weighing dishes. The samples were then dried at 110°C for 24 hours and reweighed. The water content (WC) of the sample was then computed using the following equation.

$$WC = \frac{m_{wetsoil+dish} - m_{drysoil+dish}}{m_{wetsoil+dish} - m_{dish}} \quad \text{.....(A.1)}$$

where $m_{wetsoil+dish}$ is the weight of the soil before drying including the dish, $m_{drysoil+dish}$ is the weight of the soil after drying including the dish, and m_{dish} is the weight of the dish. This value was then used to calculate the weight of dry soil used in the corresponding microbiological analysis ($*m_{dry}$) as follows:

$$*m_{dry} = (1 - WC) * \left(m_{wetsoil+bag} - m_{bag}\right) \text{.....(A.2)}$$

where $m_{wetsoil+bag}$ is the weight of the soil placed in the WhirlpakTM bag (including the weight of the bag), and m_{bag} is the weight of the bag.

In order to extract the microbes from the sand grains to a liquid, two scoops of the sand sample were removed from the sampling bags and were asceptically placed into the new sterile pre-weighed Whirl-pak bags. The weight of the Whirl-pak bags containing sand were measured to calculate the amount of each sand sample. 200 ml of sterile de-ionized water were then added to each bag. The samples were shaken vigorously to

promote the transfer of microbes toward the liquid phase. The liquid samples were then filtered using 30 :m pore size nylon net filters (Millipore, Type NY30). A predetermined volume of the liquid extract was then utilized for subsequent bacterial enumeration.

More specific details concerning the microbial analytical methods are provided below. Information concerning Quality Assurance/Quality Control (QAQC) analysis are provided in appendix B.

Microbial Analytical Methods:

Two general types of microbial analytical methods were used. The first was the membrane filter (MF) method which provides a direct count of bacteria based on the development of colonies on the surfaces of a membrane filter. The method involves filtering a given volume of the sample through a 0.45 μm pore size filter membrane (Fisherbrand, 47 mm diameter membrane) that retains the bacteria. Sample volumes used were 30 and 100 ml during the first half of the dry season monitoring. The sample volumes were later changed to 10 and 50 ml for the last half of the dry season monitoring due to excessive microbial growth on the membranes for proper quantification. Only 50 ml volumes were evaluated during wet season monitoring.

The filter holder unit was presterilized and immediately after filtration the funnel was rinsed with at least 20 ml of sterilized phosphate buffered solution. The filter funnel was re-sterilized when used to filter a different sample. The MF method was used for the analysis of fecal coliform, enterococci, and *C. perfringens*.

The second method used for microbial analysis is based upon the use of a chromogenic substrate sold by the company called IDEXX. The chromogenic substrate method in simple term utilizes enzymes that are specific to particular microbial groups. These enzymes are attached to dyes which are then released when the target microbe is present in the sample. Enumeration of the microbe population is based upon the use of a tray (Quanti-Tray/2000, IDEXX, Westbrook, Maine) which separates the sample into 49 large and 48 small test wells. The number of test wells that show the characteristic color are then counted and used in conjunction with a standardized table to provide the concentration in terms of the most probable number (MPN). Total coliform, *E. coli* and enterococci were enumerated using the chromogenic substrate method.

Specific details concerning the microbial laboratory methods used are provided below.

Enterococci using MF method (USEPA 1997): The membrane filter containing bacteria was then placed on a selective medium (mEI agar, Becton Dickinson, Sparks, MD) and incubated at 41°C for 24 hours. Colonies with a blue halo were counted as enterococci.

Fecal coliform using MF method (APHA 1995): The filter was placed on modified mFC agar (Becton Dickinson, Sparks, MD) and incubated at 44.5 ± 0.2°C for

24 hours. Colonies that were various shades of blue were counted as fecal coliform bacteria. Colors of non-fecal coliform were gray to cream-colored.

C. perfringens using MF method (USEPA 1995): The filter was placed on mCP agar plate and incubated anaerobically using a anaerobic chamber fitted with an anaerobic GasPak (BBL GasPak Anaerobic System Envelopes, Becton Dickinson, Sparks, MD) at 44.5± 0.2°C for 24 hours. The plates were exposed to ammonium hydroxide fumes after the incubation and dark pink to magenta colonies were counted as *C. perfringens*.

Total coliform and E.coli using IDEXX method (IDEXX, Westbrook, Maine): IDEXX's Colilert-18® reagents were used for the simultaneous detection of total coliform and *E.coli*. Ten milliliters of sample were poured into 100 ml sterile vessel and diluted with 90 ml of sterile deionized water. Colilert-18 reagent was added into the vessel and mixed well. The sample was poured into Quanti-Tray/2000® (IDEXX) and sealed in an IDEXX Quanti-Tray sealer® (IDEXX). The trays were incubated at 35 ± 0.5 °C for 18 hours. Test wells showing a yellow color were positive for total coliform and wells that fluoresce under ultra violet (UV) light were positive for *E. coli*.

Enterococci using the IDEXX method (IDEXX, Westbrook, Maine): Enterococci were enumerated using IDEXX's Enterolert® reagents. The method was very similar to the method used for total coliform and *E. coli* as mentioned above. Ten milliliters of sample were diluted with 90 ml of the sterile deionized water in a pre-sterilized vessel. The Enterolert reagent was added into the vessel and the sample was mixed. The sample was poured into a Quanti-Tray/2000 and sealed. Enterococci was detected by fluorescence under UV light after 24 hours of incubation at 41± 0.5°C.

Methods for Physical-Chemical Measurements:

The physical-chemical parameters measured in this study included temperature, pH, salinity, dissolved oxygen, and turbidity. Through March 16, 2001, all the physical-chemical measurements, with the exception of turbidity, were measured in the field using a YSI Probe Model 600 R (Yellow Springs, OH). The YSI probe was stolen on March 17, 2001; after this time dissolved oxygen was no longer analyzed and only temperature was measured in the field. The remaining physical-chemical parameters were analyzed at the Environmental Engineering Laboratory located at the University of Miami

YSI Probe

The YSI 600R series sonde (YSI Inc., Yellow Springs, OH) was used through March 16th to determine the pH, temperature, and specific conductivity or salinity. The readings from the probe were displayed on a handheld microprocessor (YSI model 610D). The sonde was calibrated at the initiation of the study.

Turbidity Measurements

A Turner Designs (Sunnyvale, CA) TD-40 nephelometer was utilized in the laboratory for turbidity measurements. The TD-40 was calibrated using a 2 ntu and 20 ntu formazin standard. After calibration a blank sample of 0 ntu was used to check the zero point of the instrument. Once calibrated, samples were placed into the appropriate 20 ml scintillation vials and analyzed for their turbidity.

Salinity Measurements (Laboratory)

Laboratory measurements of salinity utilized an Amber Science (Eugene, OR) model 4081 EC meter. This apparatus was calibrated using a potassium chloride solution of 35 salinity units. This solution was prepared by mixing 32.4356 g of potassium chloride into 1 kg of water. Samples were then analyzed once the instrument was calibrated.

pH Measurements (Laboratory)

Laboratory measurements of pH utilized an Orion (Beverly, MA) model 525A pH meter which was calibrated using pH 4 and pH 10 buffer solutions. The calibration was then checked with a buffer of pH 7. The pH of the samples was then taken once the instrument was calibrated.

Data Retrieval:

Readily available data obtained for this study included local rainfall and tidal information.

Rainfall and Tide Data:

Use the below NOAA web pages to obtain rainfall and tide data for the site.

For Rainfall:

http://www.rsmas.miami.edu/etc/download-weatherpak.cgi

For Tide:

http://www.co-ops.nos.noaa.gov/cgi-bin/get_pred.cgi?year=2004&stn=2498+Miami+Harbor+Entrance&secstn=Bear+Cut,+Virginia+Key&thh=%2b0&thm=50&tlh=%2b0&tlm=53&hh=*0.83&hl=*0.75

Tide prediction data were obtained through National Oceanic and Atmospheric Administration (NOAA) Homepage. The instructions for obtaining the tidal data are as follows.

1. Go to the NOAA web site http://www.noaa.gov/
2. Click a Site Map on the top of page
3. Go to Ocean Section and click Tide Prediction
4. Click a picture of Florida State or Florida in the table
5. Go to Florida Keys section and click Virginia Key
6. See the numbers in the line of Bear Cut, Virginia Key (the top line) and write down time differences at high tide and low tide and height differences at high and low tide. The Bear Cut, Virginia Key station was selected as a reference site because of its close proximity to Hobie and Crandon Beaches.
7. Click Miami Harbor Entrance
8. Find the date and calculate predict tide at Bear Cut using the number obtained at the previous page.

In order to determine the tide at Bear Cut, tidal data at the Miami Harbor entrance were used and adjusted with the corresponding time difference and height difference ratio for Bear Cut (Table A.1).

Table A.1: Tide Adjustment Coefficients for Bear Cut

Time Difference		Height Difference (feet)	
High Tide	Low Tide	High Tide	Low Tide
(+) 0:49	(+) 0:52	*0.82	*0.82

Once the times and tidal stages were adjusted, the tidal stage for any given time period was then interpolated from high an low tide using the following equation.

$$s_x = \frac{(s_2 - s_1)(t_x - t_1)}{t_2 - t_1} + s_1 \ldots\ldots\ldots \text{(A.3)}$$

where s_x is the tidal stage at the sample collection time, s_1 is the tidal stage at the preceding high or low tide, s_2 is the tidal stage at the following high or low tide, t_x is the sampling time, t_1 is the time of the preceding high or low tide, and t_2 is the time of the following high or low tide. For example, assume that a sample was collected at 10:35 am (t_x = 10.583 hours where 0.583 = 35/60) and that low tide occurred at 8:03 am (t_1 = 8.05 hours) and high tide occurred at 2:13 pm (t_2 = 14.217 hours). The tidal stage at low tide was 0.6 ft (s_1) and at high tide was 1.4 ft (s_2). Through interpolation the tidal stage at the time of sampling, s_x, was therefore computed as 0.93 ft (= [(1.4-0.6)*(10.583-8.05) / (14.217-8.05)] + 0.6).

Sanitary Survey:

In addition to the water quality monitoring, information was gathered from various agencies in a effort to locate potential sources of contamination to each of the beach sites. The locations of marinas and restaurants were noted. Infrastructure maps including information concerning the sanitary and storm sewer infrastructure were gathered along with detailed maps concerning the water treatment system at the Miami Seaquarium. The sanitary infrastructure reviewed included septic tanks and private and public sewage pump stations and force mains. Complaint investigations were also reviewed related to sewage overflows. Agencies contacted included Miami-Dade County Parks Department, City of Miami Public Works, Miami-Dade Department of Environmental Resources Management, Miami Dade Water and Sewer, and the Miami-Dade County Department of Health, Florida Department of Business and Professional Regulation / Division of Hotels and Restaurants, U.S. Coast Guard, USEPA, Village of Key Biscayne, and the Florida Department of Environmental Protection.

Statistical Analysis:

Statistical analyses were performed on the data using the "Data Analysis Tools" option of Microsoft® Excel 2000 program. For calculation purposes, the values that were either below or above detection limits were modified as indicated in Table A.2.

Table A.2: Modification of Above and Below Detection Limit Data for Statistical Analyses

Meaured Values	Value Used for Statistical Analysis
> 24,192 (IDEXX)	24,192
< 10 (IDEXX)	5
< 2 (MF)	1
< 1 (MF)	0.5
Too Numerous to Count (Fecal coliform analysis only)	200
Confluent Growth	---
No Data	---

The options used within the "Data Analysis Tools" included "descriptive statistics" which were used to obtain basic parameters including the mean, standard deviation, range, maximum, minimum, confidence limits, etc... of various groups of data. In order to evaluate the relationships between microbial concentrations and physical parameters, standard regression analysis was performed and the correlations option was used within "Data Analysis Tools". T-tests (paired two sample means) were utilized to: evaluate if IDEXX (Enterolert) provided statistically similar results as MF for enterococci and compare the concentrations of microbes in the sand samples at different shoreline levels. Paired t-tests (two-sample assuming equal variances) were applied to

determine whether differences in microbial concentrations and physical parameters were statistically significant at 95% confidence.

Appendix B

Data and Results from Various Sampling Efforts conducted at Hobie Cat Beach:

Shibata et al., 2004, conducted a pilot epidemiological and water quality study at two public beaches, Hobie Cat and Crandon, located in southern part of Biscayne, Miami, Florida (Figure B-1). Hobie Beach is approximately one mile long, relatively shallow, and characterized by poor water circulation; its shoreline is covered with seaweed over a silty and muddy floor. It is a very narrow beach, the average distance between the mean water line and the outer edge of sand and gravel is about 15 feet. It is the only beach in Miami-Dade County, where visitors can bring their pets. The beach has a history of poor water quality. During the year 2000, the beach exceeded the EPA Poor Water Quality Guideline (PWQG) for enterococci 29.2% of the times. On the other hand, Crandon Beach is located in about 2.5 miles southeast of Hobie Beach. It is about one mile long, relatively shallow, and located on the ocean side. The beach was chosen for this study because it possesses obvious contrasting characteristics in comparison with Hobie Beach. Crandon Beach has relatively good water quality and good circulation. The main objectives of the study were: evaluate the microbial water quality including soils at the selected beaches and the bay using the regulatory microbial indicators (total and fecal coliforms, *E. coli* and enterococci) and *Clostridium perfringens* (alternative microbial indicator recommended for tropical climate); conduct sanitary surveys to identify point and none point sources of fecal pollution; identify sources of microbial indicators; administer an epidemiological study to evaluate relationships between swimming related illnesses and microbial density. Findings indicate that there was no dose relationship found between density of microbes and health effects. The water quality at Crandon Beach was better than Hobie Cat Beach regardless of the season (wet vs. dry). There was no fecal pollution point source identified in the sanitary survey. Intensive spatial water quality monitoring indicated the southern tip of the shoreline at Hobie Beach appears to be the source of microbes; this finding was supported by the soil sample results collected from this end of shoreline. And the concentrations of indicator microbes were considerably lower during low tide as compared to high tide. Figure B-2 shows the distribution of enterococci during the low and high periods. The detection of those indicators in the soils/vegetation of the shoreline without a known point source fecal pollution again questions the suitability of those indicators for measuring the sanitation water quality in subtropical/tropical climates.

Wright et al., (2005) conducted a comprehensive environmental study at Hobie Cat Beach. The purpose of the study was to determine sources of enterococci to the beach waters and environmental conditions that control enterococci levels. Four sediment and water sampling and testing efforts have been made within the study site: 1) Intensive Water and Sediment Sampling Effort. Twelve sediment samples dry and wet were collected along a transect perpendicular to the water line, starting approximately 50 feet from the outer bound of the swash zone and ending approximately 75 feet from the inner bound of the swash zone into the water side. Swash zone is defined as the shoreward

section on the beach between the lowest and highest water lines during low and high tides respectively.This effort lasted 3 days. Results from this effort were significant, they indicated that sediments within the swash zone and immediately around its inner and outer boundaries have the highest concentrations of enterococci and those concentrations decrease as the distance increases into the water side and towards the opposite direction(street), see figure B-4.2) 48-Hour Water and Sediment Sampling Effort. Twenty five water and sediment samples each were collected from a pre-assigned knee-deep location at the site. Along with each water and sand sample collected, pH, temperature, tidal height, rainfall condition, site sanitary condition i.e. debris, animal and human activities around the sampling site were recorded. Two important results emerged from this sampling effort; a- it confirmed that the concentrations of enterococci are on the average 2 order of magnitude higher than those in the water column, b- concentrations of enterococci in both sediment and water consistently increase at high tide and decrease at low tide and c- rainfall events may have an increasing effect on the enterococci concentrations in water and sediments, see figures B-5 &6. 3) Transects Water and Sdiments Sampling Effort. This effort was conducted daily for a 2-week period. One hundred four and sixty nine sediments and water samples were collected and analyzed for enterococci respectively. The study site was divided by 3 transects J, K and L perpendicular to the shoreline and extend from the dry sand to the water side up to the buoys. The results from this study showed that a- on the average the enterococci concentrations in the dry sand higher than wet sand followed by water , b- the concentrations of enterococci in the water column decreased as the distance increased from the shore line into the buoys and c- at high tide the concentrations of enterococci in sediment and water samples are generally higher then that at low tide, see figures B-7,8,9,10,11,&12. And 4) Runoff Water Sampling Effort. Twenty three runoff water samples were collected from 2 natural channels east and west of transect K for a period of 2 weeks (first 2 weeks of August which is the height of the wet season in Florida). Samples were analyzed for total and fecal coliforms and enterococci. This study indicated that a- runoff water consistently contains enterococci concentrations at least two order of magnitude higher than the state and federal regulatory standards, b- on the average, total coliforms concentrations in water runoff are 2.5 times higher than fecal coliforms and 6 times higher than enterococci and c- runoff water is one of the primary non-point source of enterococci next to the sand, see figure B-13.

Finally, many important logistical, administrative and training activities either preceded or accompanied those intensive sampling efforts, including, developing and implementing safety, sampling training and analyses protocols, meetings with the epidemiological, sediment and modeling groups, governmental agencies, universities and public and private local laboratories. Those activities were essential for the success of the four sampling efforts.

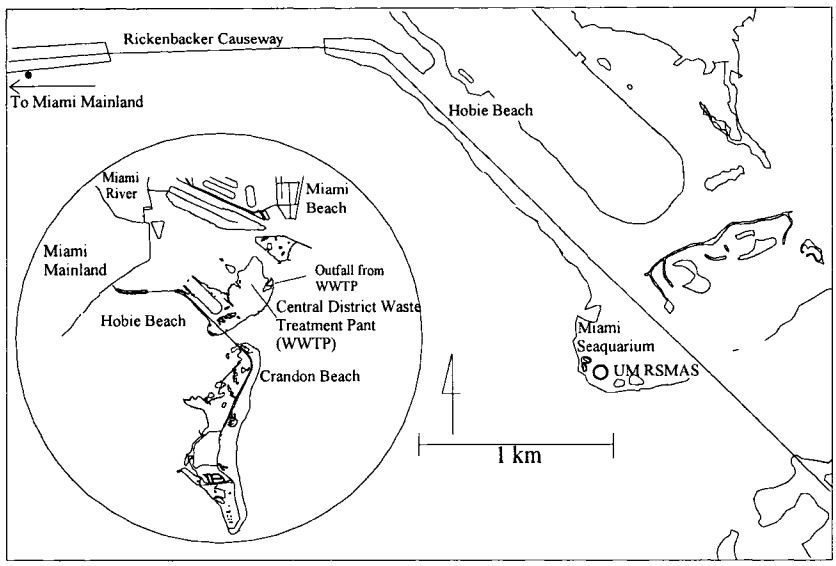

Figure B.1 Hobie and Crandon, located in southern part of Biscayne, Miami, Florida

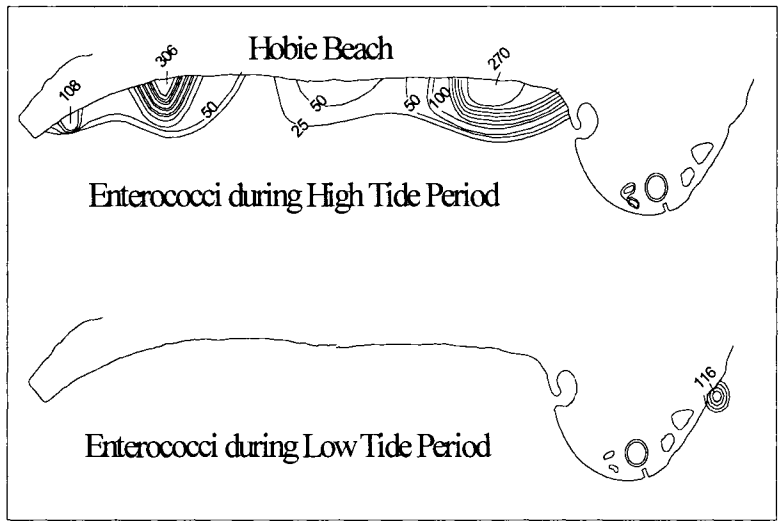

Figure B-2: Concentrations of enterococci (CFU/100ml) during high and low tide at Hobie Beach

	Over Exposed Number (%) N=27 (13%)	True Participants No Return Number (%) N=181 (87%)	Statistical Significance*
INITIAL INTERVIEW:			
Dry Month vs Wet Month	18 (9%)	57 (27%)	0.001
Hobie Beach vs Crandon Beach	20 (10%)	79 (38%)	0.003
Women	16 (8%)	82 (39%)	0.13
Mean Age± Standard Deviation	25.2±19.2	19.8±16.2	0.12
Race Ethnic WNH WH B	 1 (0.5%) 25 (13%) 0	 49 (24%) 130 (62%) 2 (1%)	0.04
Interview language (English)	20 (10%)	130 (63%)	0.51
At beach < 7 days prior	0	0	
Did not get face wet	0	0	
PHONE FOLLOW UP:			
Return to beach	27 (2%)	0	
If return, went to Hobie Beach	17 (55%)	0	
Fever	1 (0.5%)	7 (3%)	0.72
Chills	0	3 (1%)	0.66
Eye Redness	1 (0.5%)	0	0.13
Earache	0	2 (1%)	0.76
Ear Discharge	0	1 (0.5%)	0.87
Skin Rash	1 (0.5%)	10 (5%)	0.57
Infected cuts	0	0	
Nausea	0	1 (0.5%)	0.87
Vomiting	0	1 (0.5%)	0.87
Diarrhea	1 (0.5%)	5 (2%)	0.57
If Diarrhea, blood in stool	0	1 (0.5%)	0.87
Stomach pain	0	4 (2%)	0.57
Cough	2 (1%)	12 (6%)	0.57
If Cough, then phlegm	2 (1%)	1 (0.5%)	0.05
Nasal congestion	2 (1%)	8 (3%)	0.28
Sore Throat	0	5 (2%)	0.50
≥ 1 Symptom	6 (22%)	29 (16%)	0.29
Number of families	9	54	

Symptoms present AFTER visiting beach

*by Chi square or Fishers Exact Test for categorical data or t-test for continuous data

Table B.1: Description of Participants:"Over-exposed" VS Other Participant Population

Beach	Date	Number ≥ 1 Symptom People (%) [p value]*	Total Coliform	Fecal Coliform	*E coli*	Enterococcus 1	Enterococcus 2	*Clostridium perfringens*
Both	1	5/33 (15%)						
	2	3/17 (18%)						
	3	3/25 (12%)						
	4	19/97 (20%)						
	5	4/36 (11%)						
	All	Pearson Correlation (p value)	−0.79 (0.05)	−0.84 (0.04)	−0.72 (0.08)	−0.49 (0.19)	−0.84 (0.04)	−0.88 (0.03)
Hobie	1	3/17 (18%) [0.59]						
	2	3/11 (27%) [0.20]						
	3	3/14 (21%) [0.16]						
	4	9/46 (20%) [0.80]						
	5	0/11 (0) [0.16]						
	All	Pearson Correlation (p value)	−0.79 (0.06)	−0.46 (0.22)	−0.16 (0.40)	−0.03 (0.48)	−0.41 (0.25)	−0.57 (0.16)
Crandon	1	2/16 (13%)						
	2	0/6 (0)						
	3	0/11 (0)						
	4	11/51 (22%)						
	5	4/25 (16%)						
	All	Pearson Correlation (p value)	0.34 (0.29)	0.04 (0.47)	0.68 (0.10)	−0.23 (0.36)	0.38 (0.27)	−0.90 (0.20)

*Chi squared (Fishers exact test) or t-test comparison of 2 beaches

Table B.2: Symptom Correlation with Indicator Microbes

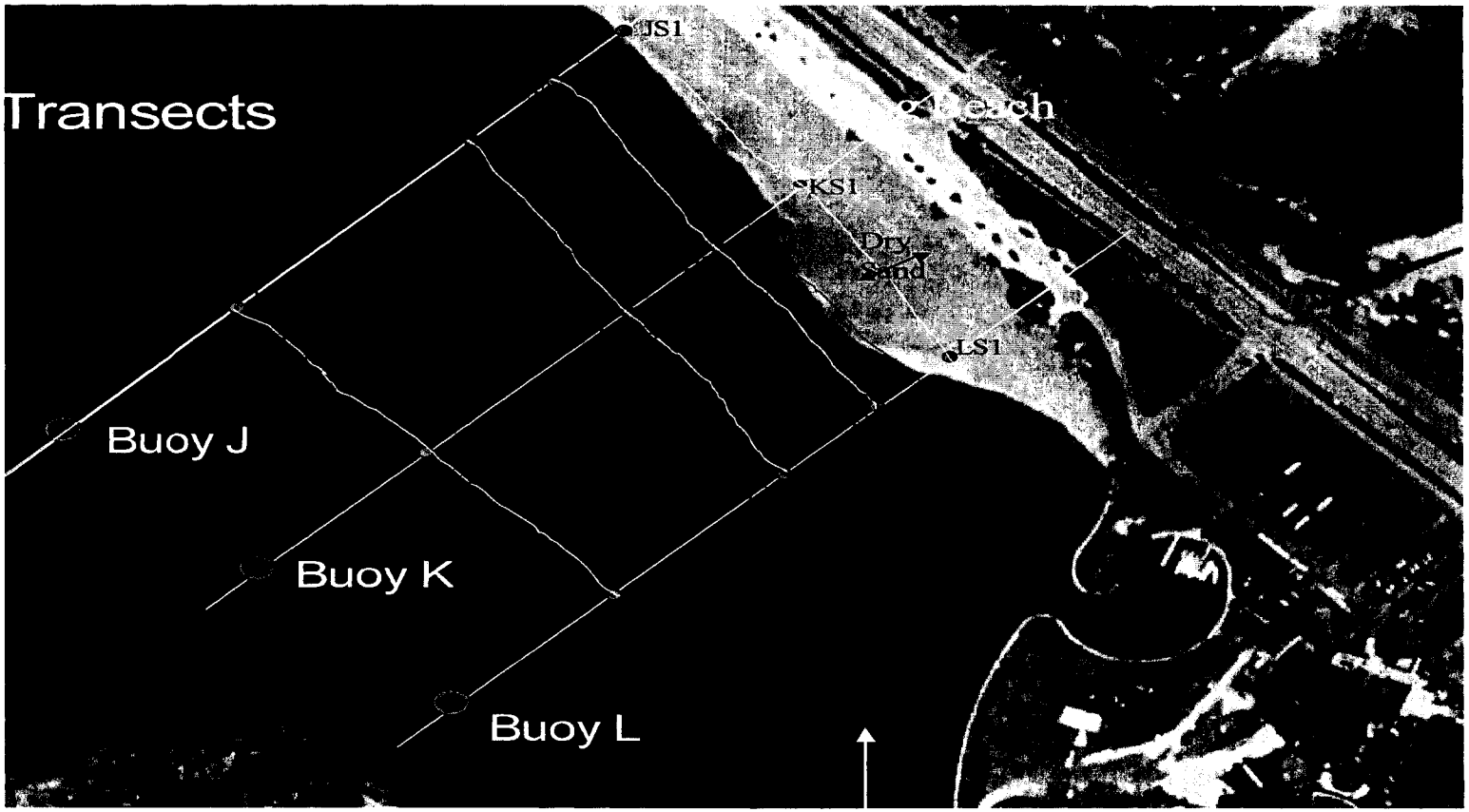

Figure B.3 Schematic of the intensive spatial and temporal of sediment and waster sampling site. S1=Dry Sand, S3=Swash Zone Sand, W1=Knee Deep Water, W2= Ambient Water, W3= Chest Deep Water Sampling Site

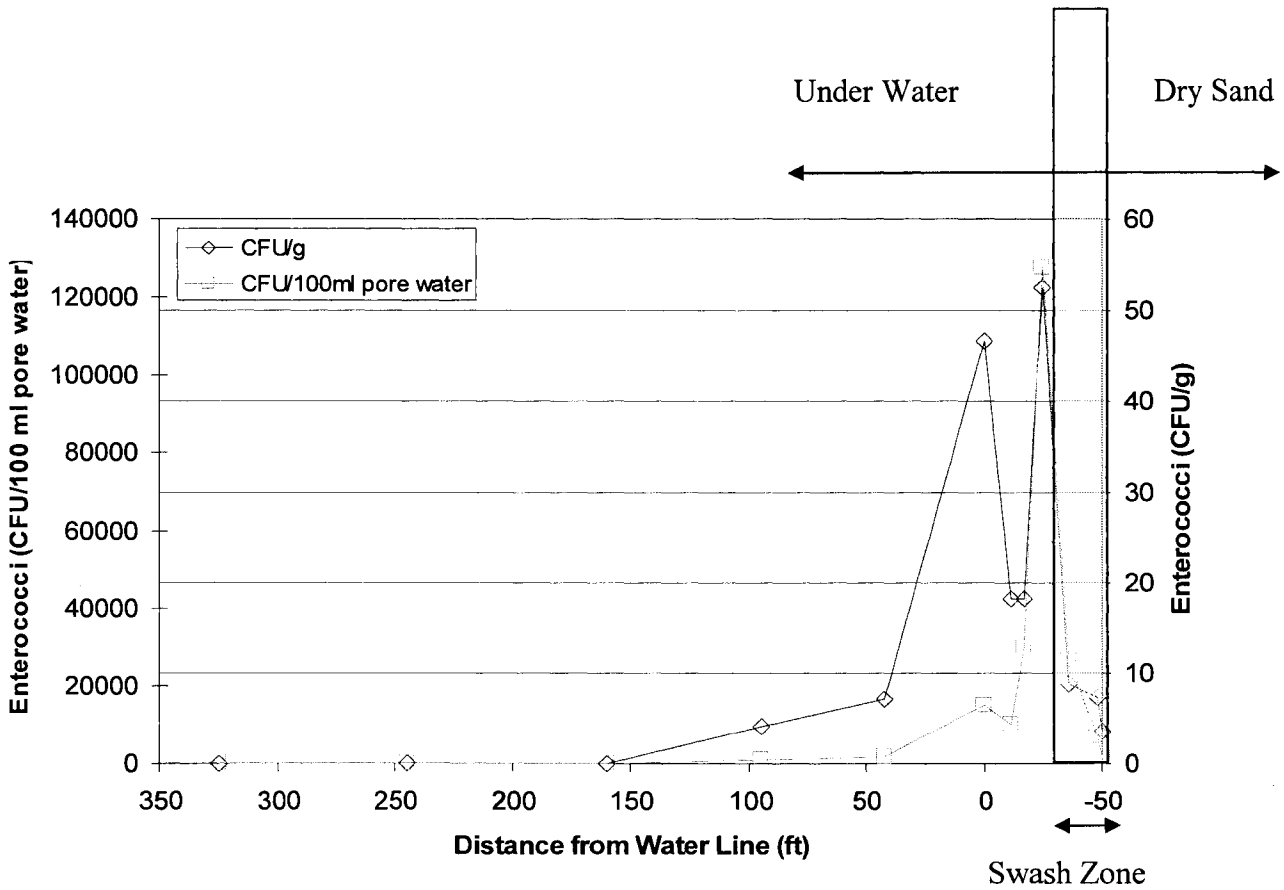

Figure B.4 Intensive Sediment Sampling Results Along Transect J

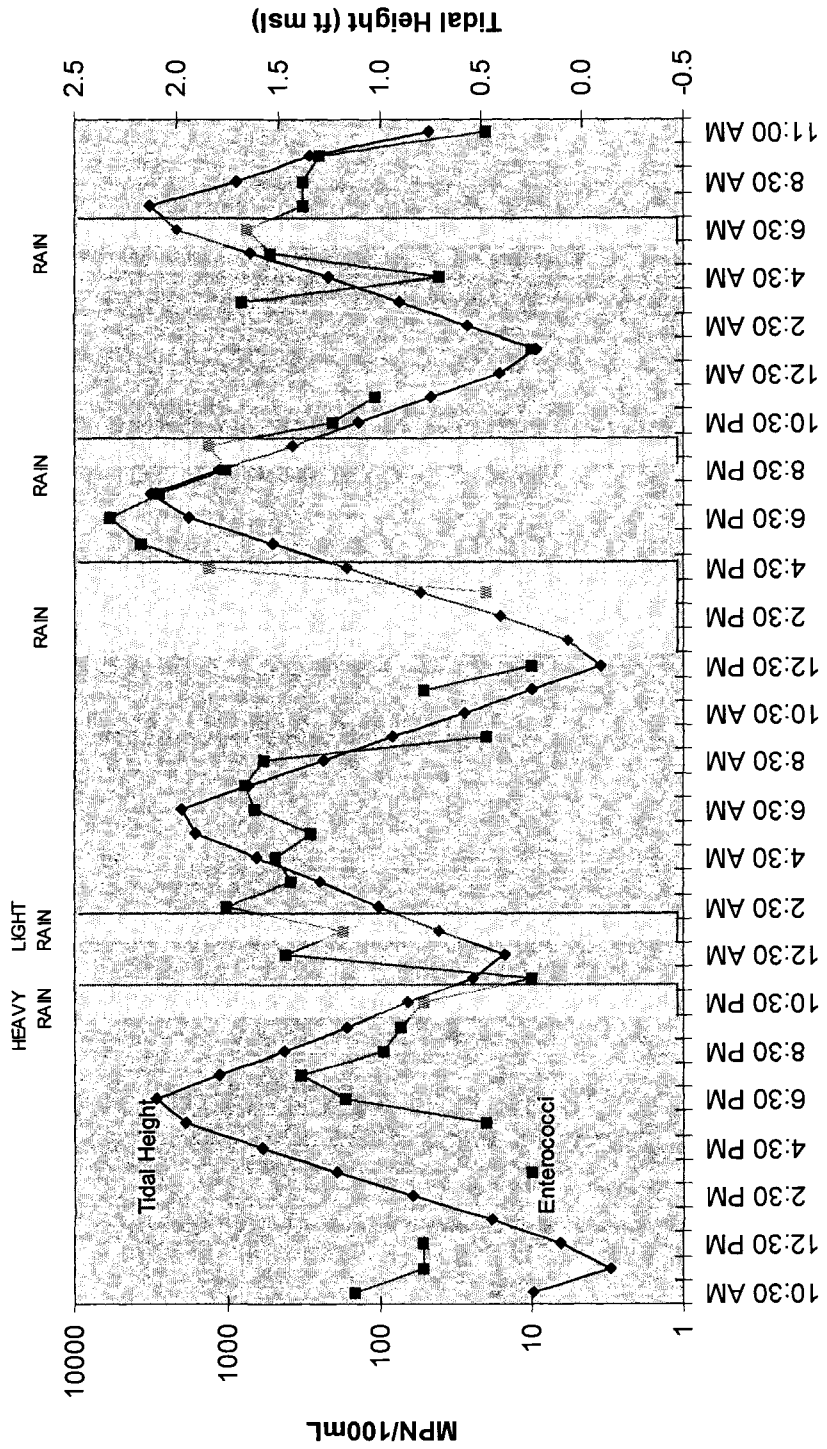

Figure B.5: 48-Hour Sampling Effort-Water

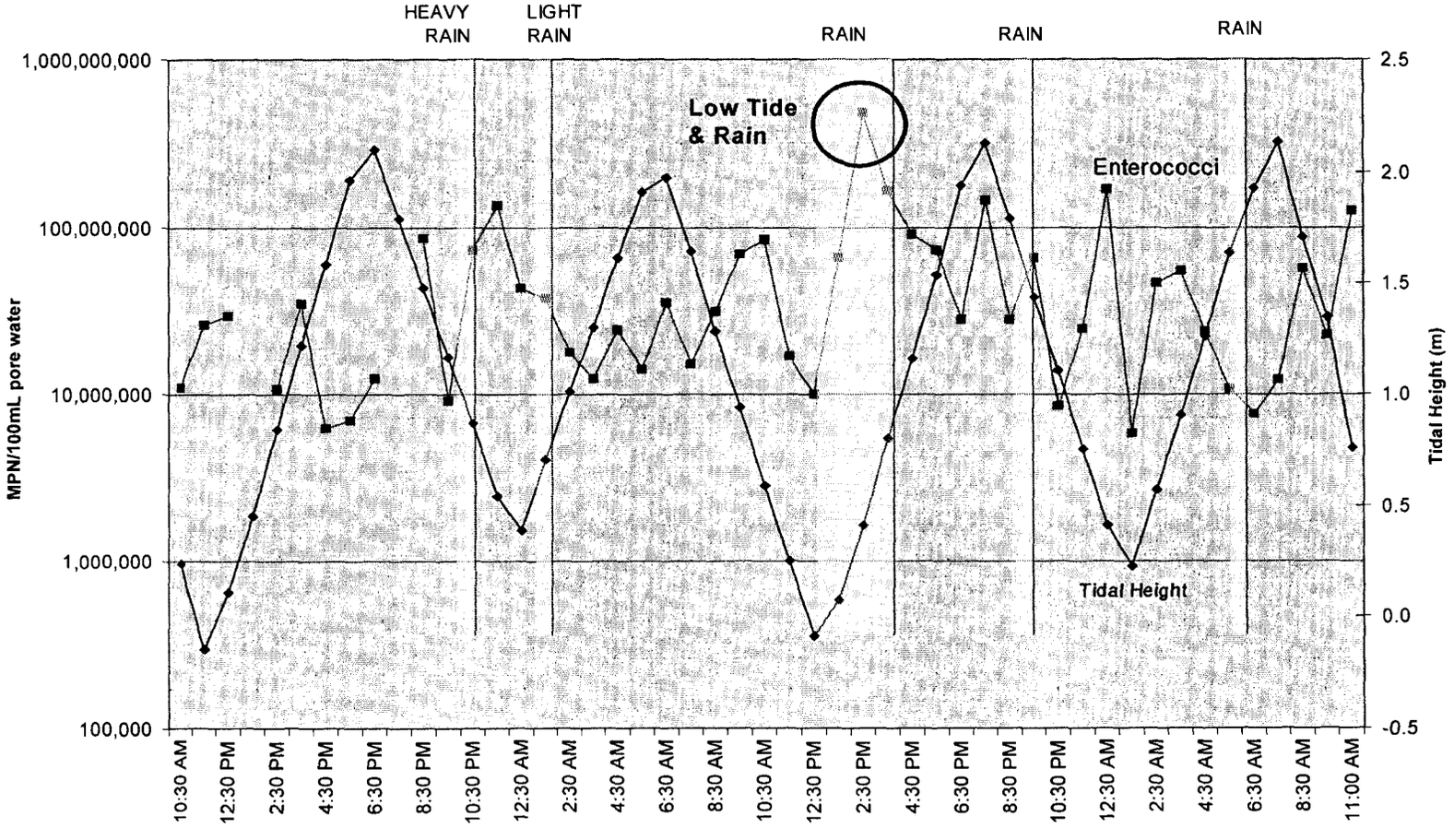

Figure B.6: 48-Hour Sampling Effort-Sediment

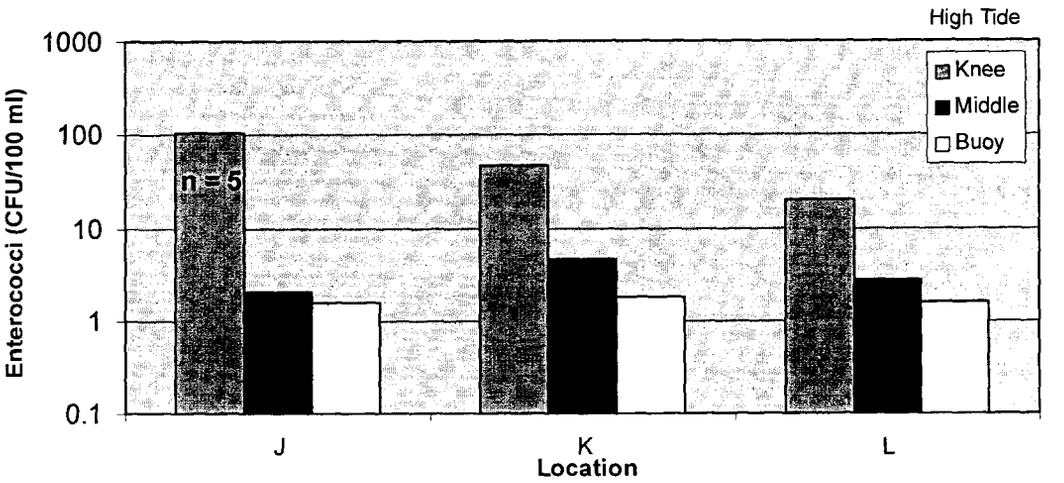

Figure B.7 Transect Water Sampling Results @ High Tide

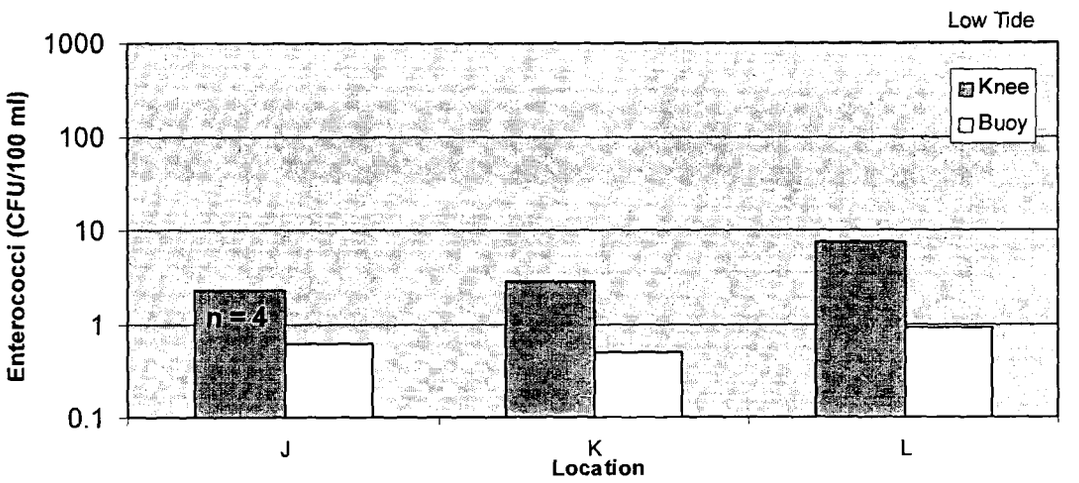

Figure B.8 Transect Water Sampling Results @ Low Tide

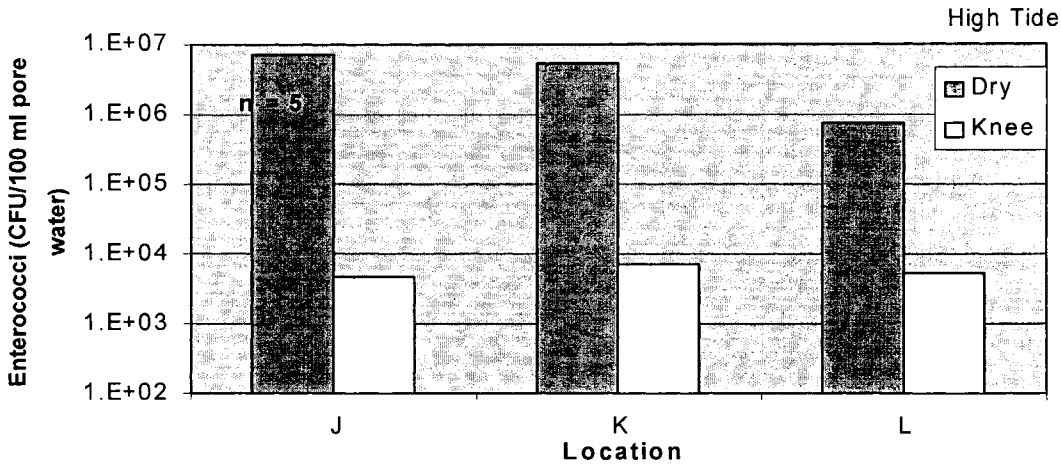

Figure B.9 Transect Sand Sampling Results @ High Tide Expressed in Pore Water Volume

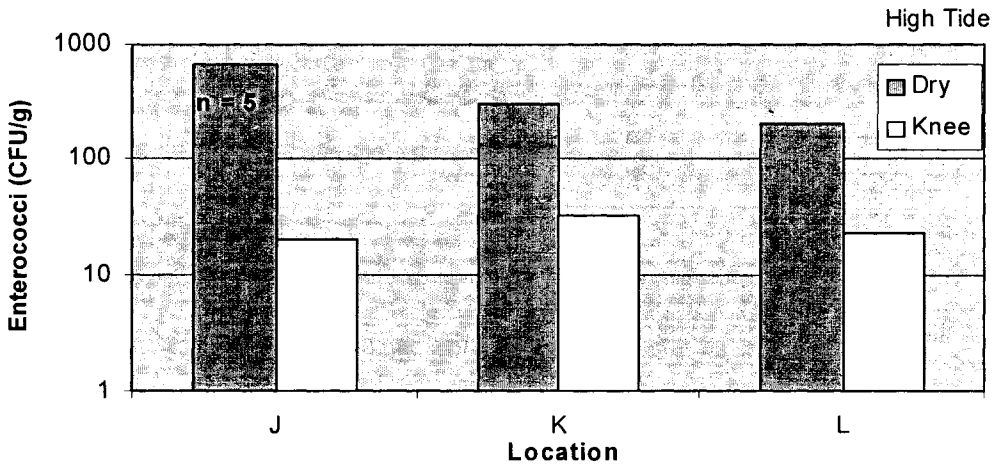

Figure B.10 Transect Sand Sampling Results @ High Tide Expressed in grams of Sand

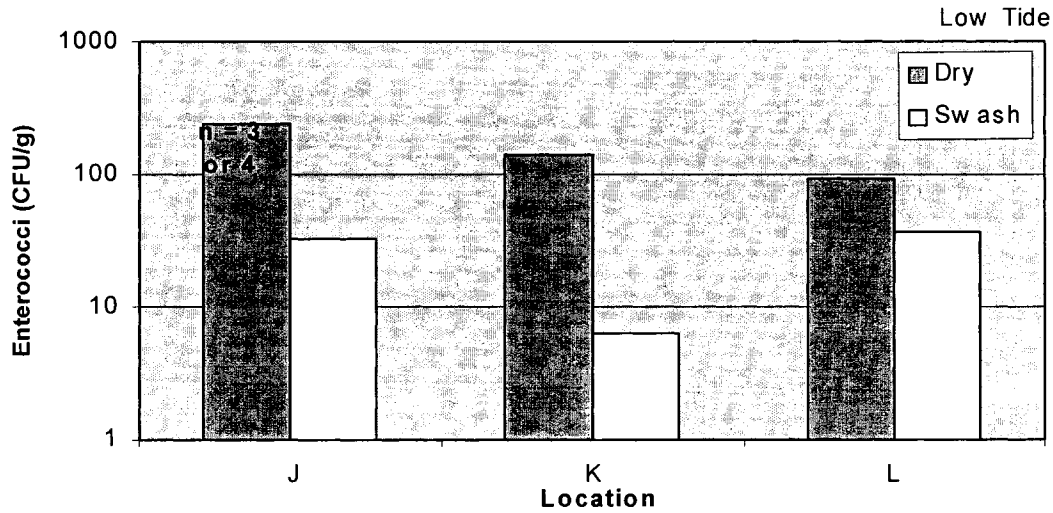

Figure B.11 Transect Sand Sampling Results @ Low Tide Expressed in grams of Sand

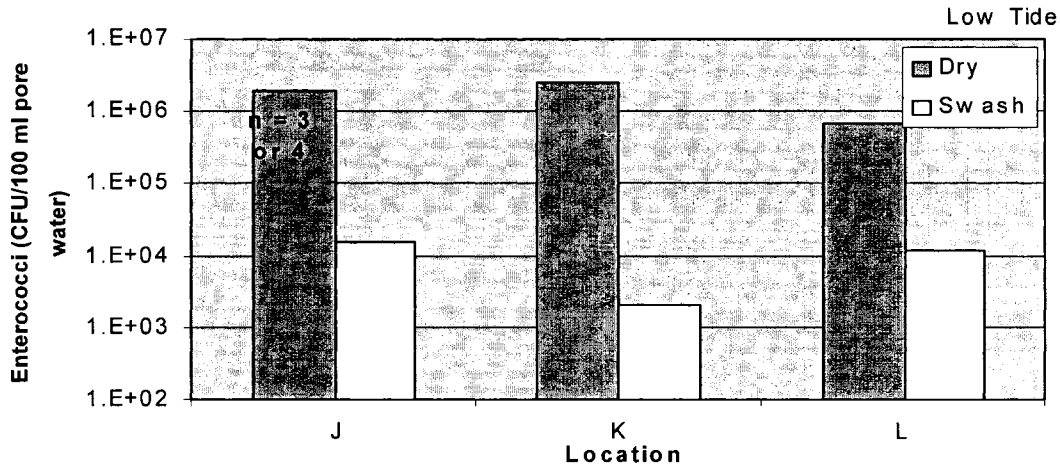

Figure B-12: Transect Sand Sampling Results @ Low Tide Expressed in Pore Water volume

Site	Date	Time collected	T. Coliform cfu/100ml	F.Coliform cfu/100ml	Enterococci cfu/100ml
K/East	08/02/04	7:15 AM	70,000	18,000	13,000
K/West	08/02/04	7:16 AM	89,000	22,000	8,000
K/West	08/02/04	7:17 AM	51,000	14,000	2,000
K/East	08/02/04	7:20 AM	79,000	11,000	6,000
K/East	08/02/04	7:22 AM	50,000	39,000	7,000
K/West	08/02/04	01/00/00	81,000	17,000	5,000
K/East	08/05/04	1:41 PM	78,000	30,000	10,000
K/West	08/05/04	1:44 PM	72,000	13,000	36,000
K/West	08/05/04	1:47 PM	54,000	5,000	19,000
K/East	08/05/04	1:50 PM	192,000	164,000	9,000
K/East	08/05/04	1:53 PM	194,000	110,000	11,000
K/West	08/05/04	1:56 PM	75,000	23,000	24,000
K/West	08/05/04	2:05 PM	198,000	154,000	49,000
K/West	08/06/04	7:32 AM	41,000	26,000	30,000
K/West	08/06/04	7:34 AM	49,000	22,000	28,000
K/East	08/06/04	7:41 AM	33,000	11,000	2,000
K/East	08/06/04	7:44 AM	35,000	19,000	3,000
K/West	08/06/04	7:49 AM	48,000	45,000	5,000
K/West	08/06/04	7:50 AM	54,000	15,000	9,000
K/East	08/06/04	7:55 AM	38,000	9,000	8,000
K/West	08/13/04	7:10 AM	235,000	27,000	7,000
K/East	08/13/04	7:15 AM	135,000	25,000	5,000
K/East	08/13/04	7:18 AM	250,000	49,000	37,000
G. Mean			77,781	24,826	9,913
Avg.			95,696	37,739	14,478

Figure B.13 Run off water sample results

Appendix C

Human Shedding Studies Supporting Information Including IRB Approval

-----Original Message-----
From: Borbolla, Jerry (PWD) [mailto:jibb@miamidade.gov]
Sent: Friday, April 23, 2004 7:39 AM
To: Solo-Gabriele, Helena M
Cc: Michael J. Moore (PWD) (E-mail); Mike Bauman (PWD) (E-mail); James Martincak (PWD) (E-mail); Svetlana Moorey (PWD) (E-mail); samir_elmir@doh.state.fl.us
Subject: RE: Research Project at Hobie Cat - Virginia Key Southside
Importance: High

Hi Helena,

Thank you informing my staff regarding your research project at Virginia Key Southside. Please be advised that we will cooperate fully with you and will provide our full support. I recommend that we meet with Svetlana and Samir in the near future to ensure proper coordination of all projects.

Should you need to contact me, please call me, at (305) 375-1925.

Best regards,

Jerry

> Mr. Jerry Borbolla, Chief
> Right-of-Way Aesthetic and Assets
> Management Division (R.A.A.M.)
> Miami- Dade County Public Works Department
> (305) 375-1925
>

-----Original Message-----
From: Solo-Gabriele, Helena M [mailto:hmsolo@miami.edu]
Sent: Thursday, April 22, 2004 2:25 PM
To: Borbolla, Jerry (PWD)
Cc: Moorey, Svetlana (PWD); Martincak, James (PWD); samir_elmir@doh.state.fl.us; Fleming, Lora E; Solo-Gabriele, Helena M
Subject: Research Project at Hobie Cat - Virginia Key Southside

Dear Mr. Borbolla,

Mr. Jimmy Martincak requested that I send a letter to your attention summarizing our research plans for Virginia Key Southside, which is also known as Hobie Cat Beach. In response to that request I have attached a letter that provides a summary of our proposed work. I have also attached a copy of the proposal that received funding. Please let me know if you have

any comments or concerns about our proposed research project. I hope that you find the attached information useful and informative. Our intent is to keep you and Mr. Martincak informed of our plans throughout the duration of our 5 year study.

I thank you for your attention to this.

Sincerely,

Helena Solo-Gabriele, Ph.D., P.E.
Associate Professor, University of Miami

7/26/05 UM IRB 20057223

Pilot Study
of
Human Microbial Input into Recreational Marine Waters
CONSENT FORM

Description of the Study:
This purpose of this Pilot Study is to see how many microbes on the skin of people at the beach add to the microbes in the beach water.

To participate in this study, you will be asked to

a) Sit for 15 minutes for 4 separate times in a small pool (approximately 15 inches deep) filled with marine water with other people. In this pool, you will be asked to dunk your head under the water 3 times for each 15 minute period.
b) After walking, sitting and lying in beach sand, you will be asked to stand in a small pool (approximately 15 inches deep) and have marine water poured over your head into the pool.

Risks:
There are no additional risks to you from participating in this study.

Rights:
You have the alternative to not participate in this study. By participating in this study, you do not give up any rights to which you would otherwise be entitled.

If you are a student, your desire not to participate, or your request to withdraw from the study, will not affect your grades or other academic standings within the University. If you are an employee of the University, your decision to participate in or to withdraw from the study will not affect your employment within the University.

Benefits:
No direct benefit can be promised to you for your participation in this study.

Confidentiality section:
Your records and results will not be identified as pertaining to you in any publication without your expressed permission. The investigator and his/her collaborators, staff and the NSF-NIEHS will consider your records confidential to the extent permitted by law. The Food and Drug Administration (FDA) and Department of Health and Human Services (DHHS) may review these research records. Your records may also be reviewed for audit purposes by authorized University of Miami employees or other agents who will be bound by the same provisions of confidentiality.

Costs:
You or your insurance company will be responsible for medical costs of participating in this program. If you have insurance, your insurance company may or may not pay for these costs. If you do not have insurance, or if your insurance company refuses to pay, you will be expected to pay.

Right to withdraw section:
Your participation in this study is voluntary. You are free to refuse to participate in the study or withdraw your consent at any time during the study. Your withdrawal or lack of participation will not prejudice further/additional medical treatment. The investigator reserves the right to remove you from the study without your consent at such time that they feel it is in the best interest for you medically or for administrative reasons.

You may ask and will receive answers to any questions during the course of the study. If you have any questions about this study, please contact Dr Lora E Fleming MD PhD. If you have questions about your rights as a research participant you may contact the University of Miami Human Subjects Research Office, at (305) 243-3195.

_____ _____
Subject's Signature Date

_____ _____
Witness' Signature Date

_____ _____
Person Obtaining Consent Date

_____ _____
Print Name of Person Obtaining Consent Date

Principal Investigator: Dr Lora E Fleming MD PhD

Daytime Telephone: 305 243 5912

Nighttime Telephone: 305 844 7977

ASSENT FOR CHILDREN 12-17 YEARS OF AGE:
This purpose of this Pilot Study is to see how many microbes on the skin of people at the beach add to the microbes in the beach water.

To participate in this study, you will be asked to

c) Sit for 15 minutes for 4 separate times in a small pool (approximately 15 inches deep) filled with marine water with other people. In this pool, you will be asked to dunk your head under the water 3 times for each 15 minute period.
d) After walking, sitting and lying in beach sand, you will be asked to stand in a small pool (approximately 15 inches deep) and have marine water poured over your head into the pool.

I agree, _____, I do not agree _____ to participate in this study. This has been explained to me by _____.

_____ _____
Signature of minor Date

_____ _____
Signature of parent Date

_____ _____
Signature of witness Date

CONSENT FOR SUBJECTS 18 YEARS OF AGE AND OLDER:

_____ _____
Signature of patient Date

_____ _____
Signature of witness Date

_____ _____
Signature of Person Obtaining Consent Date

_____
Print Name of Person Obtaining Consent

Jeb Bush
Governor

FLORIDA DEPARTMENT OF HEALTH

John O. Agwunobi, M.D., M.B.A.
Secretary

NOTIFICATION OF INSTITUTIONAL REVIEW BOARD APPROVAL

Date:	July 5, 2005
To:	Dr. Helena Solo Gabriele University of Miami Department of Civil/Environmental Engineering P.O. Box 248294 Coral Gables, Florida 33124-0630
Protocol Title:	Source Specific Sampling for Microbes
DOH IRB Number:	1491
IRB Decision:	**Approved**
Duration:	**No more than 12 Months**
Next Progress Report Due:	**On or before May 6, 2006**
Protocol Expires:	**July 4, 2006**

The Department of Health Institutional Review Board, or representative, determined your study involves no more than minimal risk and meets the criteria for expedited review. It has been granted **expedited approval** under § 45 CFR 46.110(b)(1). The study is approved for implementation for 12 months.

As a reminder, the IRB must review and approve all human subjects research protocols at intervals appropriate to the degree of risk, but not less than once per year. **You are responsible for applying for renewal of this project at least 60 days prior to the expiration date.** This approval is valid for no more than one year. Re-approval is contingent upon IRB review and approval of a Continuing Review Report prior to the anniversary or expiration date of this approval.

Approval is contingent upon continued ethical research practice and your agreement to obtain informed consent and authorization from your subjects, unless waived. Please make certain that confidentiality is maintained. You must abide by the policies and procedures of the Florida Department of Health with regard to the use of human subjects in research, and keep appropriate records concerning your subjects.

Investigators are required to notify the IRB in writing as soon as possible, but within 10 working days, of the occurrence of any adverse events, unanticipated problems, injuries, side effects, deaths, other problems involving risks to subjects, or deviations from federal or state regulations, or DOH policy.

The IRB has approved exactly what was submitted. Any revisions to this protocol or consent form, no matter how minor, must be presented to the IRB for review and approval before implementation of the changes, except where necessary to eliminate hazard to human subjects. If a change is required to

eliminate an immediate hazard, the IRB should be notified as soon as possible but no later than 10 working days.

Researchers are required to notify this IRB, in writing, in the event that this study is not implemented or when termination of this study takes place.

Research records must be maintained for three years after completion of the research; if the study involves medical treatment, it is recommended that records be maintained for eight years.

Please note that this protocol has been assigned the above-referenced DOH IRB protocol number. All inquiries and correspondence concerning this protocol must include (1) the above-referenced IRB number; (2) name of the principal investigator; and, (3) full title of study.

If you have any questions, or if we can be of any assistance, please contact the Department of Health IRB at (850) 245-4585 or toll-free in Florida (866)-433-2775. You may also visit our website at: http://www.doh.state.fl.us/execstaff/irb/

Thank you for your cooperation with the IRB.

Sincerely,

Paul Arons, M.D.
Chair, Institutional Review Board

Encl:

Date of study: July 20, 2005
Title of Study: Shedding Study
Demographics

Volunteer #	Gender	Height(Inches)	Weight(lbs)	Race	Age(Yrs)
1	M	5 8"	154	A	30
2	M	6 2"	190	H	34
3	M	5 9"	200	H	62
4	M	5 6"	175	H	51
5	M	6 4"	200	H	20
6	F	5 3"	120	H	17
7	F	5 2"	115	W	14
8	F	5 5"	140	W	17
9	M	5 6"	172	H	40
10	M	5 10"	170	B	50

Date of study: August 22, 2005
Title of Study: People/Shedding Small Pool Study
Demographics

Volunteer #	Gender	Height(Inches)	Weight(lbs)	Race	Age(Yrs)
11	Male	68	143	Peruvian	19
12	Female	65	141	White	34
13	Male	66	165	Japanese	36
14	Female	64	130	White	22
15	Male	76	190	Spanish	20

Supplemental On-Line Information for

Quantitative Evaluation of Bacteria Released by Bathers in a Marine Water

Samir M. Elmir[1,2,3], Mary E. Wright[1,2], Helena M. Solo-Gabriele[1,2,*], Amir Abdelzaher[1,2],
Lora E. Fleming[1], Gary Miller[3], Michael Rybolowik[3], Meng-Ta Peter Shih[4], Segaran P. Pillai[4], Jennifer A. Cooper[4], Elesi A. Quaye[4]

[1] NSF-NIEHS Oceans and Human Health Center, University of Miami, Rosenstiel School for Marine and Atmospheric Sciences,1801 NW 9 Avenue, Suite 200 (R-669), Miami, Florida 33136, USA

[2] University of Miami, Department of Civil, Architectural, and Environmental Engineering, P.O. Box 248294, Coral Gables, Florida, 33124-0630, USA

[3] Miami-Dade County Health Department, 1725 NW 167 Street Miami, Florida 33056, USA

[4] Florida Department of Health, Bureau of Laboratories- Miami,1350 NW 14 Street, Miami, Florida, USA

[*]**Corresponding Author:**
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Picture Panel #1. Scenes from the Large Pool Study. Top left: Set-up to transport the water pump and hose offshore. Top right: Filling the large pool with offshore water in order to initiate the first 15 minutes exposure cycle. Bottom left: Participants sitting in the large pool for a 15- minute exposure cycle. Bottom right: Sanitizing the large pool prior to subsequent cycle.

Picture Panel #2. Scenes from the Small Pool Study. Left: One of the participants rinsed of sand with offshore water. Center: Collection of water after rinsing a participant. Right: Collection of sediment after rinsing a participant.

From: Water Research [mailto:wr-eo@elsevier.com]
Sent: Tue 10/3/2006 2:13 AM
To: Solo-Gabriele, Helena M
Subject: WR5813R1: Editor's decision: accepted

Dear Dr. Solo-Gabriele,

I am pleased to inform you that the manuscript "Quantitative Evaluation of Bacteria Released by Bathers in a Marine Water" (Dr. H.M. Solo-Gabriele) has now been accepted by the editor for publication.

Your manuscript will soon be passed to the production department for further handling. Then you will receive further notice.

Thank you for considering our journal for the publication of your research.

Kind regards,
For the Editor,

Sheilagh Douma, Journal Manager
Water Research

Appendix D

Human shedding studies raw data and calculations

Large Pool Study:

Table D.1 *Staph. aureus* laboratory sample results and analysis

			Staph. aureus				
Cycle No.	**Sample ID**	**Volume Used, ml**	**No. colonies**	**CFU/100 ml**	**Mean, CFU/100ml**	**Std dev**	**COV**
	O1	50	32	64	64		
	I1A	50	10	20			
	I1B	50	7	14	13	7.0	53%
1	I1C	50	3	6			
	F1A	10	404	4040			
	F1B	10	384	3840	4187	438.8	10%
	F1C	10	468	4680			
	O2	50	1	2	2		
	I2A	50	5	10			
	I2B	50	4	8	7	3.1	42%
2	I2C	50	2	4			
	F2A	10	244	2440			
	F2B	10	270	2700	2080	858.6	41%
	F2C	10	110	1100			
	O3	50	0	<1	<1		
	I3A	50	2	4			
	I3B	50	7	14	11	5.8	54%
3	I3C	50	7	14			
	F3A	10	124	1240			
	F3B	10	88	880	1027	189.0	18%
	F3C	10	96	960			
	O4	50	0	<1	<1		
	I4A	50	3	6			
	I4B	50	5	10	9	2.3	27%
4	I4C	50	5	10			
	F4A	10	57	570			
	F4B	10	57	570	523	80.8	15%
	F4C	10	43	430			

Oi : Source sample, cycle No.

Iij* : Initial pool sample, cycle No. I, and sample loaction J

Fij*:Final pool sample, cycle No. I, and sample loaction J

* :Samples collected from three locations in pool (A,B, and C)

Table D.2 Enterococci laboratory sample results and analysis

			Enterococci				
Cycle No.	Sample ID	Volume Used, ml	No. colonies	CFU/100 ml	Mean, CFU/100ml	Std dev	COV
	O1	50	336	672	672		
	I1A	50	12	24			
	I1B	50	13	26	21	7.6	37%
1	I1C	50	6	12			
	F1A	10	42	420			
	F1B	10	43	430	400	43.6	11%
	F1C	10	35	350			
	O2	50	4	8	8		
	I2A	50	2	4			
	I2B	50	1	2	3	1.2	35%
2	I2C	50	2	4			
	F2A	10	20	200			
	F2B	10	18	180	153	64.3	42%
	F2C	10	8	80			
	O3	50	0	<1	<1		
	I3A	50	2	4			
	I3B	50	4	8	7	2.3	35%
3	I3C	50	4	8			
	F3A	10	13	130			
	F3B	10	14	140	140	10.0	7%
	F3C	10	15	150			
	O4	50	0	<1	<1		
	I4A	50	3	6			
	I4B	50	1	2	3	2.3	69%
4	I4C	50	1	2			
	F4A	10	9	90			
	F4B	10	6	60	87	25.2	29%
	F4C	10	11	110			

Oi : Source sample, cycle No.

Iij* : Initial pool sample, cycle No. I, and sample loaction J

Fij*:Final pool sample, cycle No. I, and sample loaction J

* :Samples collected from three locations in pool (A,B, and C)

Table D.3 Field observations data

Cyle#	time in	time out	depth,cm	Temp.,C	DO,%Sat.	DO, mg/l	PH	# of Initial samples	# of Final samples	# of Ocean samples
1	8:57 AM	9:12 AM	20	30.28	29.9	1.84	7.95	3*	3	1
2	9:48 AM	10:03 AM	26	30.27	10.5	0.64	7.92	3	3	1
3	10:20 AM	10:35 AM	17	31	38.1	2.33	7.25	3	3	1
4	10:50 AM	11:05 AM	18	31.26	66.9	4.09	6.82	3	3	1

Table D.4 Pool volumes data

Cyle#	water depth,ft	Pool Diameter, ft	Pool Area, ft2	Pool Volume, ft3	Pool Volume, liters
1	0.66	10	78.54	51.54	1458
2	0.85	10	78.54	67.00	1895
3	0.56	10	78.54	43.81	1239
4	0.59	10	78.54	46.38	1312

Table D.5 Calculations for the No. of enterococci (CFU) shed per person

Sampling type	enterococci, CFU/100ml	Water depth, ft	Volume of pool, liters	enterococci in pool, CFU	No.enterococci shed per person, CFU
O1	672				
O2	8				
O3	<1				
O4	<1				
I1	21	0.66	1458	123889	570619
I2	3	0.85	1895	63159	284216
I3	7	0.56	1239	82593	165186
I4	3	0.59	1312	43726	109314
F1	400		1458	5830079	
F2	153		1895	2905322	
F3	140		1239	1734448	
F4	87		1312	1136865	

Xi: sample type (source, initial, or final) and cycle No.

Table D.6 Calculations for the No. of Staph. aureus (CFU) shed per person

Sampling type	S. aureus, CFU/100ml	Water depth, ft	Volume of pool, liters	S. aureus in pool, CFU	No. S.aureus shed per person,CFU
O1	64				
O2	2				
O3	<1				
O4	<1				
I1	13	0.66	1458	145752	6087574
I2	7	0.85	1895	138950	3927238
I3	11	0.56	1239	132148	1258714
I4	9	0.59	1312	113687	675123
F1	4187		1458	61021489	
F2	2080		1895	39411331	
F3	1027		1239	12719288	
F4	523		1312	6864918	

Xi: sample type (source, initial, or final) and cycle No.

Small Pool Study :

Table D.8 Enterococci sand laboratory analysis

Sample/ Subject	Whirlpack (g)	Wet Sand + Whirlpack (g)	Dry Sand(g)	Volume filtered through 30µm (mL)	Volume filtered through .45µm (mL)	Date & Time in incubator	Date & Time out of incubator
S11	3.5447	9.4312	4.6374	100	2 6 12 20 50	05_08_22 12:30 PM	05_08_23 12:35 PM
S12	3.5529	19.5307	15.9778	190	2 6 12 20 50	05_08_22 12:30 PM	05_08_23 12:35 PM
S13	3.516	18.7069	15.1909	101	2 6 12 20 50	05_08_22 12:30 PM	05_08_23 12:35 PM
S14	3.5576	10.9215	5.6934	102	2 6 12 20 50	05_08_22 12:30 PM	05_08_23 12:35 PM
S15	3.5044	8.1951	3.575072	108	2 6 12 20 50	05_08_22 12:30 PM	05_08_23 12:35 PM

Table D.8 (continued) Enterococci sand laboratory analysis

Sample/Subject	# of colonies	CFU/mL (Cextract)	CFU/g dry sand	Avg CFU/g dry sand	STDEV CFU/g dry sand
S11	3	1.5	32.3457	26.1354	4.7174
	8	1.3333	28.7518		
	11	0.9167	19.7668		
	23	1.15	24.7984		
	58	1.16	25.0140		
S12	5	2.5	29.7287	30.2361	4.3492
	18	3	35.6745		
	28	2.3333	27.7468		
	56	2.8	33.2962		
	104	2.08	24.7343		
S13	0	0	0	5.6570	2.8391
	8	1.3333	8.8650		
	9	0.75	4.9865		
	20	1	6.6487		
	16	0.32	2.1276		
S14	1	0.5	8.9578	4.0967	4.1738
	0	0	0		
	1	0.0833	1.4930		
	2	0.1	1.7916		
	23	0.46	8.2412		
S15	5	2.5	75.5229	55.0411	17.1282
	7	1.1667	35.2440		
	22	1.8333	55.3835		
	45	2.25	67.9707		
	68	1.36	41.0845		

Table D.9 Sand laboratory analysis

Sample/ Subject	I.D.	Tin (g)	Wet sand + Tin (g)	Date & Time in oven	Date & Time out of oven	Dry sand + tin (g)	WC	STDEV of WC	AVG of WC
S11	S11-1	1.733	21.1796	05_08_22	05_08_23	17.1435	20.75%	0.66%	21.22%
	S11-2	1.7377	25.6673	11:00 AM	11:45 AM	20.4782	21.68%		
S12	S12-1	1.7763	Not processed because there wasn't				0.00%	0.00%	0.00%
	S12-2	1.7378	enough sand				0.00%		
S13	S13-1	1.7514	Not processed because there wasn't				0.00%	0.00%	0.00%
	S13-2	1.7424	enough sand				0.00%		
S14	S14-1	1.7971	10.0282	05_08_22	05_08_23	8.1825	22.42%	0.37%	22.69%
	S14-2	1.7752	6.4219	11:00 AM	11:45 AM	5.3556	22.95%		
S15	S15-1	1.7373	13.4168	05_08_22	05_08_23	10.7758	22.61%	1.66%	23.78%
	S15-2	1.7818	11.3605	11:00 AM	11:45 AM	8.9701	24.96%		

Table D.9(continued) Sand laboratory analysis

Sample/Subject	Sample collection Time	Sample Description	Whirlpack weight (g)	Whirlpack weight + Wet Sand (g)	Weight of Wet Sand (g)	Amount of Dry Sand (g)
S11	7:45 AM	Sandy water	9.1547	91.4288	82.2741	64.8157
S12	8:15 AM	A small amount of sand in water	9.1994	19.6115	10.4121	10.4121
S13	8:45 AM	Almost no sand	9.2193	18.7206	9.5013	9.5013
S14	9:10 AM	Sand	9.2742	36.8878	27.6136	21.3493
S15	9:25 AM	A little sand	9.23	60.5757	51.3457	39.1337

Appendix E:

Data from the Model Runs:

Table E.1 Calculations for the sand input function, $L_{s(t)}$, CFU/hour

Tidal Direction	t hrs	t, time interval, hrs	As(t), m2	$d_{s(t)}$, m	ρ = bulk density of sand g/m3	C_{sand}= Avg.density of enterococci, CFU/g of rdy sand	f= frequency of tidal cycles, hr^{-1}	$L_{s(t)}$, CFU/hour
High to Low Tide	0							
	1	0 to1	116	0.004	2.E+06	56	0.17	8.E+06
	2	1 to 2	231	0.004	2.E+06	56	0.17	2.E+07
	3	2 to 3	231	0.004	2.E+06	56	0.17	2.E+07
	4	3 to 4	231	0.004	2.E+06	56	0.17	2.E+07
	5	4 to 5	231	0.004	2.E+06	56	0.17	2.E+07
Low to High Tide	6	5 to 6	231	0.004	2.E+06	56	0.17	2.E+07
	7	6 to 7	231	-0.006	2.E+06	56	0.17	0.E+00
	8	7 to 8	231	-0.006	2.E+06	56	0.17	0.E+00
	9	8 to 9	231	-0.006	2.E+06	56	0.17	0.E+00
	10	9 to 10	231	-0.006	2.E+06	56	0.17	0.E+00
	11	10 to 11	231	-0.006	2.E+06	56	0.17	0.E+00
	12	11 to 12	116	-0.006	2.E+06	56	0.17	0.E+00

$L_{s(t)}=\rho * d_{s(t)} * A_{s(t)} * C_{sand} * f$, CFU/hour

f= 4 tidal cycles/24 hrs or (1/6) hr^{-1}

ρ = bulk density of sand = 1746000 g/m^3

$d_{s(t)}$= depth of beach sand erosion or deposit, m at any time t

Based on direct fiekd measurments:

Incoming Tide: $d_{s(t)}$= -0.006 m sediment erosion

Outgoing Tide: $d_{s(t)}$=+0.004 m sediment deposit

$A_{s(t)}$, m^2 = Area of exposed or covered sand within the intertidal zone

from LT to HT and from HT to LT respectively = Xt*B

C_{sand}= Average concentrations of enterococci in beach sand CFU/g of rdy sand = 56 CFU/g

Table E.2 Calculations for run-off input function, $Q_{runoff}*C_{runoff}$, CFU/hr

Tidal Direction	t hrs	t, time interval, hrs	A_{d1}, m^2	A_{d2}, m^2	A_{d3}, m^2	I, m/hr	Q_{runoff}, m^3/hr	C_{runoff}, CFU/m^3	$Q_{runoff}*C_{runoff}$, CFU/hr
High to Low Tide	0								
	1	0 to1	116	1488	372	8.E-04	1.2E+00	1.5E+08	2.E+08
	2	1 to 2	347	1488	372	8.E-04	1.3E+00	1.5E+08	2.E+08
	3	2 to 3	578	1488	372	8.E-04	1.4E+00	1.5E+08	2.E+08
	4	3 to 4	809	1488	372	8.E-04	1.4E+00	1.5E+08	2.E+08
	5	4 to 5	1040	1488	372	8.E-04	1.5E+00	1.5E+08	2.E+08
Low to High Tide	6	5 to 6	1272	1488	372	8.E-04	1.6E+00	1.5E+08	2.E+08
	7	6 to 7	1272	1488	372	8.E-04	1.6E+00	1.5E+08	2.E+08
	8	7 to 8	1040	1488	372	8.E-04	1.5E+00	1.5E+08	2.E+08
	9	8 to 9	809	1488	372	8.E-04	1.4E+00	1.5E+08	2.E+08
	10	9 to 10	578	1488	372	8.E-04	1.4E+00	1.5E+08	2.E+08
	11	10 to 11	347	1488	372	8.E-04	1.3E+00	1.5E+08	2.E+08
	12	11 to 12	116	1488	372	8.E-04	1.2E+00	1.5E+08	2.E+08

$Q_{runoff}C_{runoff} = C_{runoff}*I*(D_{c1}*A_{d1}+D_{c2}*A_{d2}+D_{c3}*A_{d3})$, CFU/hr

Qrunoff= $I\sum D_c A_d$

A_{d1}, m2 = Drainage area created within the intertidal zone by tidal activities at time t

A_{d2}, m^2 = Drainage Area between the edge of the pavement

And the HT water line, it is constant = B, m * 12.2 m

A_{d3}, m^2 = Drainage area of the paved road only to the center line

Drainage Coefficients: D_{c1} = 0.5 (Wet sand), D_{c2} = 0.7 (dry beach sand),

And Dc3 = 1.0 (pavement)

I, Average rainfall intensity, m/hr = (0.0099/24) m/hr

C_{runoff}, CFU/m^3 = Average concenterations of enteroccci in water runoff = 15,000 (CFU/100ml) *(1000,000 ml/m3)

Table E.3 Calculations for birds input function, L_b Input Function, (CFU)/(hour)

	N = # of animals	W_b = concentrations of enterococci, (CFU)/(g of dry bird feces)	U_b = grams of dry bird feces, (g)/(birdxday)	Z = WbxUb= enterococci loading rate, (CFU)/(birdxday)	L_b= NxZ= Input Function, (CFU)/(Day)	L_b Input Function, (CFU)/(hour)
Bird	1	3.80E+05	11.5	4.4E+06	4.4E+06	1.8E+05

$L_b = (N_b x W_b x U_b) x (1/24)$

Table E.4 Calculations for dogs input function, L_d Input Function, (CFU)/(hour)

Dog Size	Dog weight, Kg	N = # of animals	f = # of (animal fecal events)/(animalxday)	W_d = concentrations of enterococci, (CFU)/(g of dry animal feces)	$U_{d(Wright\ et\ al)}$, g of dry feces/day/dog
Medium	27.2	0	2	6.67E+07	51.8
Small	3.2	0	2	6.67E+07	7.6
Average	15.2	0	2	6.67E+07	29.7

Table E.4 (continued)Calculations for dogs input function, L_d Input Function, (CFU)/(hour)

$Y = (U_d/f)$ grams of dry feces/animal fecal event, (g)/(animal fecal event)	$Z=W_dxY=$ enterococci loading rate/animal fecal event, (CFU)/(animal fecal event)	$L_d = fxNxZ_1=$ Input Function, (CFU)/(Day)	L_d Input Function, (CFU)/(hour)
25.9	1.7E+09	0.0E+00	0.0E+00
3.8	2.5E+08	0.0E+00	0.0E+00
14.8	9.9E+08	0.0E+00	0.0E+00

$L_d= (N_dxW_dxU_d)x(1/24)$

Table E.5 Calculations for bathers input function, L_p=Bathers input function, CFU/hour

$N_p=$ Avg # of bathers	f_p=Avg # of 15' exposure/ bather day	$Y_{tskin}=$ Enterococci loading rate, CFU/15' exposure	L_p=Bathers input function, CFU/day	L_p=Bathers input function, CFU/hour
3	4	3.3E+05	4.0E+06	1.7E+05

$L_p=f_pxN_pxY_{tskin}$

Solution of the General mass-balance conservation equation @steady state condition:

$$C = \frac{[(Q_{tidal}C_{tida}] + Q_{runoff}C_{runoff} + Q_{parallelin}C_{in}) + \sum L]}{(Q_{parallelout} + K_bV)}$$ Equation (3-26)

For incoming tide $C_{tidal}=0$

$$C = \frac{[(Q_{runoff}C_{runoff} + Q_{parallelin}C_{in}) + \sum L]}{(Q_{parallelout} + K_bV)}$$ Equation (3-27)

For outgoing tide $C_{tidal}=C$

$$\boxed{C = \frac{[(Q_{runoff}C_{runoff} + Q_{parallelin}C_{in}) + \sum L]}{(Q_{parallelout} - Q_{tidal} + K_bV)}}$$ Equation (3-28)

Model Runs Results Using Parameters from Wright et al. (2005)

Table E.6 Intensive sampling study summer 2004

Tidal Direction	t, hrs	t, time interval, hrs	Ht, m	µHt, m	Lt, m	µLt, m	B, m	Vt, m3	Kb, hr-1	KbVt, m3/hr	Avg. (KbVt), m3/hr	dV/dt, m3/hr	Qrunoff, m3/hr
High to Low Tide	0		0.56		11.37		122	388	0.92	356.03		0.0	0.0
	1	0 to1	0.47	0.51	9.48	10.42	122	270	0.92	247.24	301.64	-118.7	0.2
	2	1 to 2	0.37	0.42	7.58	8.53	122	173	0.92	158.24	202.74	-97.1	0.2
	3	2 to 3	0.28	0.33	5.69	6.63	122	97	0.92	89.01	123.62	-75.5	0.2
	4	3 to 4	0.19	0.23	3.79	4.74	122	43	0.92	39.56	64.28	-53.9	0.2
	5	4 to 5	0.09	0.14	1.90	2.84	122	11	0.92	9.89	24.72	-32.4	0.3
Low to High Tide	6	5 to 6	0.00	0.05	0.00	0.95	122	0	0.92	0.00	4.94	-10.8	0.3
	7	6 to 7	0.09	0.05	1.90	0.95	122	11	0.92	9.89	4.94	10.8	0.3
	8	7 to 8	0.19	0.14	3.79	2.84	122	43	0.92	39.56	24.72	32.4	0.3
	9	8 to 9	0.28	0.23	5.69	4.74	122	97	0.92	89.01	64.28	53.9	0.2
	10	9 to 10	0.37	0.33	7.58	6.63	122	173	0.92	158.24	123.62	75.5	0.2
	11	10 to 11	0.47	0.42	9.48	8.53	122	270	0.92	247.24	202.74	97.1	0.2
	12	11 to 12	0.56	0.51	11.37	10.42	122	388	0.92	356.03	301.64	118.7	0.2

Variables: No. people =7, No. Dogs =1, No. Birds =0, C_{sand}=39 cfu/g dry sand, I=0.00013 m/hr

Table E.6 (Continued) Intensive sampling study summer 2004

Qtidal=(dV/dt)-Qrunoff, m3/hr	Qparallelin, m3/hr	Qparallelout, m3/hr	Cin, CFU/m3	Qparallelin*Cin, CFU/hr	Qrunoff*Crunoff, CFU/hr	(QrunoffCrunoff+QparallelinCin) CFU/hr	(Qparallelout-Qtidal+KbV) CFU/hr	(Qparallelout+KbV) CFU/hr
0.0	0	0	0	0				
-118.9	79	79	0	0	3.E+07	3.E+07	5.E+02	4.E+02
-97.3	79	79	0	0	3.E+07	3.E+07	4.E+02	3.E+02
-75.7	78	78	0	0	3.E+07	3.E+07	3.E+02	2.E+02
-54.2	77	77	0	0	4.E+07	4.E+07	2.E+02	1.E+02
-32.6	72	72	0	0	4.E+07	4.E+07	1.E+02	1.E+02
-11.1	40	40	0	0	4.E+07	4.E+07	6.E+01	4.E+01
10.5	40	40	0	0	4.E+07	4.E+07	3.E+01	4.E+01
32.1	72	72	0	0	4.E+07	4.E+07	6.E+01	1.E+02
53.7	77	77	0	0	4.E+07	4.E+07	9.E+01	1.E+02
75.3	78	78	0	0	3.E+07	3.E+07	1.E+02	2.E+02
96.9	79	79	0	0	3.E+07	3.E+07	2.E+02	3.E+02
118.5	79	79	0	0	3.E+07	3.E+07	3.E+02	4.E+02

Table E.6 (Continued) Intensive sampling study summer 2004

Ls(t), CFU/hr	Lb, birds, CFU/hr	Ld, dogs, CFU/hr	Lp, bathers, CFU/hr	∑L, CFU/hr	Ct, CFU/m3	Ct, CFU/100ml
5.E+06	0.E+00	8.E+07	4.E+05	9.E+07	2.E+05	23
1.E+07	0.E+00	8.E+07	4.E+05	9.E+07	3.E+05	33
1.E+07	0.E+00	8.E+07	4.E+05	9.E+07	5.E+05	46
1.E+07	0.E+00	8.E+07	4.E+05	9.E+07	7.E+05	66
1.E+07	0.E+00	8.E+07	4.E+05	9.E+07	1.E+06	102
1.E+07	0.E+00	8.E+07	4.E+05	9.E+07	2.E+06	239
0.E+00	0.E+00	8.E+07	4.E+05	8.E+07	3.E+06	274
0.E+00	0.E+00	8.E+07	4.E+05	8.E+07	1.E+06	125
0.E+00	0.E+00	8.E+07	4.E+05	8.E+07	8.E+05	84
0.E+00	0.E+00	8.E+07	4.E+05	8.E+07	6.E+05	58
0.E+00	0.E+00	8.E+07	4.E+05	8.E+07	4.E+05	40
0.E+00	0.E+00	8.E+07	4.E+05	8.E+07	3.E+05	29
					Overall Avg.	93

Table E.7 Intensive sampling study Winter 2005

Tidal Direction	t, hrs	t, time interval, hrs	Ht, m	μHt, m	Lt, m	μLt, m	B, m	Vt, m3	Kb, hr-1	KbVt, m3/hr	Avg. (KbVt), m3/hr	dV/dt, m3/hr	Qrunoff, m3/hr
High to Low Tide	0		0.56		11.37		122	388	0.92	356.03		0.0	0.0
	1	0 to1	0.47	0.51	9.48	10.42	122	270	0.92	247.24	301.64	-118.7	3.E-02
	2	1 to 2	0.37	0.42	7.58	8.53	122	173	0.92	158.24	202.74	-97.1	3.E-02
	3	2 to 3	0.28	0.33	5.69	6.63	122	97	0.92	89.01	123.62	-75.5	4.E-02
	4	3 to 4	0.19	0.23	3.79	4.74	122	43	0.92	39.56	64.28	-53.9	4.E-02
	5	4 to 5	0.09	0.14	1.90	2.84	122	11	0.92	9.89	24.72	-32.4	4.E-02
Low to High Tide	6	5 to 6	0.00	0.05	0.00	0.95	122	0	0.92	0.00	4.94	-10.8	4.E-02
	7	6 to 7	0.09	0.05	1.90	0.95	122	11	0.92	9.89	4.94	10.8	4.E-02
	8	7 to 8	0.19	0.14	3.79	2.84	122	43	0.92	39.56	24.72	32.4	4.E-02
	9	8 to 9	0.28	0.23	5.69	4.74	122	97	0.92	89.01	64.28	53.9	4.E-02
	10	9 to 10	0.37	0.33	7.58	6.63	122	173	0.92	158.24	123.62	75.5	4.E-02
	11	10 to 11	0.47	0.42	9.48	8.53	122	270	0.92	247.24	202.74	97.1	3.E-02
	12	11 to 12	0.56	0.51	11.37	10.42	122	388	0.92	356.03	301.64	118.7	3.E-02

Variables: No. people =4, No. Dogs =1, No. Birds =0, Csand=13 cfu/g dry sand, I=0.0000212 m/hr

Table E.7 (Continued) Intensive sampling study Winter 2005

Qtidal=(dV/dt)-Qrunoff, m3/hr	Qparallelin, m3/hr	Qparallelout, m3/hr	Cin, CFU/m3	Qparallelin*Cin, CFU/hr	Qrunoff*Crunoff, CFU/hr	(QrunoffCrunoff+QparallelinCin) CFU/hr	(Qparallelout-Qtidal+KbV) CFU/hr	(Qparallelout+KbV) CFU/hr
0.0	0	0	0	0				
-118.9	79	79	0	0	3.E+07	3.E+07	5.E+02	4.E+02
-97.3	79	79	0	0	3.E+07	3.E+07	4.E+02	3.E+02
-75.7	78	78	0	0	3.E+07	3.E+07	3.E+02	2.E+02
-54.2	77	77	0	0	4.E+07	4.E+07	2.E+02	1.E+02
-32.6	72	72	0	0	4.E+07	4.E+07	1.E+02	1.E+02
-11.1	40	40	0	0	4.E+07	4.E+07	6.E+01	4.E+01
10.5	40	40	0	0	4.E+07	4.E+07	3.E+01	4.E+01
32.1	72	72	0	0	4.E+07	4.E+07	6.E+01	1.E+02
53.7	77	77	0	0	4.E+07	4.E+07	9.E+01	1.E+02
75.3	78	78	0	0	3.E+07	3.E+07	1.E+02	2.E+02
96.9	79	79	0	0	3.E+07	3.E+07	2.E+02	3.E+02
118.5	79	79	0	0	3.E+07	3.E+07	3.E+02	4.E+02

Table E.7 (Continued) Intensive sampling study Winter 2005

Ls(t), CFU/hr	Lb, birds, CFU/hr	Ld, dogs, CFU/hr	Lp, bathers, CFU/hr	∑L, CFU/hr	Ct, CFU/m3	Ct, CFU/100ml
2.E+06	0.E+00	8.E+07	2.E+05	8.E+07	2.E+05	18
3.E+06	0.E+00	8.E+07	2.E+05	9.E+07	2.E+05	24
3.E+06	0.E+00	8.E+07	2.E+05	9.E+07	3.E+05	33
3.E+06	0.E+00	8.E+07	2.E+05	9.E+07	5.E+05	47
3.E+06	0.E+00	8.E+07	2.E+05	9.E+07	7.E+05	72
3.E+06	0.E+00	8.E+07	2.E+05	9.E+07	2.E+06	167
0.E+00	0.E+00	8.E+07	2.E+05	8.E+07	2.E+06	199
0.E+00	0.E+00	8.E+07	2.E+05	8.E+07	9.E+05	92
0.E+00	0.E+00	8.E+07	2.E+05	8.E+07	6.E+05	63
0.E+00	0.E+00	8.E+07	2.E+05	8.E+07	4.E+05	44
0.E+00	0.E+00	8.E+07	2.E+05	8.E+07	3.E+05	31
0.E+00	0.E+00	8.E+07	2.E+05	8.E+07	2.E+05	23
					Overall Avg.	68

Table E.8 48 hour sampling study 2004

Tidal Direction	t, hrs	t, time interval, hrs	Ht, m	µHt, m	Lt, m	µLt, m	B, m	Vt, m3	Kb, hr-1	KbVt, m3/hr	Avg. (KbVt), m3/hr	dV/dt, m3/hr	Qrunoff, m3/hr
High to Low Tide	0		0.56		11.37		122	388	0.92	356.03		0.0	0.0
	1	**0 to1**	0.47	0.51	9.48	10.42	122	270	0.92	247.24	301.64	118.7	4.4E+00
	2	**1 to 2**	0.37	0.42	7.58	8.53	122	173	0.92	158.24	202.74	-97.1	4.8E+00
	3	**2 to 3**	0.28	0.33	5.69	6.63	122	97	0.92	89.01	123.62	-75.5	5.1E+00
	4	**3 to 4**	0.19	0.23	3.79	4.74	122	43	0.92	39.56	64.28	-53.9	5.5E+00
	5	**4 to 5**	0.09	0.14	1.90	2.84	122	11	0.92	9.89	24.72	-32.4	5.8E+00
Low to High Tide	6	**5 to 6**	0.00	0.05	0.00	0.95	122	0	0.92	0.00	4.94	-10.8	6.1E+00
	7	**6 to 7**	0.09	0.05	1.90	0.95	122	11	0.92	9.89	4.94	10.8	6.1E+00
	8	**7 to 8**	0.19	0.14	3.79	2.84	122	43	0.92	39.56	24.72	32.4	5.8E+00
	9	**8 to 9**	0.28	0.23	5.69	4.74	122	97	0.92	89.01	64.28	53.9	5.5E+00
	10	**9 to 10**	0.37	0.33	7.58	6.63	122	173	0.92	158.24	123.62	75.5	5.1E+00
	11	**10 to 11**	0.47	0.42	9.48	8.53	122	270	0.92	247.24	202.74	97.1	4.8E+00
	12	**11 to 12**	0.56	0.51	11.37	10.42	122	388	0.92	356.03	301.64	118.7	4.4E+00

Variables: No. people =3, No. Dogs =0, No. Birds =0, Csand=56 cfu/g dry sand, l=0.000146 m/hr

Table E.8 (Continued) 48 hour sampling study 2004

Qtidal=(dV/dt)-Qrunoff, m3/hr	Qparallelin, m3/hr	Qparallelout, m3/hr	Cin, CFU/m3	Qparallelin*Cin, CFU/hr	Qrunoff*Crunoff, CFU/hr	(QrunoffCrunoff+QparallelinCin) CFU/hr	(Qparallelout-Qtidal+KbV) CFU/hr	(Qparallelout+KbV) CFU/hr
0.0	0	0	0	0				
-123.1	79	79	0	0	7.E+08	7.E+08	5.E+02	4.E+02
-101.9	79	79	0	0	7.E+08	7.E+08	4.E+02	3.E+02
-80.6	78	78	0	0	8.E+08	8.E+08	3.E+02	2.E+02
-59.4	77	77	0	0	8.E+08	8.E+08	2.E+02	1.E+02
-38.2	72	72	0	0	9.E+08	9.E+08	1.E+02	1.E+02
-16.9	40	40	0	0	9.E+08	9.E+08	6.E+01	4.E+01
4.6	40	40	0	0	9.E+08	9.E+08	4.E+01	4.E+01
26.6	72	72	0	0	9.E+08	9.E+08	7.E+01	1.E+02
48.5	77	77	0	0	8.E+08	8.E+08	9.E+01	1.E+02
70.4	78	78	0	0	8.E+08	8.E+08	1.E+02	2.E+02
92.3	79	79	0	0	7.E+08	7.E+08	2.E+02	3.E+02
114.3	79	79	0	0	7.E+08	7.E+08	3.E+02	4.E+02

Table E.8 (Continued) 48 hour sampling study 2004

Ls(t), CFU/hr	Lb, birds, CFU/hr	Ld, dogs, CFU/hr	Lp, bathers, CFU/hr	∑L, CFU/hr	Ct, CFU/m3	Ct, CFU/100ml
8.E+06	0.0E+00	0.E+00	2.E+05	8.E+06	1.E+06	**133**
2.E+07	0.0E+00	0.E+00	2.E+05	2.E+07	2.E+06	**190**
2.E+07	0.0E+00	0.E+00	2.E+05	2.E+07	3.E+06	**277**
2.E+07	0.0E+00	0.E+00	2.E+05	2.E+07	4.E+06	**416**
2.E+07	0.0E+00	0.E+00	2.E+05	2.E+07	7.E+06	**658**
2.E+07	0.0E+00	0.E+00	2.E+05	2.E+07	2.E+07	**1520**
0.E+00	0.0E+00	0.E+00	2.E+05	2.E+05	2.E+07	**2062**
0.E+00	0.0E+00	0.E+00	2.E+05	2.E+05	9.E+06	**903**
0.E+00	0.0E+00	0.E+00	2.E+05	2.E+05	6.E+06	**581**
0.E+00	0.0E+00	0.E+00	2.E+05	2.E+05	4.E+06	**380**
0.E+00	0.0E+00	0.E+00	2.E+05	2.E+05	3.E+06	**254**
0.E+00	0.0E+00	0.E+00	2.E+05	2.E+05	2.E+06	**174**
					Overall Avg.	**629**

Table E.9 Labor day study, May 31st 2005

Tidal Direction	t, hrs	t, time interval, hrs	Ht, m	µHt, m	Lt, m	µLt, m	B, m	Vt, m3	Kb, hr-1	KbVt, m3/hr	Avg. (KbVt), m3/hr	dV/dt, m3/hr	Qrunoff, m3/hr
High to Low Tide	0		0.56		11.37		122	388	0.92	356.03		0.0	0.0
	1	0 to1	0.47	0.51	9.48	10.42	122	270	0.92	247.24	301.64	-118.7	5.E-01
	2	1 to 2	0.37	0.42	7.58	8.53	122	173	0.92	158.24	202.74	-97.1	5.E-01
	3	2 to 3	0.28	0.33	5.69	6.63	122	97	0.92	89.01	123.62	-75.5	5.E-01
	4	3 to 4	0.19	0.23	3.79	4.74	122	43	0.92	39.56	64.28	-53.9	6.E-01
	5	4 to 5	0.09	0.14	1.90	2.84	122	11	0.92	9.89	24.72	-32.4	6.E-01
Low to High Tide	6	5 to 6	0.00	0.05	0.00	0.95	122	0	0.92	0.00	4.94	-10.8	6.E-01
	7	6 to 7	0.09	0.05	1.90	0.95	122	11	0.92	9.89	4.94	10.8	6.E-01
	8	7 to 8	0.19	0.14	3.79	2.84	122	43	0.92	39.56	24.72	32.4	6.E-01
	9	8 to 9	0.28	0.23	5.69	4.74	122	97	0.92	89.01	64.28	53.9	6.E-01
	10	9 to 10	0.37	0.33	7.58	6.63	122	173	0.92	158.24	123.62	75.5	5.E-01
	11	10 to 11	0.47	0.42	9.48	8.53	122	270	0.92	247.24	202.74	97.1	5.E-01
	12	11 to 12	0.56	0.51	11.37	10.42	122	388	0.92	356.03	301.64	118.7	5.E-01

Variables: No. people =30, No. Dogs =4, No. Birds =0, Csand=19 cfu/g dry sand, I=0.000307 m/hr

Table E.9 (Continued) Labor day study, May 31st 2005

Qtidal=(dV/dt)-Qrunoff, m3/hr	Qparallelin, m3/hr	Qparallelout, m3/hr	Cin, CFU/m3	Qparallelin*Cin, CFU/hr	Qrunoff*Crunoff, CFU/hr	(QrunoffCrunoff+QparallelinCin) CFU/hr	(Qparallelout-Qtidal+KbV) CFU/hr	(Qparallelout+KbV) CFU/hr
0.0	0	0	0	0				
-119.1	79	79	0	0	7.E+07	7.E+07	5.E+02	4.E+02
-97.6	79	79	0	0	7.E+07	7.E+07	4.E+02	3.E+02
-76.0	78	78	0	0	8.E+07	8.E+07	3.E+02	2.E+02
-54.5	77	77	0	0	8.E+07	8.E+07	2.E+02	1.E+02
-33.0	72	72	0	0	9.E+07	9.E+07	1.E+02	1.E+02
-11.4	40	40	0	0	9.E+07	9.E+07	6.E+01	4.E+01
10.2	40	40	0	0	9.E+07	9.E+07	3.E+01	4.E+01
31.8	72	72	0	0	9.E+07	9.E+07	6.E+01	1.E+02
53.4	77	77	0	0	8.E+07	8.E+07	9.E+01	1.E+02
75.0	78	78	0	0	8.E+07	8.E+07	1.E+02	2.E+02
96.6	79	79	0	0	7.E+07	7.E+07	2.E+02	3.E+02
118.2	79	79	0	0	7.E+07	7.E+07	3.E+02	4.E+02

Table E.9 (Continued) Labor day study, May 31st 2005

Ls(t), CFU/hr	Lb, birds, CFU/hr	Ld, dogs, CFU/hr	Lp, bathers, CFU/hr	∑L, CFU/hr	Ct, CFU/m3	Ct, CFU/100ml
3.E+06	0.E+00	3.E+08	2.E+06	3.E+08	8.E+05	80
5.E+06	0.E+00	3.E+08	2.E+06	3.E+08	1.E+06	108
5.E+06	0.E+00	3.E+08	2.E+06	3.E+08	1.E+06	149
5.E+06	0.E+00	3.E+08	2.E+06	3.E+08	2.E+06	215
5.E+06	0.E+00	3.E+08	2.E+06	3.E+08	3.E+06	329
5.E+06	0.E+00	3.E+08	2.E+06	3.E+08	8.E+06	767
0.E+00	0.E+00	3.E+08	2.E+06	3.E+08	1.E+07	952
0.E+00	0.E+00	3.E+08	2.E+06	3.E+08	4.E+06	436
0.E+00	0.E+00	3.E+08	2.E+06	3.E+08	3.E+06	295
0.E+00	0.E+00	3.E+08	2.E+06	3.E+08	2.E+06	203
0.E+00	0.E+00	3.E+08	2.E+06	3.E+08	1.E+06	144
0.E+00	0.E+00	3.E+08	2.E+06	3.E+08	1.E+06	105
					Overall Avg.	315

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