Report on 2009 National Epidemiologic and Environmental Assessment of Recreational Water Epidemiology Studies

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Chapter 1

Executive Summary

The Clean Water Act (CWA) requires EPA to develop, publish and revise ambient water quality criteria (AWQC). The Beaches Environmental Assessment and Coastal Health Act of 2000 (The Beach Act), revised the CWA to require: "studies concerning pathogen indicators in coastal recreation waters". These studies were to include: "an assessment of potential human health risks ...', "appropriate and effective indicators for improving detection in a timely manner ...", and "appropriate, accurate, expeditious and cost effective methods ... for detecting in a timely manner in coastal recrationl waters the presence of pathogens that are harmful to human health."

Since 2003, EPA's National Health and Environmental Effects Research Laboratory (NHEERL), in collaboration with the National Exposure Research Laboratory (NERL), has been conducting epidemiology studies at beach sites to study beach-goers health and to measure water quality with new and faster ways of testing for microbial indicators of health effects and water quality. Studies have been conducted at four freshwater beaches in the Great Lakes and three marine sites. These studies have demonstrated that fecal indicator bacteria measured by a faster, molecular approach (quantitative polymerase chain reaction or qPCR) to measure fecal indicator bacteria in recreational waters were associated with swimming-associated gastrointestinal illness at beach sites with nearby treated sewage discharges.

In 2008, a meeting of expert scientists [1] called for additional studies at tropical beach sites and beach sites impacted by diffuse sources such as urban run-off. As a consequence of a consent decree and settlement agreement, resulting from a lawsuit (NRDC vs. Johnson, 2008), EPA prepared to conduct two studies, one at a tropical beach site and the other at a beach site impacted by urban run-off. In the summer of 2009, NHEERL and NERL successfully carried out studies at Boquerón Beach, Puerto Rico, and Surfside Beach, South Carolina.

The study design remained nearly identical to that used at the Great Lakes and marine beach sites. In brief, on summer weekends and holidays, beach-goers were offered enrollment in the study. Those who agreed completed three

interviews: an enrollment interview, an interview upon leaving the beach and a telephone interview 10-12 days later. The second interview determined exposure to water and other activities during the beach visit. The telephone interview ascertained the occurrence of health symptoms experienced since the beach visit.

Eighteen water samples were collected and tested each day for indicators of fecal contamination: *Enterococcus* spp. and *Bacteroidales* spp. using quantitative polymerase chain reaction, and *Enterococcus* using the standard culture based method. Swimmers were defined as those who immersed, at a minimum, their body in the water. Health symptoms studied included: gastrointestinal (GI), respiratory, skin rash, earache, and eye irritations.

The health surveys and interviews began in Boquerón Beach on May 16, 2009 and concluded on August 2, 2009. A total of 15,726 individuals were enrolled. Swimmers reported higher rates of rash compared to non-swimmers but swimmers experienced the same rates of GI illness, respiratory illness, earache, and eye irritations as non-swimmers. Densities of fecal indicator bacteria were low and no single day exceeded the currently recommended EPA criteria for *Enterococcus*. In addition, many qPCR assays could not be completed because of interfering or inhibitory substances in the water sample. These two factors (good water quality and interference of the qPCR assay complicated interpretation of the health, water quality relationship. As a result of the good water quality and the interference of the qPCR signal, consistent health relationships could not be developed between fecal indicator organisms measured by qPCR and swimming-associated illness.

At Surfside Beach, the health surveys and interviews began on June 7, 2009 and concluded on September 7, 2009. A total of 11,159 individuals were enrolled. Swimmers reported higher rates of rash GI illness and earache compared to non-swimmers but experienced similar rates of other illnesses. Only one day exceeded the currently recommended EPA criteria for Enterococcus. In addition, lower levels of *Enterococcus* and *Bacteroidales* measured by qPCR were observed than at previous beach sites. Overall, statistically significant trends between swimming-associated health effects and fecal indicator bacteria levels of *Enterococcus* were not observed but some positive trends were observed between the fecal indicator bacteria *Enterococcus* and GI illness.

Despite successful completion of an epidemiology study at a tropical beach site and a beach site impacted by urban runoff, several questions have been raised by these findings. The most notable of these are an evaluation of the possible reasons and potential remedies for the interference with the PCR assay observed at Boquerón Beach.

In summary, at Boquerón Beach despite successful enrollment in the 2009 NEEAR study in Puerto Rico, health relationships with indicators of water quality could not be established due to the good water quality and matrix interference with the qPCR signal.

Consistent health relationships between fecal indicator organisms and swimming-associated illness were also not established at Surfside Beach. This may have been the result of the good water quality since only one day exceeded the currently recommended EPA criteria for Enterococcus. Results could also be due

to the lack of human inputs impacting the beach.

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Chapter 2

Introduction and Purpose

2.1 Purpose

During the summer of 2009, the United States Environmental Protection Agency conducted epidemiology studies at two beach sites: A site impacted primarily by "non-point" source pollution or "urban runoff" (Surfside, North Carolina) and a tropical beach site with a nearby treated sewage discharge (Boquerón Beach, Puerto Rico). This report presents the results of these studies. The report focuses on indicator bacteria and health relationships for which previous associations have been established in fresh water and marine water beach sites. Previous studies have shown associations between *Enterococcus* spp. measured by quantitative polymerase chain reaction (qPCR) in marine and fresh waters and *Bacteroidales* spp. measured by qPCR in marine waters [2, 3, **?**].

2.2 Background

Fecal indicator bacteria in recreational waters can indicate the potential presence of a broad range of pathogenic microorganisms which are infectious in humans, resulting in gastrointestinal and other symptoms. As early as the 1950s, studies observed associations with the occurrence of high levels of coliform bacteria and an increased risk of gastrointestinal disturbances [4].

In 1972, the EPA initiated a long-term recreational water quality research program that examined the relationship between water quality and swimming-associated acute infectious disease. The first phase of the program, from 1972 to 1978, was conducted at multiple marine bathing beaches in New York, Louisiana and Massachusetts. A direct linear relationship between swimming-associated gastrointestinal illness and water quality which was indexed by the density of *Enterococcus* in the water was observed [5]. From 1978-1982, the EPA recreational water quality research program was directed at freshwater bathing areas. The freshwater studies were conducted in Pennsylvania and Oklahoma. Relationships between swimming-associated gastrointestinal illness and two bacterial

indicators, *Enterococcus* and *E. coli* were observed [6].

Numerous other studies of recreational water quality and swimmers' health were conducted in the decades following the first series of EPA studies. Comprehensive reviews have concluded that the literature generally supports the findings of EPA's studies: that swimming in fecally-polluted water was associated with a higher rate of gastrointestinal illnesses in swimmers when compared to non-swimmers [7, 8, 9]. A recently published review also showed that swimmers exposed to marine water at high levels of several indicator bacteria experienced a significant increase in skin-related symptoms compared to non-swimmers [10]. Several studies [11, 12, 13, 14] observed associations between indicator bacteria and respiratory illness.

2.2.1 The National Epidemiologic and Environmental Assessment of Recreational Water Study

One drawback of the currently recommended approaches to monitor and test for these fecal indicator bacteria in recreational waters is that the tests require at least 24 hours to obtain results [15]. Since 2002, the National Health and Environmental Effects Research Laboratory (NHEERL) and the National Exposure Research Laboratory (NERL) of EPA's Office of Research and Development (ORD) have been conducting research at beach sites across the United States to develop and validate better and faster ways to measure water quality and to develop associations between these measures and swimming associated illnesses.

Between 2003 and 2007, EPA initiated The National Epidemiologic and Environmental Assessment of Recreational Water Study (The NEEAR Water Study) designed to examine associations between swimming associated illnesses and novel and faster approaches to measuring recreational water quality. Studies were conducted at four freshwater and three marine beach sites (See Figures 2.1 and 2.2) with a nearby treated sewage discharge in the continental United States. Over 20,000 beach goers were enrolled and over 2,000 water samples collected and tested. Results from the four freshwater beach sites at the Great Lakes indicated associations between estimates of *Enterococcus sp.* measured by quantitative polymerase chain reaction (qPCR) [16, 17] and swimming-associated gastrointestinal illness [2, 3]. This finding represents a potential advantage to managing health risks at beach sites since results can be obtained by qPCR in under 3 hours, compared to at least 24 hours for culture based methods. Results from marine sites have yet to be published (papers in preparation), but preliminary results, which have been presented at scientific conferences [**?**], support the findings reported at freshwater beaches. The 2008 publication from the freshwater beach sites is included as an Appendix to this report (Appendix E).

With a few notable exceptions [18, 13, 19, 14] the vast majority of epidemiological investigations of water quality and health effects in recreational waters, including those conducted by the US EPA have been conducted at sites with nearby treated sewage discharges. Fecal contamination from runoff could be from a diverse mixture of sources including domestic animal, wildlife, and treated and untread human sources. Furthermore the nature of sources impact-

Figure 2.1: Freshwater beaches

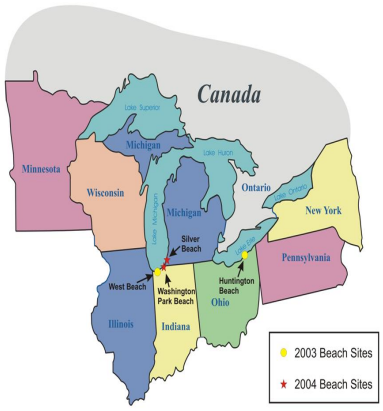


Figure 2.2: Marine beaches

ing runoff may be highly variable. Due to the complex and variable nature of this type of fecal contamination, it can be challenging to make consistent epidemiological linkages at runoff impacted sites. For example, a study in California observed an increased risk of illness related to swimming near storm drains and stormwater discharge [14], but other studies at beach sites which were impacted by runoff or other sources of pollution failed to find strong associations with illness [19]. A study in Mission Bay found an association between GI illness and male-sepcific coliphage occurrence, but this was based on few numbers of swimmers and infrequent detection of coliphage [18]

Several zoonotic pathogens which can cause mild to severe illness in humans could theoretically be transmitted from animal feces to humans via recreational water exposure including *Campylobacter sp.*, *Salmonella sp.*, pathogenic *E. coli*, and *Cryptosporidium*. However, there are few documented reports of such trans-

mission. Most outbreaks of these potentially zoonotic pathogens in recreational waters are usually attributed to person-person transmission [20, 21]. Recent risk assessments have indicated that with the possible exception of cattle feces, human-derived pollution probably has a higher risk than fecal contamination from other animal sources [22, 23]. Associations derived from indicator bacteria measured at sites with nearby treated sewage discharge have been applied to runoff impacted sites with the presumption they may be equally or more protective of health. Some have raised concerns that such studies may not be representative of recreational sites with impacts from runoff or other diffuse sources [24]. Others have noted the wide variability in observed associations between indicators and pathogens and raised concerns regarding the ability to generalize indicator health-effects associations to diverse types of beaches [25].

Few studies have been conducted at "tropical" sites where some have suggested that reliance on traditional fecal indicator bacteria for recreational water quality monitoring may not be appropriate since they may grow or survive in tropical soil [26, 27, 28].

EPA assembled a group of scientists with expertise in recreational water, monitoring and related issues in 2007 to "identify research and science needs for developing scientifically defensible new or revised...recreational ambient water quality criteria (AWQC) in the near-term" [1]. The expert panel called for, among other research, epidemiological investigations at tropical beach sites as well as sites impacted primarily be runoff [24].

2.2.2 Research question

During the summer of 2009, EPA conducted studies at two additional beach sites. One was impacted primarily by "urban runoff" and the second was again located near a treated sewage discharge but in a "tropical" climate.

The studies were designed to address the following research question:

> Is there an association between novel and faster measures of recreational water quality and swimming-associated illness at
>
> 1. A beach site primarily impacted by urban "runoff"?
> 2. A beach site in a tropical region?

It is not among the primary goals of this report to address associations among water quality indicators or between water quality indicators and environmental factors. It is anticipated this will be the focus of future efforts. It was also not a primary goal to address potential other associations between illness and non-fecal indicators of water quality or environmental factors such as turbidity and rainfall. It is also anticipated this may be the focus of future efforts.

Chapter 3

Methods

3.1 Site selection

3.1.1 Urban runoff impacted beach site

The following criteria were used to select a site for an epidemiology study at an urban-runoff impacted beach site:

- Generally meet State or local water quality standards for recreational beach water.
- Have a minimum exceedance rate of 15 percent of samples.
- Source of contamination is primarily from runoff.
- Can provide raw monitoring data for fecal coliform or enterococci for 2006 and 2007.
- The beach is subjected to a minimum of one rain event per month, and both rain frequency and magnitude can be readily documented.
- The swimming season > 90 days
- The attendance is > 300 beach goers per weekend day
- Beach is not included in the list of beaches studied under the NEEAR beach study
- Beach is located in a county with population density > 100 per square mile

Additional requirements were: variability in water quality, and sufficient population size to conduct an epidemiology study. Unlike previous studies, which were designed to be combined since they were all located near treated sewage discharges, it was important to enroll sufficient beach goers so the urban

runoff impacted beach site could be examined as a stand-alone site. It was also desirable to have regular rainfall during the summer beach season. Since water quality at beach sites impacted by runoff is often linked to rainfall, this criterion would ensure the beach site would receive some storm water flow during the study.

Previous experience has indicated that approximately 5,000 individual subjects are usually sufficient to observe an association between water quality and swimming-associated illness. Assuming a minimum 20 days of study, enrollment of 300 beach goers would result in a sample size of 6,000.

The search for a beach site meeting these criteria was conducted in 2007/2008. Initially, regional EPA beach managers were contacted to identify sites which potentially met these criteria. From this initial list of 179 beaches, historical monitoring data and attendance information were requested from states and local authorities. Additional screening reduced the potential sites to ten. For these additional details were obtained regarding the accuracy of the estimates for beach-goer attendance, monitoring data, land use information. The absence of regular human point-source impacts from septic systems, combined sewers was further investigated to confirm the sites were primarily impacted by runoff. Five beaches (three in South Carolina and two in Florida) were selected and targeted for additional water quality monitoring between December 2009 and January 2010 with targeted monitoring occurring after a "rain event" (defined as > 0.25 inches of rain in the 12 hour period prior to sampling).

3.1.2 Tropical beach site

EPA evaluated potential locations in U.S. states and territories (below 28 degrees latitude) in Puerto Rico, Hawaii, Guam and South Florida. Efforts were focused on finding a beach with proximity to a treated sewage discharge to allow for a comparison of health risks posed by treated sewage discharge in a tropical climate versus a temperate climate.

The following criteria were used to identify beach sites in a tropical region:

- Beach waters must be influenced by effluent from a wastewater treatment plant
- The attendance is > 300 beach goers per weekend day
- Beach water quality should be variable within local guideline limits

In addition to the above criteria, it was also desirable to select a beach site which was mostly attended by a local population. Although tourists could be at higher risk from swimming-associated illness, they are also likely to be at higher risk from gastrointestinal infections and subsequent diarrhea or other symptoms from other exposures. "Traveller's diarrhea" can affect up to 50% travellers to some destinations [29]. Such a high proportion of illnesses caused by exposures other than swimming could limit the ability to detect an association with water quality. Local and regional EPA beach representatives were contacted to identify potential beach sites meeting these criteria. Local officials responsible for

beach management were also contacted to request additional information. Ultimately, three beach sites in Puerto Rico were selected and were visited to obtain additional information on beach attendance and estimates of the proportion of local attendees compared to tourists.

3.2 Epidemiologic study design

3.2.1 General study design

The study was a prospective cohort study with an abbreviated follow-up period, designed using an approach similar to numerous previous studies. Sites were selected such that they had sufficient variability in water so that relationships between illness and water quality could be developed without a control or pristine beach.

The goal was to approach and offer enrollment to all beach-goers between 11:00 AM and 5:00 PM. The health survey was administered in three parts: enrollment, exit interview, and telephone interview. Interviewers approached beach-goers on weekends and holidays during the summer. An adult (18 years or older) answered questions for other household members. The beach interview included questions about demographics, swimming and other beach activities, consumption of raw or undercooked meat or runny eggs, chronic illnesses, allergies, acute health symptoms in the past 3 days, contact with sick persons in the past 48 hours, other swimming in the past week, and contact with animals in the past 48 hours. The telephone interview was conducted 10-12 days after the beach visit, and an adult 18 years of age or older answered questions for other household members who visited the beach. The telephone interview consisted of questions about health symptoms experienced since the beach visit; and other swimming or water related activities, contact with animals, and consumption of high-risk foods since the beach visit. Economic and physical burdens experienced as a result of each illness were also obtained (for example, days missed from work, money spent on medications). Bilingual (English-Spanish) interviewers were available.

Consent process The study protocol and questionnaire were reviewed by the Institutional Review Board (IRB) for the Centers for Disease Control and Prevention and approved by the EPA Human Subjects Review Official. A waiver of written informed consent for the enrollment process was obtained and was justified by the following: 1) the study involved no more than minimal risk, 2) potential enrollees would be apprised of the projects purpose and requirements and will have ample opportunity to defer from being enrolled, 3) only adult beach-goers were interviewed about both their individual exposure information and as surrogates for information on their children's activities (adults had their children with them to assist them in estimating child exposure, particularly for 12-17 year olds), 4) no sensitive questions were asked, and 5) identifying information was only being collected to allow completion of the telephone interviews

and payment of incentives. Personal identifiers were unlinked from the data following completion of the phone interviews. Contact information/mailing lists were only retained if the families have indicated that they would like to receive information about the program or for results of the study. All mailing lists were certified as destroyed following completion of the study.

Consent brochure This document served as the verbal consent form and included information about the benefits of participation (incentives and public health improvements for beach users), potential disadvantages of participation (time), absence of health risk, confidentiality, information dissemination, contact information for investigators, IRB, and contractor (email address for project, phone number, and a website).

Paper reduction act The questionnaires and study protocol were published in the Federal Register and public comment was requested. The comments and the estimated burden of the questionnaire were reviewed and approved by the Office of Management and Budget. The Office of Management and Budget (OMB) number for this study is 2080.0068.

Incentives Incentives were provided to participants to encourage completion of the beach questionnaire on enrollment day (e.g., tote bag, cooler, or beach-related item). Upon completion of the follow-up phone interviews a 25$ check was issued to each household.

Eligibility criteria Potential enrollees had to meet the following criteria

1. have a household member at least 18 years of age (for Puerto Rico, 21 years of age)
2. participate in the study and complete the telephone interview
3. have not participated in the study within the prior 28 days. Participants were allowed to re-enroll in the study after 28 days.

Questionnaire design The beach interview and telephone interview are basically identical to those used in the freshwater studies, and similar to those being used by other investigators [18]. Electronic replications of the Questionnaires are provided in Appendix A although the actual format differs since all information was obtained using a hand held computer. When possible, questions were designed to be compatible with the Centers for Disease Control, National Center for Infectious Diseases FoodNet survey. Similar questions have been used in previous studies of waterborne disease [30, 31, 32, 33]. Respiratory symptoms have been adapted from the Epidemiology Standardization Project of the American Thoracic Society and the Division of Lung Diseases [34].

Beach enrollment and exit interview The recruitment goal was to approach, all beach-goers on study days during the designated study period (weekends and designated holidays). Adult family beach-goers were approached for initial enrollment throughout the day. Interviewers confirmed at least one household member was 18 years or older and then obtained verbal informed consent. Further information on family make-up/membership and contact information was obtained. After completion of enrollment, families were encouraged to visit project work sites near beach exits when they left, to complete the beach questionnaire. All contacts on the beach were given either a flag or colored tape to signify they had been approached. At the exit interview, the information collected included the day's activities, food and water consumption and water exposure (extent, time, duration, and location) and other activities and exposures. The questionnaire obtained individual level information on health status and characteristics such as age, sex, race, ethnicity, housing characteristics, family characteristics and behaviors. Respondents were given an inexpensive gift (cooler or tote bag) following completion of the exit interview.

Telephone interview Study participants were contacted by phone 10-12 days after visiting the beach. An adult caregiver, preferably the same one interviewed at the beach, was asked a series of questions about family members swimming activities, other exposures, health status, and the severity of any illnesses reported since the initial beach visit. Questions covered enteric and non-enteric illness (gastrointestinal, respiratory, ear, eye, skin irritations, and urinary tract infections).

Data entry, management, and security A computer-assisted personal interview device (CAPI) equipped with a template of the questionnaire was used to collect the information at the enrollment and exit interviews. This device is a lightweight hand-held tablet computer tolerant of extreme environmental conditions. The CAPI program flagged missing items prior to terminating the interview and also flagged erroneous responses to allow the interviewer to obtain the correct information while interviewing the household. All data were kept in locked cabinets or in password-protected computers. Networks were protected with a firewall prevent unauthorized access to agency networks. Personal identifying information was stored separately from questionnaire and telephone survey information and all personal information was removed from analytical databases. Following completion of the final follow-up survey at the end of the study, participant personal identifiers were unlinked from the data.

The phone interview was conducted using a computer-assisted telephone interview (CATI) system. The CATI system automatically flags missing or erroneous data prior to termination of the interview. Telephone interviews were conducted from secured research facilities. Each of these devices reduced data entry errors that result from data transfer in traditional paper-based studies.

Quality control EPA developed and implemented a Quality Assurance Project Plan (QAPP) prior to the conduct of any data collection. In addition, EPA sent a Quality Assurance Team to perform a Technical Systems Review at each beach to assure the plan was being adhered to and also to review procedures carried out at the beach. The QAPP for survey data collection (Appendix B) and water collection and testing (Appendix D) are attached. At each site, prior to initiating data collection, EPA investigators conducted a "dry run" to review and correct procedures with study staff. For this exercise, water samples were collected, delivered to the local laboratory, processed and stored. Sample labeling and identification procedures were reviewed, as was proper ancillary data collection procedures. Each study day, an EPA employee, trained in the goals of the study, questionnaire administration ,water sample collection, ancillary data collection and water sample processing was available on site to answer questions from study staff, the study participants, and to handle other inquiries. On site EPA staff also ensured ancillary data and water samples were properly collected, and water samples were properly processed and stored during the conduct of the study.

Illness definitions

We considered the following health endpoints consistent with those we previously reported[2, 3].

"Gastrointestinal illness" (GI illness) was defined as any of the following: (1) diarrhea (three or more loose stools in a 24-hour period); (2) vomiting; (3) nausea and stomachache; 4 nausea or stomachache, and interference with regular activities (missed regular activities as a result of the illness).

"Upper respiratory illness" (URI) was defined as any 2 of the following: sore throat, cough, runny nose, cold, or fever.

"Rash" was defined as a rash or itchy skin.

"Eye irritations" were defined as either eye infection or watery eye.

"Earache" was defined as earache, ear infection, or runny ears.

Diarrhea was also considered as a stand alone outcome because it is a commonly used definition of gastroenteritis in population-based surveillance [35, 36].

Participants ill within 3 days before their beach visit were excluded from analysis of the health outcome related to their baseline symptoms.

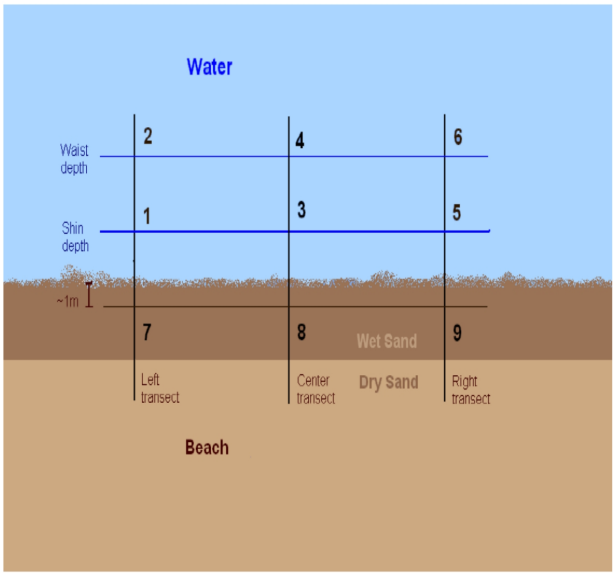
3.3 Water sample and ancillary data collection

3.3.1 Sampling locations

The goal of water sample collection and testing with regard to the epidemiological study was to characterize the water quality to which swimmers were

exposed. The approach to sample collection was the same as in previous years of the study. Three sampling transects were identified which encompassed the majority of the beach site. At each of the three sampling transects, samples were collected at 8:00 AM, 11:00 AM and 3:00 PM at two depths, "shin" (0.3 meters) and waist (1 meters). On a given study day a total of 18 samples were collected (when all samples could be collected successfully). Figure 3.1 illustrates the water sampling scheme.

Figure 3.1: Water sample locations



3.3.2 Composite sampling

In addition to standard "beach" samples, in 2009 composite samples were collected. Each study day an additional nine composite samples were collected as described below:

- 300 mL portions from the 3 bottles collected at points 1, 3, and 5 were combined to form a shin composite samples.
- 300 mL portions from the 3 bottles collected at points 2, 4, and 6 were combined to form a waist composite samples.
- 150 mL from each bottle collected at points 1-6 was combined to form a total composite samples.

Results may be informative in assessing how well compositing samples compares to individual sample results and establishing monitoring protocols. How-

ever, in this report averages of individual samples were used to establish health effects associations and for descriptive statistics.

3.3.3 Additional samples

Additional samples were collected during the epidemiological study but were not directly related to the goals of the current study and report, and as a result, the most of the results and interpretation of the results relating to these samples will be reported elsewhere.

- "Contaminated" sites. Four additional samples each day were collected near near the source of runoff and treated sewage effluent. These samples were collected primarily to inform modeling as part of a related but separate effort led by EPA-NERL in Athens.
- Sand samples. Sand samples were collected at three sites at 8:00 AM. These samples were tested and analyzed for indicators of fecal contamination.
- Cyanobacteria samples. Samples were collected from the waist depth location at each of three transects (sampling points 2, 4, and 6, see Figure 3.1) at the 11:00 AM sampling time at the tropical beach site only.

3.3.4 Sample collection and processing

All water samplers were provided a detailed training by EPA and Westat prior to initiating the study.

Sampling bottles were prepared and labeled using a unique identifier prior to the sampling time (see Appendix D) for detailed discussion). Sampling transects were identified using fixed landmarks and GPS coordinates.

Water samples were collected according to establish protocols [37]. Samples were collected in waist-high (1 m deep) and shin-high (0.3 m deep) water by serially immersing (2) capped 1000-mL presterilized, polypropylene bottles to the appropriate sample depth, removing the lids and allowing them to fill, raising them out of the water, and emptying them slightly to allow approximately 1 inch of head space before replacing the lids. Samples were taken about 1 foot (0.3 m) under the surface of the water in waist-high water, and shin-high samples were taken 6 inches (0.15 m) above the bottom of the water. The samples collected near the bottom were taken with care so as not to introduce additional sand/solids/debris into the samples. GPS coordinates were recorded at the location of each sample.

Following collection, all samples were placed in coolers and maintained on ice during transport and at 1 - 4℃during the time interval before they were analyzed or shipped.

3.3.5 Ancillary data collection

The measurements shown in Table 3.1 were collected at each sampling time (8:00 AM, 11:00 AM, and 3:00 PM). The measures collected included date, time, geographical position (latitude and longitude) air temperature, water temperature, cloud cover, ultra violet radiation (UV), rainfall, wind speed, wind direction, water current direction, wave height, bather density, number of boats, number of animals/birds, debris presence, pH, and turbidity. Additional detail is provided in the Quality Assurance Project Plan (Appendix B).

Table 3.1: Ancillary Measurements Recorded at each Sampling

Measurement	**Description**	**Units/Format**	**Comments**
GPS Measurements	Garmin GPS 76 device	Degrees W and Degrees N	± 3 meters
Air Temperature	Thermometer at fixed location	Celsius	
Water Temperature	Thermometer at center waist and shin transect depth	Celsius	
Cloud Cover	Per-cent cloud cover	S (0%), MS (20-50%), C (50-70%), MC (70-99%), O	Field Team Consensus
Rainfall	Rain gauge and weather station. Supplemented with NOAA data	Inches	
Wind Speed	Wind gauge	Miles per hour	
Wind Direction	Compass direction measured on wind gauge	N, NE, E, SE, S, SW, W, NW	Field Team Consensus
Current Wind Direction	Described in relation to shoreline facing out	Descriptive (onshore, right, etc.)	Field Team Consensus
Wave Height	Meter stick measurement at central sampling point	Meters	± 2 meters
Bather Density	Count of bathers in the water	<20, 20-100, 100-200, >200	Field Team Consensus
Boats	Count of boats in the water, 500 M of sampling area	None, 1-5, 5-10, 10-20, 20-30, >30	Field Team Consensus
Animals	Animals 20 M of the sampling area	Description and count of each animal type	Field Team Consensus
Debris	Description debris in water	None, Very Little, Little, Lots with description	Field Team Consensus
pH	Each sample measured after processing [37]	pH units	± 0.3
Turbidity	Each sample measured after processing [37]	Nephlometric Turbidity Units (NTUs)	Range dependent see Standard Methods 2130B
Salinity and Conductivity	Each sample measured after processing [37]	parts per thousand (salinity); microSiemens or milliSiemens (Conductivity)	
UV Reading	Hand-held UV Device	$\mu W/cm^2$	$\pm 1 \mu W/cm^2$
Tide	NOAA http://tidesandcurrents.noaa.gov/tides09/	7-point scale	1=high tide, 7=low tide

3.4 Water sample analysis

Water samples were analyzed for the following indicators of fecal contamination:

- *Enterococcus* by EPA Method 1600 [38]
- *Enterococcus* by quantitative polymerase chain reaction (qPCR) [16, 17]
- *Bacteroidales* by qPCR [17]

Water samples were analyzed for *Enterococcus* by Method 1600 using 100, 10, and 1 ml volumes. Results were converted to CFU per 100 ml equivalents. The best count was selected according to a standard operating procedure shown in Appendix C. Analysis of samples by Method 1600 was begun within 6 hours of collection, and the filtration and plating was completed within 8 hours of collection. Samples were filtered through 0.4 μm polycarbonate membrane filters for qPCR analysis within 6 hours of collection, and the QPCR filters were frozen at a minimum of -20 ℃ and sent on dry ice by overnight express to EMSL Analytical, Inc. (Westmont, New Jersey) for *Enterococcus* and *Bacteroidales* quantification. See Appendix D for discussion of quality control measures.

No additional samples were collected for the determination of pH and turbidity. These measurements were made from the same samples used for membrane filtration after these analyses were completed to prevent contamination.

An additional set of filters were sent to USEPA NERL laboratories in Cincinnati where additional qPCR analyses were conducted for *Clostridia* spp. [39] and *E. coli* (unpublished) and human specific *Bacteroidales* markers. Testing and analyses for these indicators were prioritized separately since health relationships in fresh or marine waters have not been previously established.

3.4.1 Quantitative polymerase chain reaction

The details of the qPCR assay used in this study including primer and probe sequences have been previously described [16, 17, 40]. In brief, the filter samples were extracted to recover total DNA and the DNA extracts were subjected to qPCR analysis by the basic procedures described previously [16]. Briefly, cells were suspended from the filters and lysed in a bead mill (BioSpec, Bartlesville, OK) for 60 seconds at maximum speed and the debris were removed by centrifugation. The published DNA extraction procedure was modified slightly by increasing the total volume of extraction buffer, containing 0.2 $\mu g/mL$ salmon sperm DNA (Sigma, St. Louis, MO) in AE buffer (Qiagen,Valencia, CA) from 0.3 ml to 0.6 ml and decreasing the dilution of recovered supernatants prior to analysis from 10-fold to 5-fold.

Polymerase chain reaction (PCR) amplification of a specific DNA sequence was carried out using the TaqMan PCR product detection system. The reactions were performed in a thermal cycling instrument (Smart-Cycler System, Cepheid, Sunnyvale, CA) that automated the detection and quantitative measurement of the fluorescent signals produced by probe degradation during each cycle of amplification.

Salmon testes DNA was added to the extraction buffer as a source of reference target sequences to estimate the relative efficiency of total DNA recovery from the water sample filters compared to the calibrator samples. Cycle Threshold (CT) values from the qPCR assay for these sequences were also used to identify potential PCR inhibition caused by the water filter extracts [41]. Five-fold dilutions of the water filter and calibration sample extracts were routinely analyzed and water filter extracts giving salmon DNA assay CT values that were > 3 CT units higher than the mean values from the calibration extracts were reanalyzed after additional 5-fold dilutions. Salmon DNA assays were performed in separate reaction tubes.

Calibrator samples (six replicates), consisting of clean polycarbonate filters amended with known cell quantities of *Enterococcus faecalis* (ATCC# 29212) and *Bacteroides thetaiotaomicron* (ATCC# 29741), and negative control samples (six replicates), consisting of clean filters only, were extracted in the same manner with each batch of test samples. Cells used in the calibrator samples originated from laboratory grown cultures and were enumerated as previously described [16, 17].

Estimation of Calibrator Cell Equivalents

Estimates of qPCR Calibrator Cell Equivalents (CCE) were based on the Comparative Cycle Threshold Method [42], consistent with previous publications [3, 2, 16, 17, 40] qPCR Calibrator Cell Equivalents (CCE) were determined using only test sample and average batch calibration sample target organism assay CT values ("delta-CT") [17] and also after corrections using CT values from the salmon reference assays ("delta delta-CT") [3, 2, 16, 17].

The delta-delta CT computational approach is derived from the comparative cycle threshold (CT) method [42]. This approach employs an arithmetic formula to determine the ratio of target sequence quantities in DNA extracts from test sample filters relative to those in similarly-prepared DNA extracts from calibrator sample filters containing a known quantity of target organism cells based on the difference in CT values obtained from qPCR analyses of these samples. Similar comparisons of CT values from qPCR assays for an exogenous target sequence from salmon sperm DNA, added in equal quantities to both the test and calibrator sample filters before DNA extraction, were used both as a reference to normalize results for differences in the amount of total DNA recovered from each sample (e.g., caused by test sample effects on DNA recovery) and as a sample processing control (SPC) to signal potentially non-quantifiable test sample results caused by PCR inhibition or low DNA recoveries[16]. The calculation can be expressed by the following equations:

$$CT_{\Delta,\Delta} = \Delta C_{T,target} - \Delta C_{T,ref} \tag{3.1}$$

and

$$CCE_{\Delta,\Delta} = N_{calibrator} \times A^{-CT_{\Delta,\Delta}} \tag{3.2}$$

where:

- $\Delta C_{T,target}$ is the difference between the CT from the sample target (e.g., *Enterococcus* and the average CT of the batch calibrator
- $\Delta C_{T,ref}$ is the corresponding difference for the salmon sperm reference sequence
- $N_{calibrator}$ is the known number of cells in the calibrator sample
- A is the amplification factor for the assay.

Ideally A=2 but typically it is in the range 1.9 2.0 with values less than 2 resulting from less than 100% replication of the target sequence at each cycle. In practice, A is either assumed to be 2 or is estimated based on the slope of a standard curve[42]. For both the *Enterococcus* and *Bacteroidales* assays, values for A were assumed to be 2 because this value was within the 95% confidence intervals of the slope values obtained by the laboratory from repeated qPCR analyses of serially diluted genomic DNA standards.

For the delta-CT calculation, the $\Delta C_{T,ref}$ above is excluded from the calculation and the salmon assay is used as a pass-fail control.

See previous manuscripts [17, 16, 40] for a more detalied discussion and description of these calculations. The delta delta-CT calculation provides quantitative adjustment for partial inhibition [40, 16], but there is some evidence that the salmon reference assay may over correct the CCE quantitation due to a higher sensitivity to matrix inhibitory effects whereas delta-CT may lead to underestimations [17]. Therefore, both calculation methods were used to determine whether health effects associations substantially affected by the calculation approach used. For both approaches, if samples failed the salmon CT criterion described above even after 5-fold dilution, the sample was considered potentially significantly inhibitory [40] and results were replaced with mean of valid samples collected at the same location, depth and time.

The lower detection limit was defined as the upper 95% CT bound of the pooled standard curve data that was generated from repeated analyses of serially diluted genomic DNA extracts from the calibrator bacterial strains during the study period. Target sequence concentrations in these genomic DNA extracts were determined as previously described [40]. CT values were restricted at this upper bound for all CCE calculations. Unless otherwise indicated, one-half the calculated CCE was used for non-detects where there was no detection after 45 cycles. Previously, we compared several different approaches to assign values for these samples [3] and demonstrated the associations with illness were not strongly affected by the approach used. We again used several approaches for these values including using one-half the estimated detection limit, a maximum-likelihood estimate, and a regression on order statistics estimate. The purpose for applying several approaches was to determine to what extent, if any, the approach used to estimate results below the limit of detection affected the interpretation of the results. Results are reported in qPCR CCE per 100 ml.

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3.5 Data analysis

An overview of data management and analysis is shown in Figure 3.2. Stata version 10.1 [43] was used for data management and regression modeling and R version 11.1 [44] was used for graphics and preparation of tables and summary statistics.

3.5.1 Data management, quality and data cleaning

Prior to initiating any analyses, all variables were tabulated, recoded as necessary and examined for missing values and non-response. When possible, additional information was obtained to reduce or eliminate missing values, or to verify and correct values suspected to be incorrect. Duplicates and potential repeated enrollees within the 28 day time window were identified by reviewing duplicated names, birth dates and addresses within the 28 day time frame. When duplicates were identified, only the first response was retained.

Electronic summaries of water quality results were received approximately on a weekly basis. Upon completion of the study, databases summarizing water quality results were prepared by the contractor (Westat, Inc.). These final draft databases were reviewed for missing data, duplicate, accuracy and completeness. Outlying observations, such as excessively high counts, missing data, high or low CT values, were checked against the original reports and corrected where necessary. For qPCR results, all counts were recalculated using the approaches described in Section 3.4.1.

Water quality measures were reduce and summarized in order to assign exposures to swimmers. Several exposure indices were evaluated, including the overall daily average, averages based on time, sample location and depth as well as averages specific to an individual swimmer's reported swimming location and time of exposure. Once water quality data bases were checked and cleaned, summary exposure indices were created and merged to the health/survey database by date of interview, and/or sampling time and/or sample/swimming location.

Referring to Figure 3.1 for the sample location layout, swimming locations were designed to coincide with samples collected from each transect. Subjects were asked where they spent most of the time swimming. Those reporting swimming mostly in location 1 would be assigned samples 1 and 3 (swimming location 1), those in location 2 assigned samples 2 and 4 (swimming location 1) and those in location 3 assigned samples 3 and 6 (swimming location 3).

Environmental, ancillary and weather station data were summarized to allow merging and combining with the health data file. Precipitation from on-site weather stations were combined to represent rainfall on the current day, the previous 24 hours (1-day lag), and the previous 24-48 hours (2-day lag). For measures such as numbers of bathers on the beach, boats in the water and

animals in the water, single daily summaries were created using the average of the three observations. The weather-station data were combined with the observation data described in Table 3.1 to create a single database with one observation per day which could then be merged, by date, to the health data file.

3.5.2 Questionnaire data

Univariate frequency tables were created for most variables in the surveys (in the interest of space all tables are not included in this report). The mean, median, standard deviation and range were determined for continuous variables such as age. Continuous variables were also categorized based on quartiles, or, in the case of age, in specific categories of interest. Race was reduced into a single variable, which included Hispanic/Latino ethnicity as a category because many respondents refused to report a race after indicating they were Hispanic/Latino. Respondents reporting "yes" to more than one race category were categorized as multiethnic.

Bivariate tabulations were conducted for most exposures, covariates and health outcomes. Bivariate tabulations were examined for significant or strong relationships between outcomes, exposures and covariates. Chi-square tests were conducted to evaluate the association between categorical variables, or when expected cell counts were few (<5), Fischer's Exact Tests were conducted.

3.5.3 Water quality data

Descriptive statistics and graphical summaries (e.g., box-plots) were used to describe and evaluate the distribution of water quality measures. Boxplots were drawn according to the default settings in R [44], where the lower and upper boundaries of the box indicate the first and third quartiles respectively, and line within the box indicates the median. The lines or "whiskers" show the largest observations that fall within 1.5 times the box size from the first or third quartle. Points beyond these values ("outliers") are represented as dots beyond the upper and lower lines. Water quality measures were log-transformed to reduce the strong right-skew present in the raw counts. Regression models were used to assess the variability in the distribution of water quality measures as a function of beach, collection time, sample location and sample depth.

3.5.4 Environmental and ancillary data

Principal components analysis is a useful way to reduce a group of highly correlated measures into a few components that represent the important features of the original group of variables. Numerous meteorological and other environmental factors were collected each day at each beach. These factors may be important determinants of both water quality as well as predictors of health effects, and therefore may also be important to consider in models designed to examine the association between water quality and health. Because numerous

measures were collected, principal components analysis was used to reduce and summarize these environmental measures. The two principal components that accounted for the majority of the variability in these measures were then used as covariates in regression models. Measures included in the principal components analyses were: tide stage, precipitation, ultraviolet intensity, wave height, wind speed, wind direction, cloud cover, numbers of bathers in the water, numbers of boats in the water, air and water temperature. For Boquerón Beach the first two components explained 40% of the total variation and were characterized by measures relating to beach population (air temperature, bathers, boats) and measures relating to water conditions (tide stage, wave height and wind direction), respectively. For Surfside Beach the first two components explained 31% of the total variation and were characterized by tide and precipitation, and measures of sunlight and temperature (cloud cover, UV, temperature, bathers), respectively. Examples include bather density, 24-hour rainfall, air temperature, water temperature, wind direction and speed, wave height, and presence of birds and animals on the beach.

3.5.5 Associations between water quality and illness

Regression models were the primary method used to determine the strength and the significance of the relationship between the indicator measures and health effects. The statistical analysis focused on describing and quantifying the relationship between estimates of fecal indicator organisms and the risk of illness among swimmers. It was expected that the risk of illness among swimmers should increase with increasing exposure to fecal indicator organisms. Logistic regression models were used to quantify and describe this relationship. The outcome was a binary indicator of a health endpoint and the primary predictor variable was the density of the fecal indicator organisms. The swimmer only model is described as follows:

$$log\left(\frac{p}{(1-p)}\right) = \alpha + \beta_1 X_1 + \beta_i X_i \ldots + \beta_j X_j \tag{3.3}$$

where p is the probability of illness, X_1 is the $\log_{10}$ transformed water quality density, and $X_i \ldots X_j$ are covariates included to reduce potential bias in the association between water quality and illness.

Robust estimates of variance were used to account for the non independence of observations within households [45, 46, 47, 48]. Covariates which could affect the relationship between exposure to varying degrees of water quality and illness, or those which were potentially strongly associated with the health outcomes were considered for inclusion in regression models. These included age, sex, race, contact with animals, contact with other persons with diarrhea, number of other visits to the beach, any other chronic illnesses (GI, skin, asthma), digging in sand, and consumption of raw or undercooked meat. For URI, rash, and eye symptoms, use of insect repellent and sun block were also included. We accounted environmental measures by including the two principal components described above (see Section 3.5.4) as covariates in regression models. To avoid

missing data on days when one or more of these measures was not available, we used best-subset regression to impute the missing principal components [49]. For graphical presentations, adjusted probabilities of illness were predicted from logistic regression models holding covariates constant at their mean value.

If an association between indicator density and illness was observed among swimmers, a regression model incorporating non-swimmers was fit as described below:

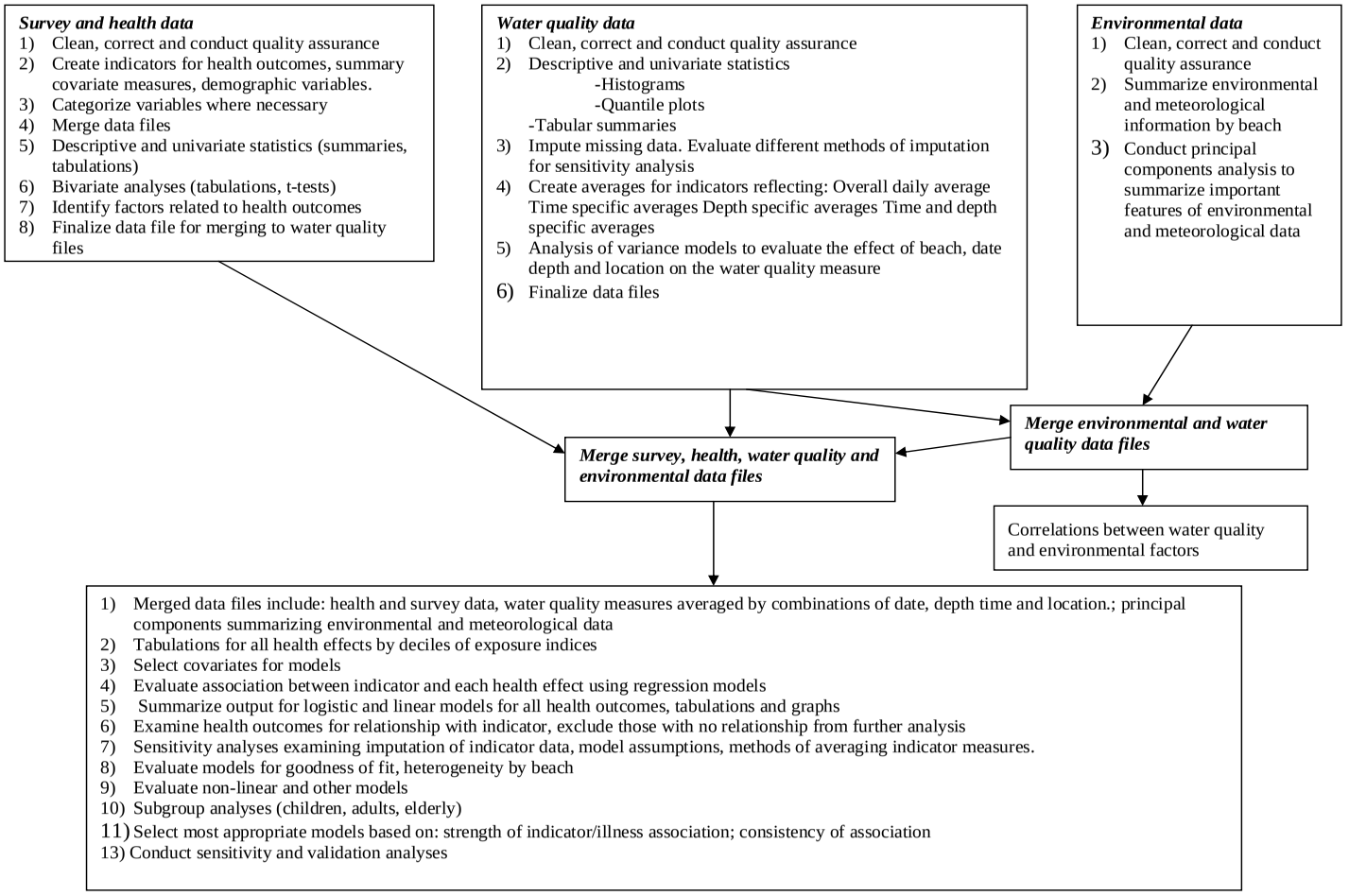
$$log\left(\frac{p}{(1-p)}\right) = \alpha + \beta_1 X_1 X_2 + \beta_2 X_2 + \beta_i X_i \ldots + \beta_j X_j \qquad (3.4)$$

Where X_1 is the log-transformed indicator density, X_2 is a 1/0 indicator of swimming status and $X_i \ldots X_j$ are covariates. This model allows a comparison of risks among swimmers compared to non-swimmers [18]. The combination: $\exp(\beta_1 + \beta_2)$ represents the ratio of the risk of illness (in terms of odds) among swimmers at 1-log exposure compared to the risk of illness among non-swimmers.

For each analysis, the set of covariates was reduced through a change-in-estimate procedure [50], where the exposure of interest was the regression coefficient for fecal indicator organisms density. A criterion of a 5% change in the coefficient was used. The selection procedure generally reduced the number of covariates to fewer than five. When data were sufficient (at least 30 cases of illness among swimmers), we conducted separate analysis for the age categories 0 to 10 years, 11 to 54 years, and 55 years and older. The age groups were selected consistent with our previous report [3]. The grouping of children 10 and under is consistent with the youngest three age groups recently recommended by the US EPA [51].

In addition to considering indicator exposure as a continuous measure, categorical variables were also created. For this analysis, indicator exposure was classified into 3 or 4 categories based on tertiles or quartiles of exposure. Comparisons were made against swimmers in the lowest exposed quartile as well as against non-swimmers. For *Enterococcus* CFU measured by EPA Method 1600, categorical comparisons were made using the currently recommended criteria for marine waters (geometric mean of 35 CFU per 100 ml) [52].

Figure 3.2: Data processing, management and analysis



Chapter 4

Results

4.1 Surfside Beach

4.1.1 Final site selection

Percent urban land use (determined using 2001 National Land Cover Data, http://www.epa.gov/mrlc/nlcd-2001.html) and percent sample exceedance based on previous three years of data for the five beaches selected for additional monitoring are shown in Table 4.1. Each of these sites were determined to meet the criteria of a runoff beach described in Section 3.1.1. In Table 4.1, the headings "Rainfall" and "Baseline" refer to the range of results from additional monitoring at the five sites conducted during the fall and winter of 2008. Rainfall samples were collected 12 hours following 0.25 inches of rainfall. Additional details of the monitoring program are provided in Appendix F.

Increased densities of *Enterococcus* were observed following rain events at several of the selected beaches, indicating the likely influence of the runoff discharges on the beach sites. The sites were further evaluated to assess the logis-

Table 4.1: Land Use, *Enterococcus* Historical Exceedance and Additional Monitoring for Urban Runoff Beach Sites

Beach site	**% Urbanized**	**% Exceedance**[1]	**Baseline**[2]	**Rainfall**[3]
Surfside Beach	89%	45%	23-27	285-346
Canes Patch Beach	72%	52%	147-177	112-150
Withers Swash	58%	54%	56-79	400-446
Florida Shores	78%	15%	16-26	21-24
Silver Beach	94%	13%	10-14	3-13

1: Percent samples exceeding recreational water quality criteria

2: Range of *Enterococcus* colony forming units per 100 ml during non-rainfall events

3: Range of *Enterococcus* colony forming units per 100 ml following >0.25 inches of rainfall in previous 12 hours

tical feasibility of conducting an epidemiology study. Critical factors were the size of the beach going population, cooperation from local officials and the availability of parking. Ultimately, based on these criteria, Surfside Beach, South Carolina, south of Myrtle Beach was selected for the runoff beach site.

4.1.2 Site description

The selected beach site, Surfside Beach, South Carolina, is located south of Myrtle Beach (Figure 4.1). The beach is directly affected by "swashes" (narrow channels of water) which receive runoff and discharge directly to the beach.

The Town of Surfside Beach, South Carolina is located in Horry County and contains 2 square miles of land. It is located on the southern side of Myrtle Beach within the same county. The beach is approximately 2 miles in length and has many public use access points. There are 12 metered street parking locations and 3 of them also have restrooms and shower facilities. A picnic shelter can also be found at the 3rd Avenue South beach access. Lifeguards are located at 10 street locations. Beach regulations prohibit alcohol year round and no animal access May 15 through September 15 making this a "family friendly" beach. Surfside is in a region with a temperate climate. Average precipitation for May-September is 5.66 inches. Day time temperatures range from 82°F to 91°F (July).

There is minimal hotel commercialization on the ocean front with only two hotel chains that accommodate 157 rooms and 133 rooms each. A variety of other stores include beachwear retail, restaurants, and watersport activities. Additionally, there is a pier on Main Street with multiple types of access. There is paid access requiring a payment of $ 1 per person for walking only. There is also pier fishing access for $ 4-12.50 daily

The beach is sandy (fine sand), gently sloping and open to the ocean. Approximate wave height noted during the study was about 3 feet, becoming larger and rough during storms. The beach is well-attended though generally not as crowded as nearby Myrtle Beach. Typical activities beach-goers engage in include swimming, playing on the beach, occasional surfing and boogie boarding.

It was the conclusion of a report commissioned by the South Carolina Department of Health and Environmental Control (SCDHEC) that many of the beaches in the region are adversely impacted by swashes and runoff. While this report did not address Surfside Beach specifically, it described general concerns regarding runoff impacts. The following is an excerpt from this report [53]:

> A Beach Monitoring workgroup, consisting of Department personnel and coastal municipal and county leaders, was initiated in response to concerns regarding stormwater inputs in South Carolina's surf zone. The consensus of the workgroup was that a voluntary baseline surf water quality project should be conducted to evaluate whether South Carolina needs to implement an ocean beach bacteria sampling program. Results of the study indicated that stormwater inflows via swashes and drain pipes are responsible for the observed

high levels of bacteria in surf during wet weather. Recommendations from the workgroup include the following: Do not swim or allow children to play in swashes or stormwater. In areas with swashes or stormwater outfalls, do not swim in the ocean during rainfall. Educate and advise the public about the health risks of swimming. Maintain a state/local partnership to regularly monitor surf in areas with beach stormwater discharges during swimming season. Reduce bacteria inputs to surface waters from residences and parks. Prevent and control sources of pathogens to beaches from stormwater discharges and nonpoint sources. The findings of the workgroup support the posting of permanent signs at specific beach swashes and storm drain outfalls. A voluntary surf water quality monitoring program, with SCDHEC oversight and supported by local coastal municipalities and counties, continues.

The beach site and sampling locations are shown in Figures 4.2 and 4.3.

Figure 4.1: Surfside Beach, South Carolina

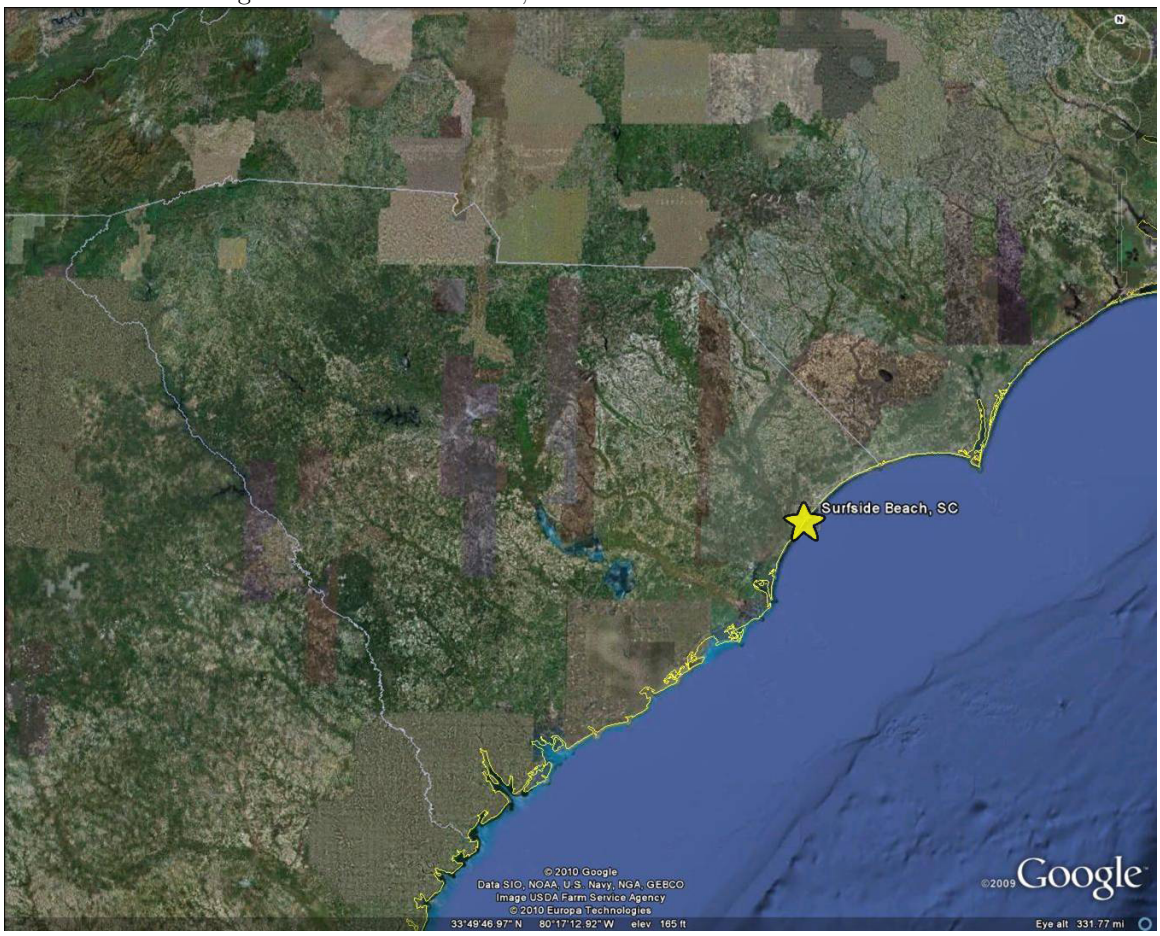


Figure 4.2: Surfside Beach, South Carolina. Swash and Contaminated Site Sample Locations

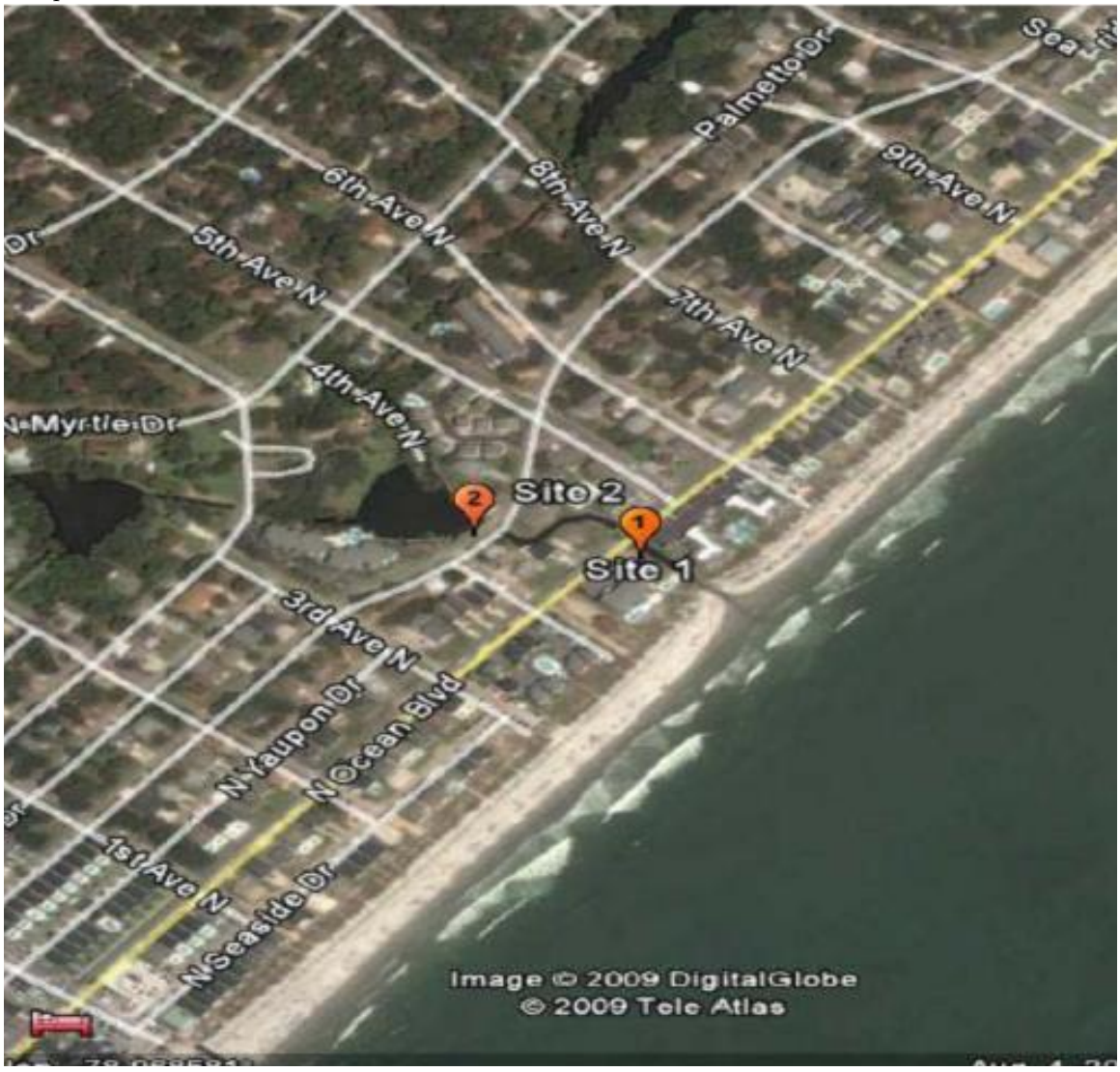


Figure 4.3: Surfside Beach, South Carolina. Swash and Beach Site Sample Locations

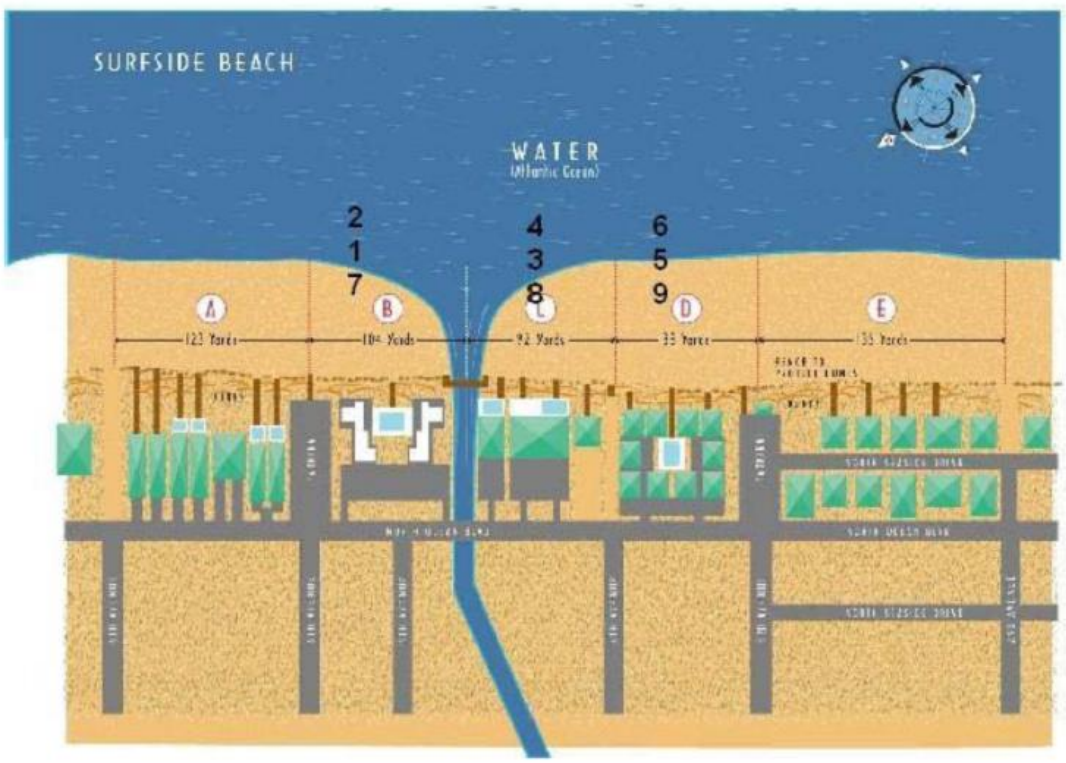


Figure 7: Schematic of Surfside Beach

4.1.3 Health survey and respondent characteristics

Enrollment

The health surveys and interviews began in Surfside Beach on June 7, 2009 and concluded on September 7, 2009. The study was conducted on a total of 29 days. A total of 14,970 individuals from 8161 households were offered enrollment. Of these, 1,097 households were ineligible because they either completed the study within the previous 28 days or there was no adult 18 years of age or older. Of those eligible, a total of 12,553 individuals from 5,835 households agreed to participate and completed the first interview. 11,675 individuals (93%) from 5,436 households (93%) returned to complete the second interview as they were leaving the beach for the day.

After accounting for probable duplicates, ineligible observations, and those who did not complete the final telephone interview, the final dataset consisted of a total of 11,159 individuals from 5205 households. This represented 64% of those households initially approached and 95% of those completing the beach interview.

Note that in the following descriptive tables, any deviation of the total from 11,159 is due to missing responses, except for tables for incident illness where respondents with baseline symptoms are also excluded.

Respondent characteristics and demographics

Basic demographic characteristics of the enrolled subjects are shown in Table 4.2. The study population was predominantly white. There were slightly more females than males, and approximately 12% were children age 11 or under.

Baseline health conditions and illnesses are shown in Table 4.3. The most frequently reported chronic health condition was allergies, reported by 16% of subjects, followed by chronic skin conditions and asthma, reported by approximately 5%. Other health conditions and symptoms in the previous 24 hours were reported infrequently.

Table 4.2: Basic demographics, Surfside Beach

	N	%
Days of Study		
Total	29	100.00
Interviews		
Total	11159	100.00
Sex		
Male	4953	44.57
Female	6159	55.43
Total	11112	100.00
Age		
0-4	647	5.88
5-11	1291	11.73
12-19	1401	12.73
20-34	2304	20.93
35 and over	5365	48.74
Total	11008	100.00
Race		
White	10513	94.48
Black	235	2.11
Asian	51	0.46
Am. Indian	17	0.15
Hispanic	253	2.27
Multi-racial	24	0.22
Other	34	0.31
Total	11127	100.00
Annual Visits to Beach		
1-5	4280	38.36
6-10	2472	22.16
Over 10	4405	39.48
Total	11157	100.00

Table 4.3: Baseline illness and other health conditions, Surfside Beach

	N	%
Chronic GI illness		
No	10871	97.42
Yes	288	2.58
Total	11159	100.00
Allergies		
No	9291	83.26
Yes	1868	16.74
Total	11159	100.00
Asthma		
No	10624	95.21
Yes	534	4.79
Total	11158	100.00
Chronic skin condition		
No	10602	95.01
Yes	557	4.99
Total	11159	100.00
GI symptoms in past 3 days		
No	10957	98.20
Yes	201	1.80
Total	11158	100.00
Vomiting in past 3 days		
No	11084	99.33
Yes	75	0.67
Total	11159	100.00
Sore throat in past 3 days		
No	10739	96.24
Yes	420	3.76
Total	11159	100.00
Skin rash in past 3 days		
No	10899	97.67
Yes	260	2.33
Total	11159	100.00
Earache in past 3 days		
No	11027	98.82
Yes	132	1.18
Total	11159	100.00
Eye infection in past 3 days		
No	11123	99.68
Yes	36	0.32
Total	11159	100.00

4.1.4 Swimming exposure

Swimming and related exposures are shown in Table 4.4. Compared to our previous studies at freshwater sites in the Great Lakes [2, 3], a high proportion of subjects reported swimming exposure. Over 80% of subjects had at least some exposure to water, over 70% immersed their body and nearly 60% immersed their head.

Factors associated with body immersion and head immersion swimming exposure are shown in Table 4.5 and 4.6, respectively. Swimming exposure was associated with younger age, and was highest among those 5-10 years of age among whom 80% immersed their head in the water. Among those age 55 and older, only 45% immersed their head. Other factors associated with head immersion swimming exposure were male gender and unknown animal contact. Those with chronic GI illness and asthma were slightly less likely to immerse their head than those without these conditions. Swimming exposure was also associated with less frequent visits to Surfside Beach.

Table 4.4: Swimming and related exposures, Surfside Beach

	N	%
Any contact with water		
No	1748	15.74
Yes	9358	84.26
Total	11106	100.00
Body immerison in water		
No	3032	27.30
Yes	8073	72.70
Total	11105	100.00
Head immersion in water		
No	4700	42.32
Yes	6406	57.68
Total	11106	100.00
Swallowed water		
No	8979	81.02
Yes	2103	18.98
Total	11082	100.00
Swam 1 week before beach visit		
No	6081	54.50
Yes	5076	45.50
Total	11157	100.00
Swam after beach visit		
No	3974	35.80
Yes	7128	64.20
Total	11102	100.00
Dug in sand		
No	7115	64.06
Yes	3991	35.94
Total	11106	100.00
Body buried in sand		
No	10665	96.03
Yes	441	3.97
Total	11106	100.00

Table 4.5: Factors associated with swimming exposure (body immersion), Surfside Beach

	Non-swimmer		Waders		Swimmer		P-value[1]
	N	%[2]	N	%[2]	N	%[2]	
Age category							
0-4	73	11.37	86	13.40	483	75.23	
5-10	26	2.02	51	3.97	1209	94.01	
11-19	127	9.12	75	5.39	1190	85.49	
20-54	342	14.91	223	9.73	1728	75.36	
55 and over	1157	21.66	825	15.45	3359	62.89	<0.001
Sex							
Male	676	13.72	385	7.82	3865	78.46	
Female	1067	17.40	883	14.40	4182	68.20	<0.001
Race							
Non-white	92	15.01	59	9.62	462	75.37	
White	1653	15.80	1213	11.60	7594	72.60	0.2464
Visits to this beach							
1 or less	525	12.33	433	10.17	3299	77.50	
2-5	418	17.01	273	11.11	1766	71.88	
6 or more	805	18.34	578	13.17	3006	68.49	<0.001
Chronic GI illness							
No	1704	15.75	1244	11.50	7871	72.75	
Yes	44	15.38	40	13.99	202	70.63	0.4301
Skin condition							
No	1672	15.85	1213	11.50	7664	72.65	
Yes	76	13.67	71	12.77	409	73.56	0.3021
Asthma							
No	1678	15.87	1215	11.49	7678	72.63	
Yes	70	13.13	69	12.95	394	73.92	0.1771
Undercooked meat							
No	1419	15.50	1072	11.71	6663	72.79	
Yes	329	16.86	212	10.87	1410	72.27	0.2293
Unfamiliar animals							
No	1650	16.19	1182	11.60	7359	72.21	
Yes	98	10.72	102	11.16	714	78.12	<0.001
Others with GI illness							
No	1615	15.86	1182	11.61	7387	72.54	
Yes	133	14.44	102	11.07	686	74.48	0.4220

1: Pearson's Chi-square test of independence
2: Row percentages add to 100%

Table 4.6: Factors associated with swimming exposure (head immersion), Surfside Beach

	Non-swimmer		Waders		Swimmer		P-value[1]
	N	%[2]	N	%[2]	N	%[2]	
Age category							
0-4	73	11.37	184	28.66	385	59.97	
5-10	26	2.02	121	9.41	1139	88.57	
11-19	127	9.12	193	13.86	1072	77.01	
20-54	342	14.91	609	26.56	1342	58.53	
55 and over	1157	21.66	1806	33.81	2379	44.53	<0.001
Sex							
Male	676	13.72	874	17.74	3377	68.54	
Female	1067	17.40	2058	33.56	3007	49.04	<0.001
Race							
Non-white	92	15.01	151	24.63	370	60.36	
White	1653	15.80	2788	26.65	6020	57.55	0.3841
Visits to this beach							
1 or less	525	12.33	1051	24.68	2682	62.99	
2-5	418	17.01	654	26.62	1385	56.37	
6 or more	805	18.34	1247	28.41	2337	53.25	<0.001
Chronic GI illness							
No	1704	15.75	2852	26.36	6264	57.89	
Yes	44	15.38	100	34.97	142	49.65	0.0039
Skin condition							
No	1672	15.85	2785	26.40	6093	57.75	
Yes	76	13.67	167	30.04	313	56.29	0.1098
Asthma							
No	1678	15.87	2788	26.37	6106	57.76	
Yes	70	13.13	164	30.77	299	56.10	0.0418
Undercooked meat							
No	1419	15.50	2442	26.67	5294	57.83	
Yes	329	16.86	510	26.14	1112	57.00	0.3224
Unfamiliar animals							
No	1650	16.19	2743	26.91	5799	56.90	
Yes	98	10.72	209	22.87	607	66.41	<0.001
Others with GI illness							
No	1615	15.86	2694	26.45	5876	57.69	
Yes	133	14.44	258	28.01	530	57.55	0.3956

1: Pearson's Chi-square test of independence
2: Row percentages add to 100%

4.1.5 Health effects

Incident illness

Incident health effects are presented among subjects without reporting illness at baseline. The overall incidence of the health outcomes studied are shown in Table 4.7. As observed previously [3], GI illness was the most commonly reported illness, with approximately 6% reporting GI illness in the 10-12 days following the beach visit. Following GI illness, the most frequently reported illnesses were respiratory illness (5%) and rash (4%). Eye irritations and earaches were reported by only about 2% of subjects.

Table 4.7: Incident illness among all subjects (excluding those with baseline illness), Surfside Beach

	N	%
GI illness		
Not ill	10177	93.85
Ill	667	6.15
Total	10844	100.00
Respiratory illness		
Not ill	10183	95.32
Ill	500	4.68
Total	10683	100.00
Rash		
Not ill	10412	96.03
Ill	430	3.97
Total	10842	100.00
Eye irritations/infections		
Not ill	10864	98.17
Ill	202	1.83
Total	11066	100.00
Earache		
Not ill	10730	97.83
Ill	238	2.17
Total	10968	100.00

Factors associated with incident illness

Non-swimming risk factors associated with the health outcomes studied are shown in Tables 4.8- 4.12.

GI illness GI illness was most frequent among young children (8% among those 0-4 years) and least frequent among those 55 and over (5%). Other factors associated with GI illness were female gender, chronic GI condition, unknown animal contact and contact with other ill people (Table 4.8).

Respiratory illness Respiratory illness was most frequent among young children (8% among those 0-4 years) and least frequent among those 55 and over (4%). Other factors associated with respiratory illness were non-white race, asthma, unknown animal contact and contact with other ill people (Table 4.9).

Skin rash Skin rash was most frequent among children 5-10 years of age (6%) and least frequent among those 55 and over (3%). Other factors associated with skin rash were non-white race, infrequent visits to Surfside Beach, chronic skin conditions, unknown animal contact and contact with other ill people (Table 4.10).

Earaches Earaches were most frequent among children 5-10 years of age (4%) and least frequent among those 55 and over (2%). Other factors associated with earaches were non-white race, infrequent visits to Surfside Beach, chronic skin and GI conditions, unknown animal contact and contact with other ill people (Table 4.11).

Eye irritations Eye irritations were the only symptom which was unassociated with age. Factors associated with eye irritations were female gender, non-white race, asthma, consumption of undercooked or raw meat, and contact with other ill people (Table 4.12).

With the exception of eye irritations, all outcomes were associated with unknown animal contact and occurred more frequently among children 0-10 and least frequently among those 55 and over. Consumption of undercooked meat and raw fish were not associated with any of symptoms (with the exception of eye irritations which were associated with undercooked meat consumption).

Table 4.8: Factors associated with GI illness, Surfside Beach

	Not Ill		Ill		P-value[1]
	N	%[2]	N	%[2]	N
Age category					
0-4	574	91.84	51	8.16	
5-10	1189	94.14	74	5.86	
11-19	1285	93.86	84	6.14	
20-54	2038	92.38	168	7.62	
55 and over	4952	94.61	282	5.39	0.0012
Sex					
Male	4557	94.62	259	5.38	
Female	5574	93.20	407	6.80	0.0025
Race					
Non-white	565	95.28	28	4.72	
White	9581	93.76	638	6.24	0.1584
Visits to this beach annually					
1 or less	3894	93.99	249	6.01	
2-5	2247	93.70	151	6.30	
6 or more	4034	93.79	267	6.21	0.8803
Chronic GI illness					
No	9952	94.06	628	5.94	
Yes	225	85.23	39	14.77	<0.001
Chronic skin condition					
No	9675	93.88	631	6.12	
Yes	502	93.31	36	6.69	0.6576
Asthma					
No	9705	93.96	624	6.04	
Yes	471	91.63	43	8.37	0.0407
Raw or undercooked meat					
No	8418	94.01	536	5.99	
Yes	1759	93.07	131	6.93	0.1333
Raw fish					
No	9489	93.95	611	6.05	
Yes	688	92.47	56	7.53	0.1237
Contact with unfamiliar animals					
No	9375	94.15	582	5.85	
Yes	802	90.42	85	9.58	<0.001
Others with GI illness					
No	9442	94.43	557	5.57	
Yes	735	86.98	110	13.02	<0.001

1: Pearson's Chi-square test of independence
2: Row percentages add to 100%

Table 4.9: Factors associated with respiratory illness, Surfside Beach

	Not Ill		Ill		P-value[1]
	N	%[2]	N	%[2]	
Age category					
0-4	563	92.45	46	7.55	
5-10	1161	95.09	60	4.91	
11-19	1281	95.74	57	4.26	
20-54	2061	94.11	129	5.89	
55 and over	4971	96.00	207	4.00	<0.001
Sex					
Male	4564	95.64	208	4.36	
Female	5572	95.02	292	4.98	0.1448
Race					
Non-white	541	92.96	41	7.04	
White	9610	95.44	459	4.56	0.0079
Visits to this beach annually					
1 or less	3927	95.78	173	4.22	
2-5	2254	94.55	130	5.45	
6 or more	4000	95.31	197	4.69	0.0764
Chronic GI illness					
No	9934	95.38	481	4.62	
Yes	249	92.91	19	7.09	0.0810
Chronic skin condition					
No	9684	95.36	471	4.64	
Yes	499	94.51	29	5.49	0.4234
Asthma					
No	9711	95.42	466	4.58	
Yes	471	93.27	34	6.73	0.0333
Raw or undercooked meat					
No	8404	95.40	405	4.60	
Yes	1779	94.93	95	5.07	0.4134
Raw fish					
No	9479	95.36	461	4.64	
Yes	704	94.75	39	5.25	0.5024
Contact with unfamiliar animals					
No	9380	95.60	432	4.40	
Yes	803	92.19	68	7.81	<0.001
Others with GI illness					
No	9396	95.63	429	4.37	
Yes	787	91.72	71	8.28	<0.001

1: Pearson's Chi-square test of independence
2: Row percentages add to 100%

Table 4.10: Factors associated with rash, Surfside Beach

	Not Ill		Ill		P-value[1]
	N	%[2]	N	%[2]	N
Age category					
0-4	594	95.81	26	4.19	
5-10	1167	94.19	72	5.81	
11-19	1281	94.89	69	5.11	
20-54	2138	96.05	88	3.95	
55 and over	5093	96.81	168	3.19	<0.001
Sex					
Male	4649	96.45	171	3.55	
Female	5718	95.70	257	4.30	0.0518
Race					
Non-white	556	93.92	36	6.08	
White	9826	96.16	392	3.84	0.0089
Visits to this beach annually					
1 or less	3934	94.75	218	5.25	
2-5	2344	97.54	59	2.46	
6 or more	4132	96.43	153	3.57	<0.001
Chronic GI illness					
No	10147	96.06	416	3.94	
Yes	265	94.98	14	5.02	0.4492
Chronic skin condition					
No	9955	96.24	389	3.76	
Yes	457	91.77	41	8.23	<0.001
Asthma					
No	9933	96.10	403	3.90	
Yes	479	94.66	27	5.34	0.1335
Raw or undercooked meat					
No	8600	96.07	352	3.93	
Yes	1812	95.87	78	4.13	0.7417
Raw fish					
No	9681	96.02	401	3.98	
Yes	731	96.18	29	3.82	0.9015
Contact with unfamiliar animals					
No	9605	96.42	357	3.58	
Yes	807	91.70	73	8.30	<0.001
Others with GI illness					
No	9590	96.32	366	3.68	
Yes	822	92.78	64	7.22	<0.001

1: Pearson's Chi-square test of independence
2: Row percentages add to 100%

Table 4.11: Factors associated with earache, Surfside Beach

	Not Ill		Ill		P-value[1]
	N	%[2]	N	%[2]	
Age category					
0-4	609	97.28	17	2.72	
5-10	1202	95.70	54	4.30	
11-19	1332	97.23	38	2.77	
20-54	2219	98.23	40	1.77	
55 and over	5222	98.36	87	1.64	<0.001
Sex					
Male	4757	97.88	103	2.12	
Female	5927	97.79	134	2.21	0.7948
Race					
Non-white	588	98.66	8	1.34	
White	10111	97.79	229	2.21	0.2014
Visits to this beach annually					
1 or less	4109	97.97	85	2.03	
2-5	2376	97.58	59	2.42	
6 or more	4243	97.83	94	2.17	0.5656
Chronic GI illness					
No	10459	97.89	225	2.11	
Yes	271	95.42	13	4.58	0.0089
Chronic skin condition					
No	10211	97.93	216	2.07	
Yes	519	95.93	22	4.07	0.0031
Asthma					
No	10219	97.85	225	2.15	
Yes	510	97.51	13	2.49	0.7236
Raw or undercooked meat					
No	8849	97.92	188	2.08	
Yes	1881	97.41	50	2.59	0.1911
Raw fish					
No	9980	97.80	224	2.20	
Yes	750	98.17	14	1.83	0.5926
Contact with unfamiliar animals					
No	9866	97.95	206	2.05	
Yes	864	96.43	32	3.57	0.0039
Others with GI illness					
No	9859	97.96	205	2.04	
Yes	871	96.35	33	3.65	0.0021

1: Pearson's Chi-square test of independence
2: Row percentages add to 100%

Table 4.12: Factors associated with eye infection/irritation, Surfside Beach

	Not Ill		Ill		P-value[1]
	N	%[2]	N	%[2]	
Age category					
0-4	628	99.05	6	0.95	
5-10	1258	98.51	19	1.49	
11-19	1367	98.42	22	1.58	
20-54	2231	97.81	50	2.19	
55 and over	5233	98.05	104	1.95	0.1922
Sex					
Male	4832	98.53	72	1.47	
Female	5985	97.87	130	2.13	0.0129
Race					
Non-white	584	97.01	18	2.99	
White	10248	98.24	184	1.76	0.0428
Visits to this beach annually					
1 or less	4167	98.49	64	1.51	
2-5	2394	97.79	54	2.21	
6 or more	4301	98.08	84	1.92	0.1062
Chronic GI illness					
No	10586	98.20	194	1.80	
Yes	278	97.20	8	2.80	0.3077
Chronic skin condition					
No	10327	98.19	190	1.81	
Yes	537	97.81	12	2.19	0.6287
Asthma					
No	10347	98.23	186	1.77	
Yes	516	96.99	16	3.01	0.0547
Raw or undercooked meat					
No	8965	98.33	152	1.67	
Yes	1899	97.43	50	2.57	0.0094
Raw fish					
No	10110	98.23	182	1.77	
Yes	754	97.42	20	2.58	0.1348
Contact with unfamiliar animals					
No	9971	98.22	181	1.78	
Yes	893	97.70	21	2.30	0.3249
Others with GI illness					
No	9984	98.38	164	1.62	
Yes	880	95.86	38	4.14	<0.001

1: Pearson's Chi-square test of independence
2: Row percentages add to 100%

Swimming exposure and incident illness

All subjects Adjusted Cumulative Incidence Ratios (aCIRs) comparing the risk of illness among swimmers compared to non-swimmers for body immersion and head immersion swimming exposures are shown together with the crude (unadjusted) percentages of incident illness in Tables 4.13 and 4.14, respectively. The risk for each illness group among swimmers with head immersion is also shown graphically in Figure 4.4. Skin rash was significantly elevated among swimmers who immersed their body, head or swallowed water. Earaches and GI illness were significantly elevated among swimmers who immersed their head.

Table 4.13: Incident illness by body immersion, Surfside Beach

	Number ill	%[1]	aCIR[2] (p-value)
GI			
Non-swimmer	79	4.67	
Swimmer	508	6.47	
Total	587	6.15	1.23(0.118)
Upper respiratory			
Non-swimmer	73	4.36	
Swimmer	373	4.83	
Total	446	4.74	1.07(0.6385)
Rash			
Non-swimmer	38	2.23	
Swimmer	354	4.52	
Total	392	4.11	1.61(0.0058)
Earache			
Non-swimmer	24	1.39	
Swimmer	201	2.54	
Total	225	2.33	1.5(0.0624)
Eye irritation			
Non-swimmer	27	1.56	
Swimmer	146	1.82	
Total	173	1.78	1.25(0.3228)

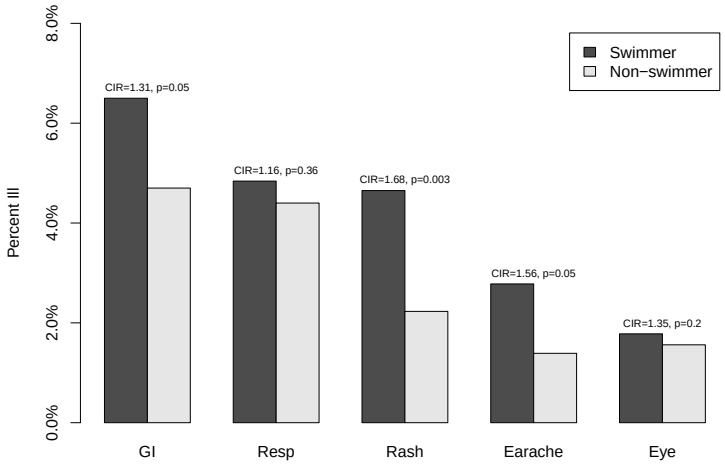
1: Percentage of those in row category with symptom (row percentage). Number and percent not ill not shown
2: Adjusted Cumulative Incidence Ratio

Table 4.14: Incident illness by head immersion, Surfside Beach

	Number ill	%[1]	aCIR[2] (p-value)
GI			
Non-swimmer	79	4.67	
Swimmer	405	6.5	
Total	484	6.11	1.31(0.0497)
Upper respiratory			
Non-swimmer	73	4.36	
Swimmer	296	4.84	
Total	369	4.73	1.16(0.358)
Rash			
Non-swimmer	38	2.23	
Swimmer	288	4.65	
Total	326	4.12	1.68(0.0034)
Earache			
Non-swimmer	24	1.39	
Swimmer	175	2.78	
Total	199	2.48	1.56(0.0456)
Eye irritation			
Non-swimmer	27	1.56	
Swimmer	113	1.78	
Total	140	1.73	1.35(0.24)

1: Percentage of those in row category with symptom (row percentage). Number and percent not ill not shown
2: Adjusted Cumulative Incidence Ratio

Figure 4.4: Incident illness by swimming status (head immersion), Surfside Beach



CIR: Adjusted cumulative incidence ratio comparing proportion of illness among swimmers compared to non swimmers

4.1.6 Water quality

General water quality parameters (Turbidity, pH and water temperature) for Surfside Beach are shown in Table 4.15. Turbidity was slightly higher at shin depth than waist depth, and also higher at the 3:00 PM sampling time. At least some rainfall occurred on 8 of the 29 study days. Twelve of the 29 study days had rainfall in the prior 24 hours and 14 had rainfall in the prior 48 hours. Maximum rainfall on the study days or within 48 hours was 1.17 inches.

Table 4.15: water quality parameters, Surfside Beach

	N	Min	Median	Max	Mean	SD
Turbidity, NTU[1]						
All Samples	510	1.10	3.50	11.33	4.04	1.86
By Depth						
-Shin	255	1.23	3.55	11.33	4.17	1.90
-Waist	255	1.10	3.40	10.34	3.90	1.80
By Collection Time						
-08:00	174	1.10	3.42	10.34	4.02	1.89
-11:00	174	1.20	3.53	9.48	4.07	1.86
-15:00	162	1.53	3.50	11.33	4.02	1.82
pH						
All Samples	509	6.70	8.00	8.30	7.97	0.24
By Depth						
-Shin	255	6.80	8.00	8.20	7.97	0.23
-Waist 2	254	6.70	8.00	8.30	7.98	0.24
By Collection Time						
-08:00	174	7.00	8.00	8.20	7.95	0.23
-11:00	173	6.70	8.00	8.20	7.96	0.25
-15:00	162	7.10	8.10	8.30	8.01	0.22
Salinity, parts per thousand						
All Samples	507	20.70	35.90	39.00	35.37	2.34
Conductivity, milliSiemens						
All Samples	509	5.00	55.30	60.10	51.19	7.52
Water Temperature (waist depth), ℃						
All Samples	69	21.10	27.30	29.40	27.26	1.42
By Collection Time						
-08:00	24	21.10	26.65	27.80	26.26	1.39
-11:00	23	25.30	27.40	28.60	27.21	0.90
-15:00	22	26.00	28.70	29.40	28.40	1.02

1: Nephelometric Turbidity Units

Enterococcus Method 1600

A total of 510 samples were tested and quantified for *Enterococcus* colony forming units using EPA Method 1600. Results are shown in Table 4.16.

Table 4.16: *Enterococcus* CFU[1] ($\log_{10}$) per 100 ml at Surfside Beach

	Min[2]	Median	Max	Mean	SD	N	Below Detect
All Samples	-1.00	0.48	2.81	0.47	0.73	510	59
By Depth							
-Shin	-1.00	0.60	2.81	0.51	0.74	255	28
-Waist	-1.00	0.48	2.73	0.42	0.72	255	31
By Collection Time							
-08:00	-1.00	0.85	2.81	0.86	0.68	174	6
-11:00	-1.00	0.48	2.03	0.35	0.68	174	24
-15:00	-1.00	0.30	1.75	0.17	0.66	162	29
By Swim Location[3]							
-Location 1	-1.00	0.60	2.81	0.50	0.74	170	19
-Location 2	-1.00	0.48	2.59	0.41	0.74	170	23
-Location 3	-1.00	0.48	2.65	0.48	0.70	170	17

1: Colony Forming Units, Measured by EPA Method 1600

2: Minimum value set to 0.1 CFU per 100 ml, or -1 $\log_{10}$ CFU per 100 ml

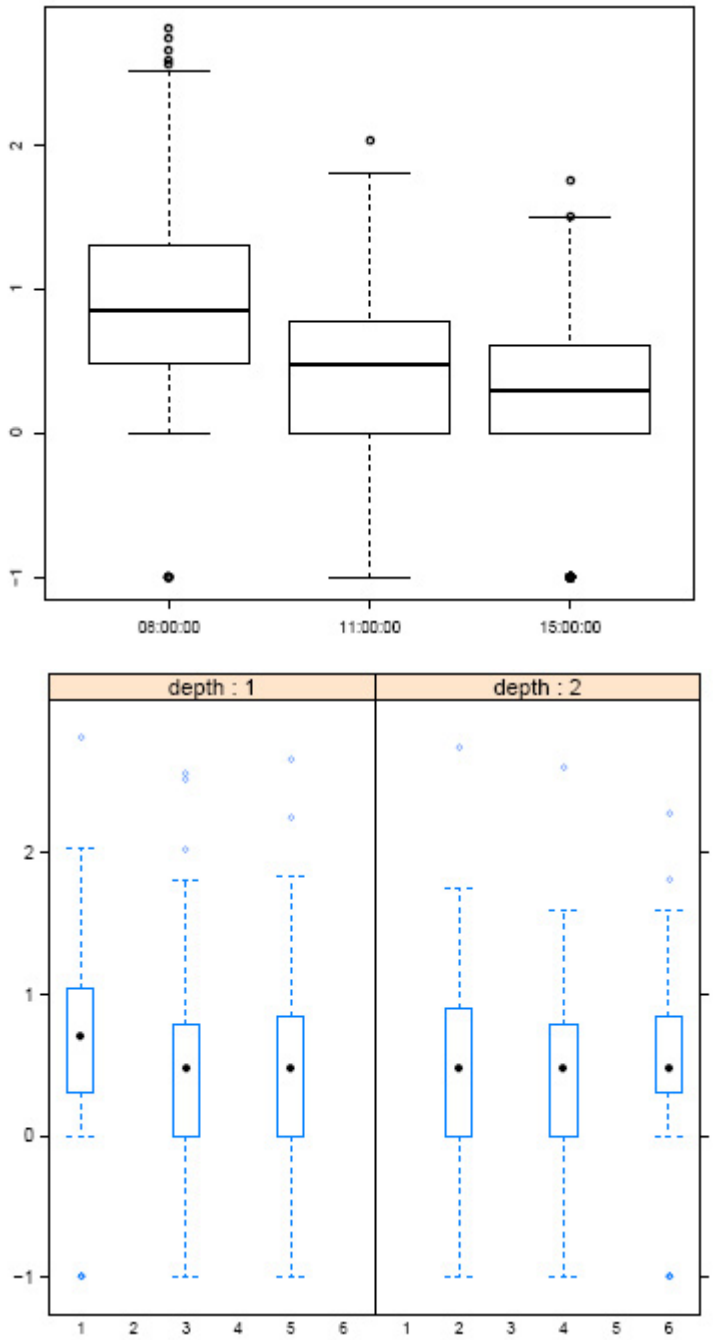
3: See Figure 4.3. Location 1 is the left transect (samples 1 and 3), 2 center (2 and 4), 3 right (3 and 6)

As measured by *Enterococcus* CFU, water quality was exceptionally good at Surfside Beach. The overall geometric mean of all samples was 3 CFU per 100 ml. CFU declined over time ($p<0.0001$) with the highest concentrations at 8:00 AM (Geometric mean=7 CFU per 100 ml) and lowest occurring at 3:00 PM (Geometric mean=1.5 CFU per 100 ml), consistent with what has been reported previously [3]. Only slightly higher densities were also observed at shin depth than waist depth. In contrast at the previously studied Great Lakes beach sites, indicator densities were consistently higher at shin depth [3]. Within depth, CFU densities also did not vary significantly by sample location ($p=0.06$). Enterococcus CFU densities are illustrated graphically in Figure 4.5.

Fifty-nine samples (11%) showed no detection by Method 1600 and were assigned a uniformly low value of 1 CFU per 1000 ml for analysis.

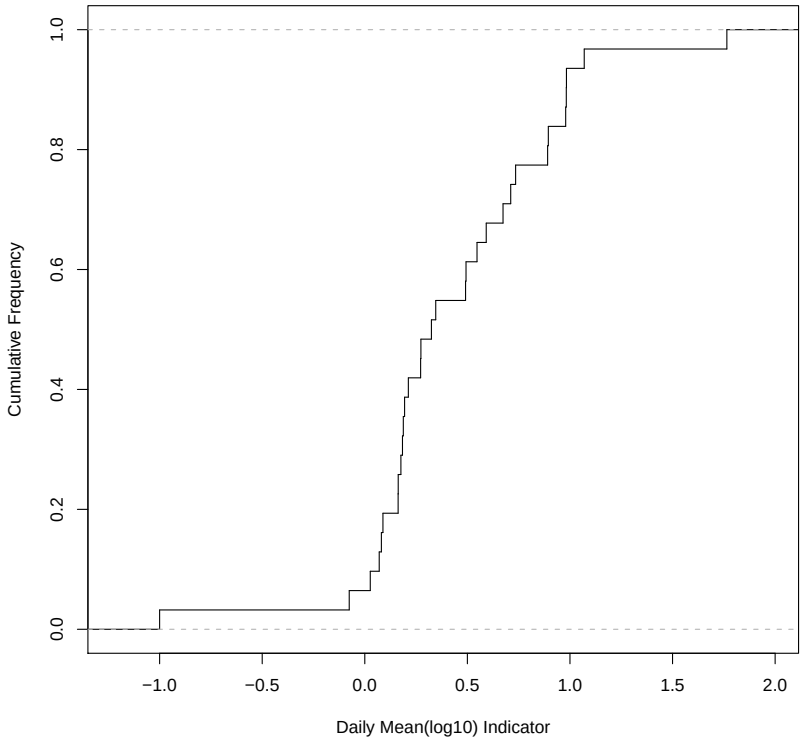
Enterococcus CFU exceeded the EPA recommended geometric mean criterion of 35 CFU per 100 ml standard [52] only one day of the 29 days studied, June 7 (58 CFU/100 ml). A cumulative frequency plot of the daily average $\log_{10}$ densities is shown in Figure 4.6.

Figure 4.5: *Enterococcus* colony forming units ($\log_{10}$) per 100 ml, Surfside Beach



See Fig 4.3 for sampling locations. Depth 1 refers to Shin depth, depth 2 to waist depth samples

Figure 4.6: Cumulative frequency plot. Daily average *Enterococcus* colony forming units ($\log_{10}$) per 100 ml, Surfside Beach



Enterococcus qPCR Calibrator Cell Equivalents (CCE)

A total of 514 water samples were tested for *Enterococcus* by qPCR. Results for the delta-delta CT method are shown in Table 4.17 and for the delta-CT method in Table 4.18. A relatively high proportion of samples were not detected by qPCR (N=167, 32%), possibly reflecting the over all high water quality at the beach. Very few samples (N=4, <1%) were out of range of the positive Salmon control assay.

Table 4.17: *Enterococcus* qPCR Calibrator Cell Equivalents (CCE), delta-delta CT method ($\log_{10}$), Surfside Beach

	Min	Median	Max	Mean	SD	N	Below Detect[1]	Control Fail[2]
Surfside Beach								
All Samples	0.91	1.77	5.04	2.01	0.78	514	167(33%)	4(1%)
By Depth								
-Shin	0.91	1.80	4.82	2.05	0.80	257	81(32%)	4(1%)
-Waist	1.07	1.73	5.04	1.97	0.77	257	86(33%)	0(0%)
By Collection Time								
-08:00	1.17	2.18	5.00	2.37	0.89	174	34(33%)	2(1%)
-11:00	0.91	1.76	5.04	1.98	0.76	174	57(20%)	2(1%)
-15:00	1.07	1.54	4.20	1.67	0.46	166	76(46%)	0(0%)
By Swim Location[3]								
-Location 1	0.91	1.81	5.00	2.02	0.76	170	49(29%)	1(1%)
-Location 2	1.17	1.78	5.04	2.07	0.82	172	53(31%)	1(1%)
-Location 3	1.03	1.68	4.34	1.95	0.77	172	65(38%)	2(1%)

1: Number of samples passing salmon criteria with no detection after 45 cycles

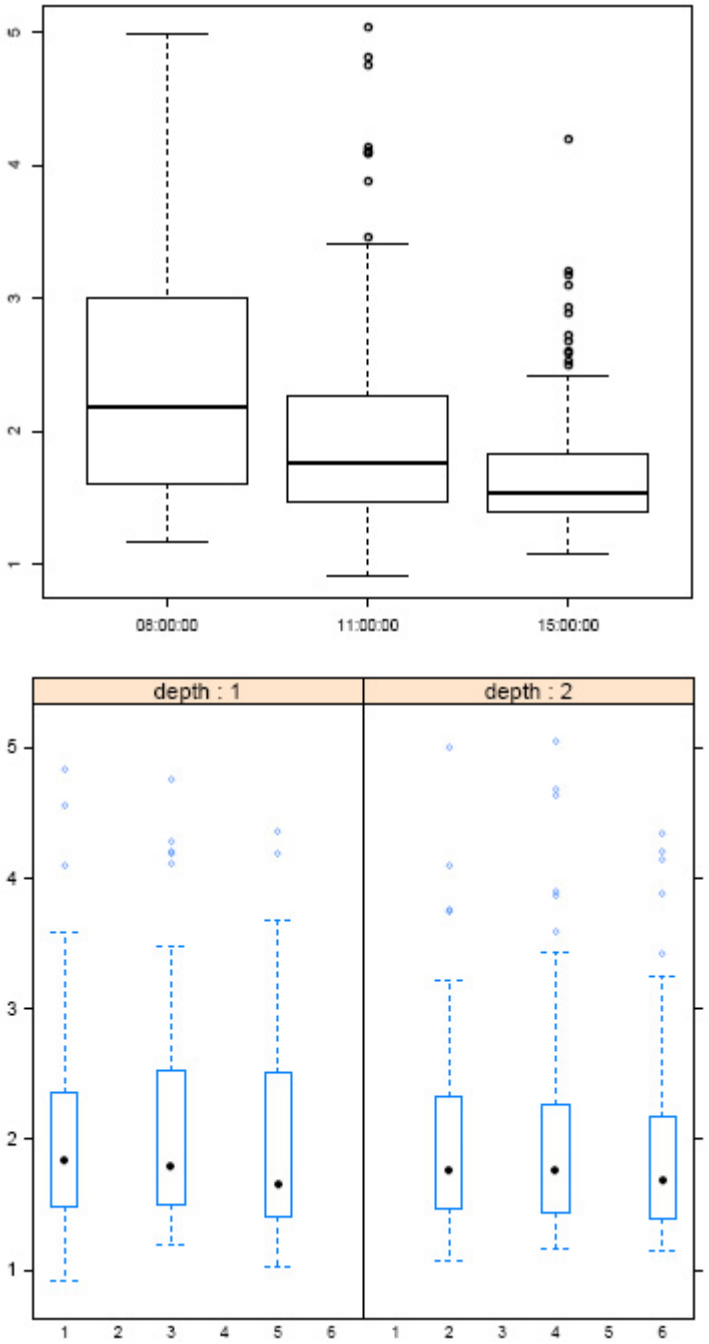
2: Number of samples where salmon assay fails cycle threshold criterion (see Sections 3.4.1 and 3.4)

3: See Figure 4.3. Location 1 is the left transect (samples 1 and 3), 2 is (2 and 4), 3 is right (3 and 6)

As with *Enterococcus* CFU, *Enterococcus* CCE declined over the course of the day, with highest estimated CCEs occurring at the 8:00 AM sampling time and the lowest at 3:00 PM ($p<0.0001$). No differences were observed by sample depth or sample location (Figures 4.7 and 4.8)

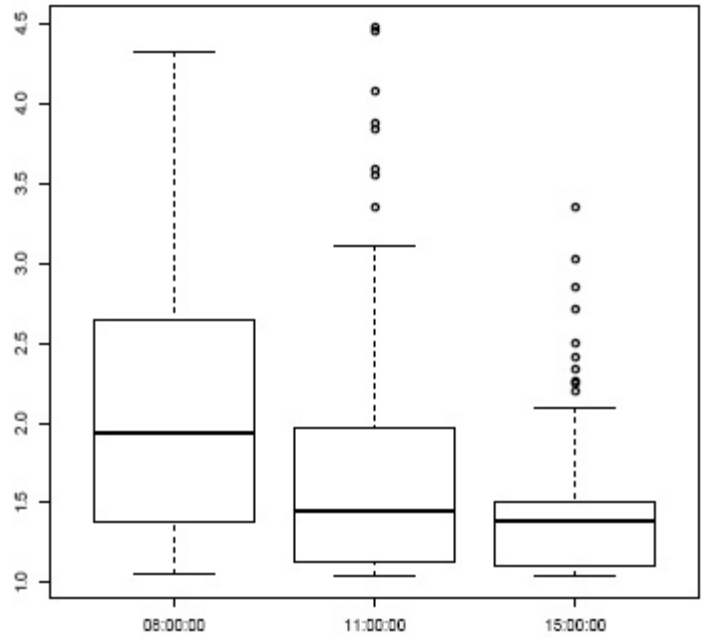
CCEs calculated using the delta-delta CT approach showed a wider range of sample estimates (8-109,648 CCE/100 ml) compared to the delta CT approach (11-30,200 CCE/100 ml) and resulted in higher CCE values (All sample geometric means of 102 and 55 CCE/100 ml). Both approaches showed higher estimated *Enterococcus* densities compared to the culturable method. On average, the ratios of CFU to CCE were 0.13 and 0.21 for the delta-delta and delta CT calculations, respectively The average ratios of CFU to CCE are shown in Table 4.19. A cumulative distribution plot of the daily average *Enterococcus* CCE (delta-delta CT) is shown in Figure 4.9.

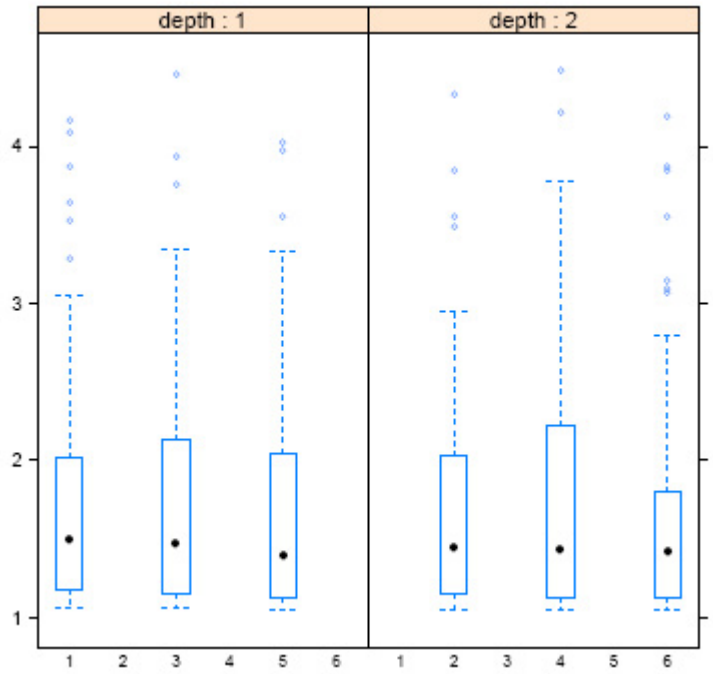
Figure 4.7: *Enterococcus* calibrator cell equivalents ($\log_{10}$) per 100 ml, delta-delta CT method, Surfside Beach



See Fig 4.3 for sampling locations. Depth 1 refers to Shin depth, depth 2 to waist depth samples

Figure 4.8: *Enterococcus* calibrator cell equivalents ($\log_{10}$) per 100 ml, delta CT method, Surfside Beach





See Fig 4.3 for sampling locations. Depth 1 refers to shin depth, depth 2 to waist depth samples

Figure 4.9: Cumulative frequency plot. Daily average *Enterococcus* CCE (delta-delta CT) ($\log_{10}$) per 100 ml, Surfside Beach

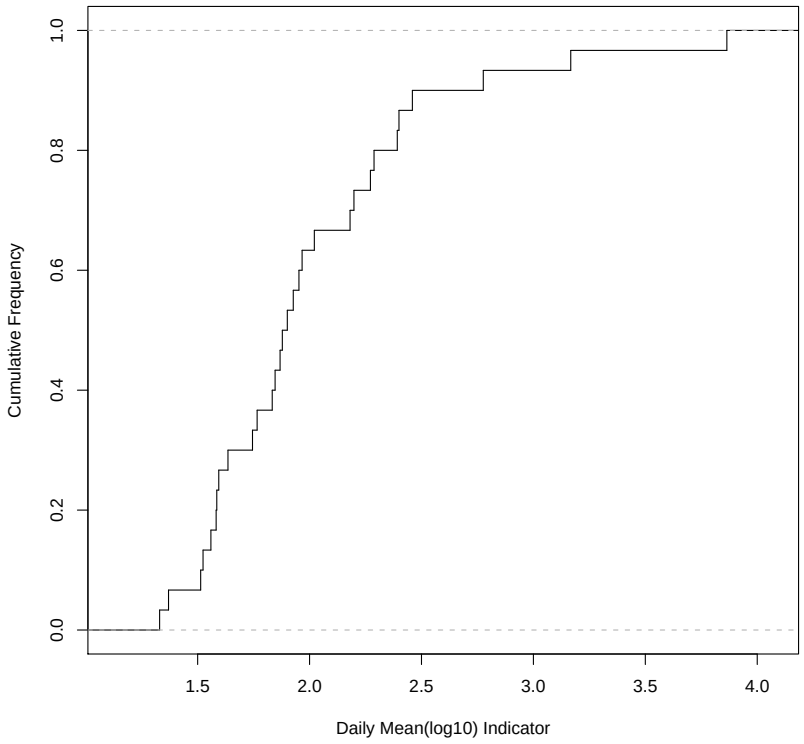


Table 4.18: *Enterococcus* qPCR Calibrator Cell Equivalents (CCE), delta CT method ($\log_{10}$), Surfside Beach

	Min	Median	Max	Mean	SD	N	Below Detect[1]	Control Fail[2]
Surfside Beach								
All Samples	1.05	1.43	4.48	1.74	0.74	514	167(33%)	4(1%)
By Depth								
-Shin	1.05	1.45	4.46	1.76	0.76	257	81(32%)	4(2%)
-Waist	1.05	1.43	4.48	1.71	0.73	257	86(33%)	0(0%)
By Collection Time								
-08:00	1.06	1.94	4.32	2.09	0.86	174	34(20%)	2(1%)
-11:00	1.05	1.45	4.48	1.69	0.71	174	57(33%)	2(1%)
-15:00	1.05	1.38	3.35	1.41	0.41	166	76(46%)	0(0%)
By Swim Location[3]								
-Location 1	1.05	1.48	4.32	1.75	0.72	170	49(30%)	1(1%)
-Location 2	1.05	1.46	4.48	1.78	0.78	172	53(31%)	1(1%)
-Location 3	1.05	1.41	4.19	1.68	0.73	172	65(38%)	2(1%)

1: Number of samples passing salmon criteria with no detection after 45 cycles

2: Number of samples where salmon assay fails cycle threshold criterion (see Sections 3.4.1 and 3.4)

3: See Figure 4.3. Location 1 is left transect (samples 1 and 3), 2 center (2 and 4), 3 right (3 and 6)

Table 4.19: Ratio of Enterococcus CFU to *Enterococcus* CCE[1]. Surfside Beach.

	Min	Median	Max	Mean	SD	N
delta-delta CT						
All Samples	0.0000	0.0381	4.6429	0.1251	0.3173	504
By Depth						
-Depth 1	0.0000	0.0412	4.6429	0.1303	0.3760	250
-Depth 2	0.0001	0.0367	1.5391	0.1200	0.2469	254
By Collection Time						
-08:00	0.0000	0.0343	2.7389	0.1764	0.3613	172
-11:00	0.0001	0.0349	4.6429	0.1146	0.3787	172
-15:00	0.0001	0.0404	1.1958	0.0811	0.1379	160
delta-CT						
All Samples	0.0000	0.0745	7.0699	0.2082	0.4917	504
By Depth						
-Shin	0.0000	0.0804	7.0699	0.2252	0.5838	250
-Waist	0.0003	0.0715	2.8680	0.1915	0.3804	254
By Collection Time						
-08:00	0.0000	0.0668	3.4349	0.2970	0.5716	172
-11:00	0.0003	0.0740	7.0699	0.1899	0.5719	172
-15:00	0.0003	0.0804	2.2007	0.1325	0.2170	160

CFU: Colony forming units, CCE: Calibrator cell equivalents

1: Sample to sample ratios. qPCR samples which failed QC excluded

Bacteroidales qPCR Calibrator Cell Equivalents (CCE)

Results of monitoring for *Bacteroidales* for the delta-delta CT and the delta CT methods are shown in Tables 4.20 and 4.21. Fewer samples (61) were below detection for *Bacteroidales* than for *Enterococcus* CCE and measures of estimated CCEs were considerably higher (Geometric means: 575/100 ml and 295/100 ml for the delta-delta and delta CT methods respectively).

Collection time was associated with *Bacteroidales* CCE ($p<0.0001$), but in contrast with *Enterococcus* CCE and CFU, *Bacteroidales* increased over time, with highest CCE occurring at the 3:00 PM sampling time. Estimated *Bacteroidales* CCE were also higher at shin depth for the delta-CT CCE ($p=0.008$) but the difference was not as apparent for CCE calculated using delta-delta CT ($p=0.08$) (Figures 4.10 and 4.10.

A cumulative distribution plot of the daily average *Bacteroidales* CCE (delta-delta CT) is shown in Figure 4.12.

Table 4.20: *Bacteroidales* qPCR Calibrator Cell Equivalents (CCE), delta-delta CT method ($\log_{10}$), Surfside Beach

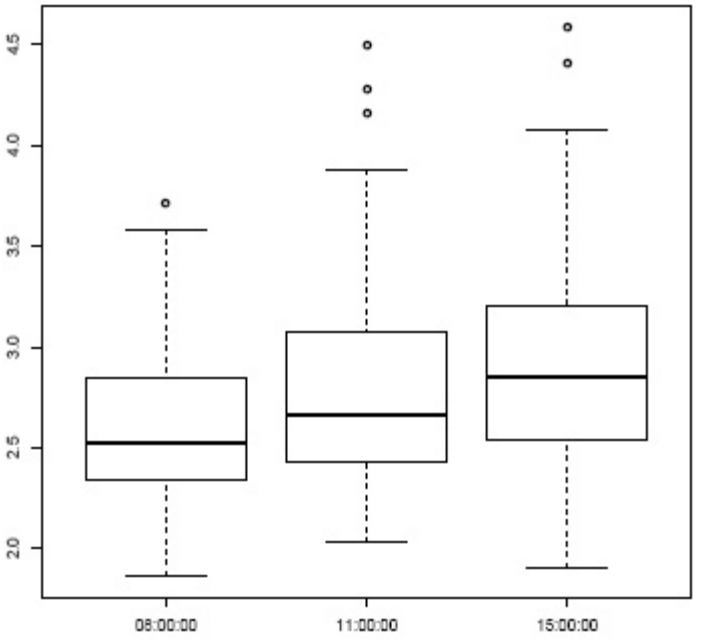
	Min	Median	Max	Mean	SD	N	Below Detection[1]	Control Fail[2]
Surfside Beach								
All Samples	1.87	2.67	4.58	2.76	0.46	514	61(11%)	4(1%)
By Depth								
-Shin	1.91	2.71	4.58	2.80	0.49	257	28(11%)	4(1%)
-Waist	1.87	2.62	4.49	2.71	0.44	257	33(13%)	0(0%)
By Collection Time								
-08:00	1.87	2.52	3.71	2.60	0.37	174	37(22%)	2(1%)
-11:00	2.03	2.66	4.49	2.76	0.46	174	13(8%)	2(1%)
-15:00	1.91	2.85	4.58	2.92	0.51	166	11(7%)	0(0%)
By Swim Location[3]								
-Location 1	1.94	2.70	4.08	2.74	0.39	170	15(9%)	1(1%)
-Location 2	1.87	2.64	4.49	2.75	0.48	172	26(15%)	1(1%)
-Location 3	1.91	2.65	4.58	2.78	0.52	172	20(12%)	2(1%)

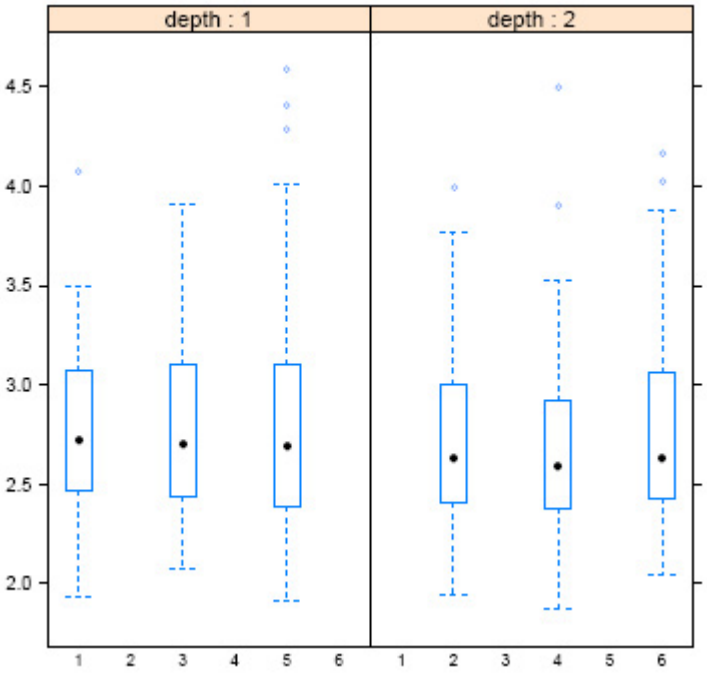
1: Number of samples passing salmon criteria with no detection after 45 cycles

2: Number of samples where salmon assay fails cycle threshold criterion (see Sections 3.4.1 and 3.4)

3: See Figure 4.3. ocation 1 is the left transect (samples 1 and 3), 2 center (2 and 4), 3 right (3 and 6)

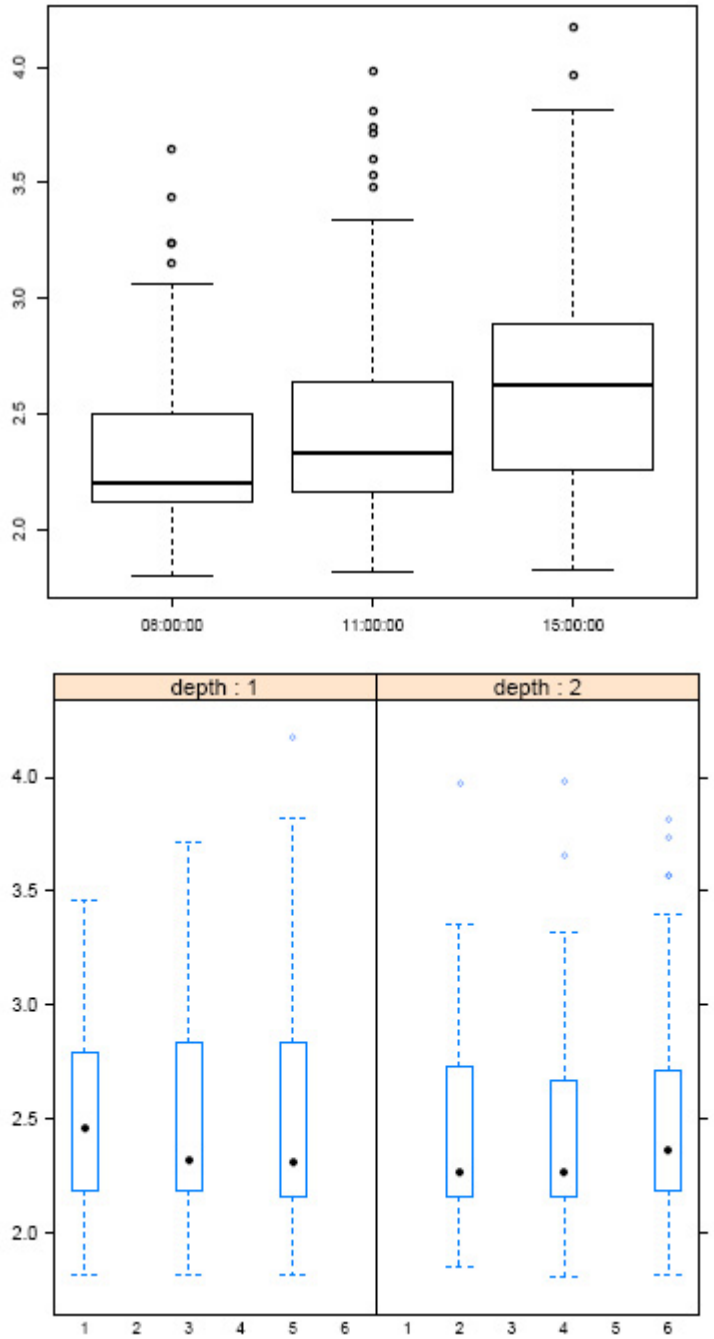
Figure 4.10: *Bacteroidales* calibrator cell equivalents ($\log_{10}$) per 100 ml, delta CT method, Surfside Beach





See Fig 4.3 for sampling locations. Depth 1 refers to Shin depth, depth 2 to waist depth samples

Figure 4.11: *Bacteroidales* calibrator cell equivalents ($\log_{10}$) per 100 ml, delta CT method, Surfside Beach



See Fig 4.3 for sampling locations. Depth 1 refers to Shin depth, depth 2 to waist depth samples

Figure 4.12: Cumulative frequency plot. Daily average *Bacteroidales* CCE (delta-delta CT) ($\log_{10}$) per 100 ml, Surfside Beach

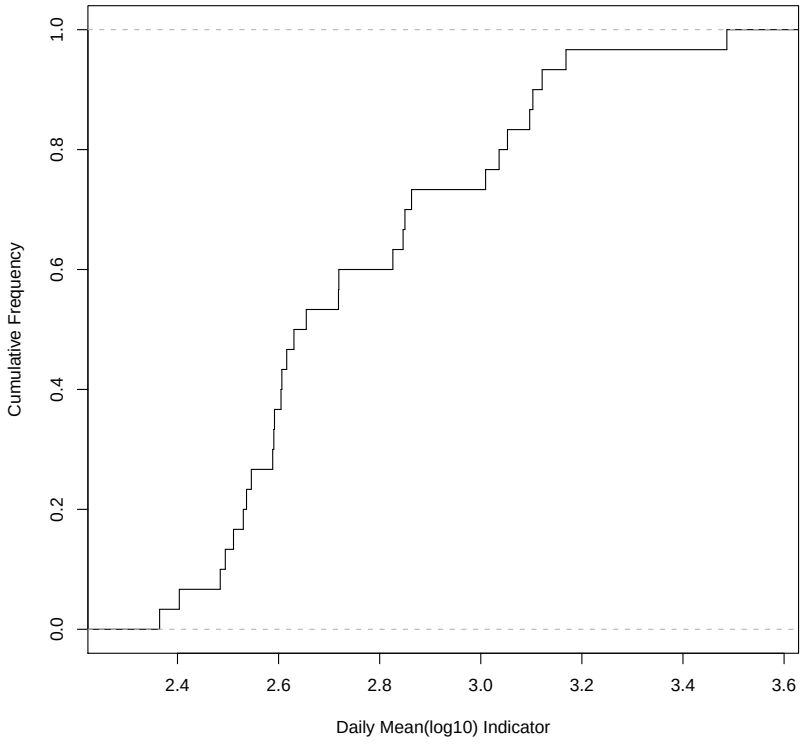


Table 4.21: *Bacteroidales* qPCR Calibrator Cell Equivalents (CCE), delta CT method ($\log_{10}$), Surfside Beach

	Min	Median	Max	Mean	SD	N	Below Detect[1]	Control Fail[2]
Surfside Beach								
All Samples	1.80	2.34	4.17	2.47	0.44	514	61(11%)	4(1%)
By Depth								
-Shin	1.82	2.34	4.17	2.49	0.46	257	28(11%)	4(2%)
-Waist	1.80	2.34	3.98	2.44	0.42	257	33(13%)	0(0%)
By Collection Time								
-08:00	1.80	2.20	3.64	2.31	0.37	174	37(22%)	2(1%)
-11:00	1.82	2.33	3.98	2.46	0.43	174	13(8%)	2(1%)
-15:00	1.83	2.63	4.17	2.64	0.46	166	11(7%)	0(0%)
By Swim Location[3]								
-Location 1	1.82	2.37	3.97	2.46	0.38	170	15(9%)	1(1%)
-Location 2	1.80	2.30	3.98	2.45	0.45	172	26(15%)	1(1%)
-Location 3	1.82	2.36	4.17	2.49	0.48	172	20(12%)	2(1%)

1: Number of samples passing salmon criteria with no detection after 45 cycles

2: Number of samples where salmon assay fails cycle threshold criterion (see Sections 3.4.1 and 3.4)

3: See Figure 4.3. Location 1 is the left transect (samples 1 and 3), 2 center (2 and 4), 3 right (3 and 6)

Swash water quality

Average salinity at the swash sites (see Figure 4.2) was lower than the beach sites ($p<0.00005$, average of 11 parts per thousand compared to 35, Table 4.22 and Table 4.15) suggesting the swash was influenced by runoff. Swash water had poorer water quality than beach water. The geometric mean of *Enterococcus* CFU at the two swash sampling sites was 224 CFU/100 ml. *Enterococcus* delta-CT and delta-delta CT CCE geometric means were 2,951 and 7,244 CCE/100 ml, respectively. *Bacteroidales* delta CT and delta-delta CT CCE geometric means were 10,715 and 27,542, both considerably higher than measures at the beach. Water quality measures in the swash are summarized in Table 4.22. Each of the indicator bacteria measures were higher in the swash than at the beach ($p<0.00001$).

Although a detailed modeling if water quality parameters was beyond the scope of this report, there was also evidence of an association between the water quality in the swash and at the beach. Average *Enterococcus* CFU from the swash were correlated with the daily average beach samples, most strongly with swash samples from location 2, furthest upstream from the beach site (see Figure 4.13, $r=0.66$, $p=0.0001$).

4.1.7 Associations among water quality measures and environmental measures

Sample to sample correlations for water quality measures, turbidity, pH and salinity are shown in Figure 4.14. Pairwise Spearman correlation coefficients and their associated p-values are shown in Table 4.23. Also shown are Spearman correlation coefficients for days with rain in the previous 24 hours and days without rain in the previous 24 hours (Table 4.24 and Table 4.25). On days where rainfall occurred in the previous 24 hours, better correlations were seen between *Enterococcus* CFU and the qPCR indicators. Also, turbidity was positively correlated with *Bacteroidales* on these days, but not on days without rainfall.

While there were good correlations between the delta and delta-delta CT calculations, other measures of water quality only correlated moderately. Turbidity was significantly correlated with all measures, but correlations were weak with slightly stronger correlations between *Enterococcus* ($r=0.23$-0.24) than *Bacteroidales*. *Enterococcus* and *Bacteroidales* were not well correlated.

Additional associations between information collected at each sampling time and the the average of the water quality measures from the same time period are shown in Tables 4.26- 4.28. Bathers in the water, ultraviolet intensity, and water temperature were inversely associated with *Enterococcus* CCE and CFU, but these same measures (in addition to wind speed) were positively associated with *Bacteroidales* CCE. Higher tide stage at 8:00 AM was also associated with lower *Bacteroidales* CCE.

Associations between water quality measures and rainfall are shown in Tables 4.29- 4.31. Whereas positive associations were observed between rainfall

Table 4.22: Fecal indicator bacteria and water quality parameters at Surfside Beach swash sites.

	Min	Median	Max	Mean	SD	N	Below Detect[1]	Control Fail[2]
Enterococcus CFU[3]								
All Samples	0.30	2.41	3.94	2.35	0.76	152	NA	NA
By Location[4]								
-Location 1	0.30	2.38	3.94	2.30	0.79	77	NA	NA
-Location 2	0.30	2.41	3.85	2.40	0.73	75	NA	NA
Enterococcus CCE[5]								
All Samples	1.86	3.91	6.11	3.86	0.81	162	2	0
By Location[4]								
-Location 1	1.87	3.76	5.64	3.75	0.72	81	1	0
-Location 2	1.86	4.03	6.11	3.96	0.89	81	1	0
Bacteroidales CCE[5]								
All Samples	2.63	4.47	5.92	4.44	0.65	162	3	6
By Location[4]								
-Location 1	2.65	4.34	5.52	4.30	0.58	81	1	0
-Location 2	2.63	4.65	5.92	4.58	0.70	81	2	6
Salinity[6]								
All Samples	1.00	9.90	31.70	10.94	7.15	109	NA	NA
By Location[4]								
-Location 1	1.10	11.10	31.70	12.56	7.26	54	NA	NA
-Location 2	1.00	9.50	28.20	9.35	6.73	55	NA	NA
Turbidity[7]								
All Samples	1.56	3.00	9.30	3.67	1.82	104	NA	NA
By Location [4]								
-Location 1	1.60	3.08	9.30	4.14	2.19	52	NA	NA
-Location 2	1.56	2.93	8.94	3.20	1.20	52	NA	NA

1: No detection after 45 cycles

2: Salmon assay fails cycle threshold criterion (see Sections 3.4.1 and 3.4)

3: Colony Forming Units, Measured by EPA Method 1600

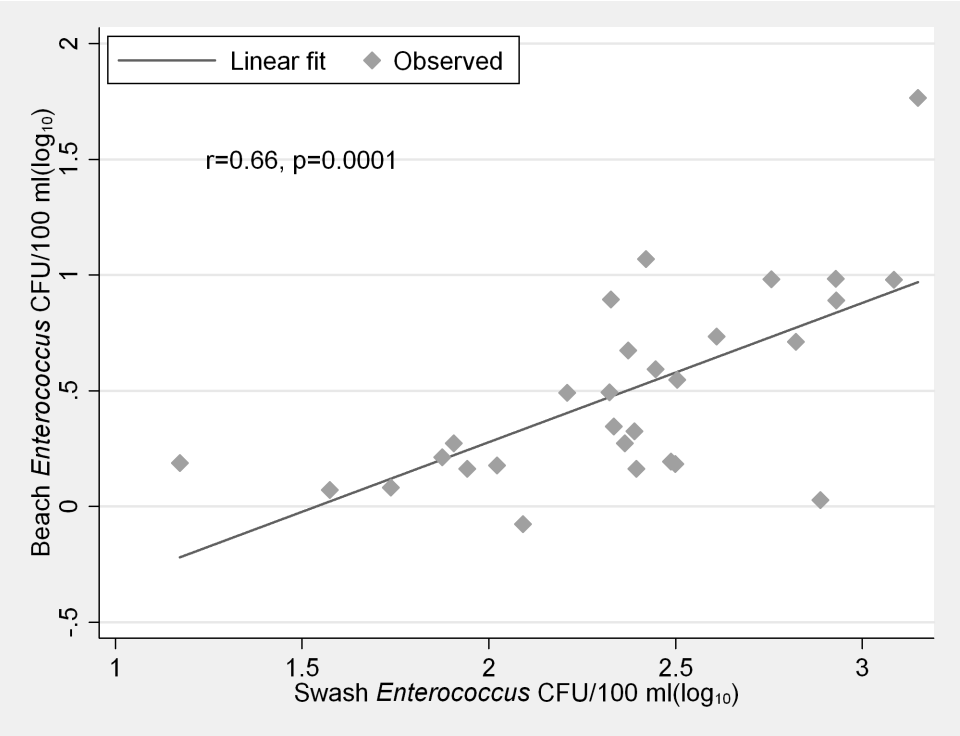
4: See Figure 4.3. Location 1 is left transect (samples 1 and 3), 2 center (2 and 4), 3 right (3 and 6)

5: Calibrator Cell Equivalents calculated using the delta delta-CT method

6: Parts per thousand

7: Nephelometric Turbidity Units (NTU)

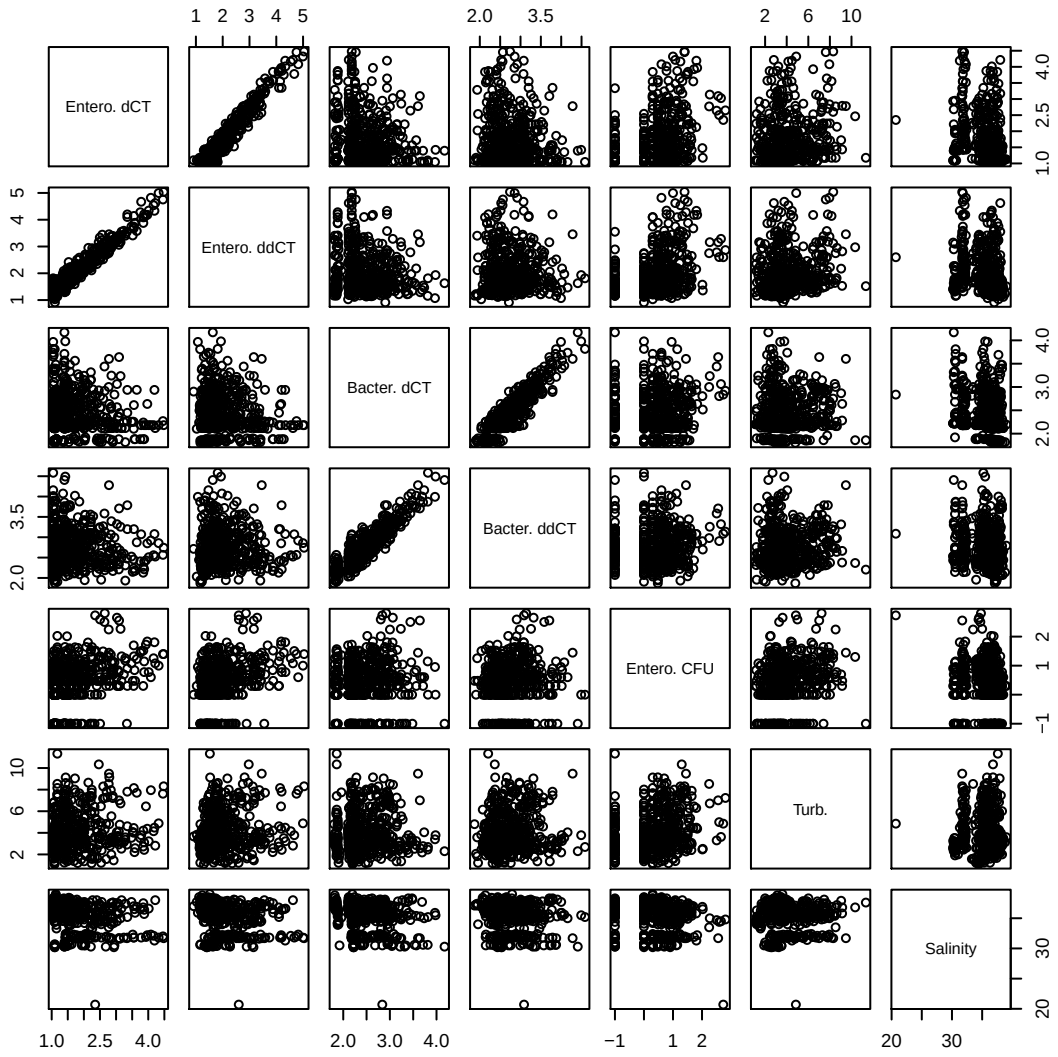
Figure 4.13: Relationship between daily average *Enterococcus* CFU[1] at beach sites and daily average *Enterococcus* CFU from swash sample location 2[1]



1: See See Fig 4.3 for sampling locations.

CFU=Colony Forming Units per 100 ml($\log_{10}$)

Figure 4.14: Multivariate plot of fecal indicator bacteria and water quality parameters



dCT=delta CT (qPCR Calibrator Cell Equivalents); ddCT=delta-delta CT (qPCR Calibrator Cell Equivalents); CFU=Colony Forming Units
Entero.=*Enterococcus*; Bacter.=*Bacteroidales*
Turbidity measured in Nephelometric Turbidity Units (NTU). Salinity in parts per thousand (ppt)

Table 4.23: Spearman pairwise correlation coefficients for water quality parameters, Surfside Beach

	Entero. dCT	Entero. ddCT	Bacter. dCT	Bacter. ddCT	Entero.CFU	Turb.	Salinity
Entero. dCT	1	0.913*	0.001	-0.006	0.369*	0.228*	-0.317*
Entero. ddCT	0.913*	1	-0.028	0.105*	0.344*	0.245*	-0.315*
Bacter. dCT	0.001	-0.028	1	0.862*	0.046	0.095*	-0.247*
Bacter. ddCT	-0.006	0.105*	0.862*	1	0.046	0.106*	-0.162*
Entero. CFU	0.369*	0.344*	0.046	0.046	1	0.228*	-0.15*
Turb.	0.228*	0.245*	0.095*	0.106*	0.228*	1	-0.053
Salinity	-0.317*	-0.315*	-0.247*	-0.162*	-0.15*	-0.053	1

*p<0.05

dCT=delta CT (qPCR Calibrator Cell Equivalents); ddCT=delta-delta CT (qPCR Calibrator Cell Equivalents)

CFU=Colony Forming Units; Entero.=*Enterococcus*; Bacter.=*Bacteroidales*

Turbidity measured in Nephelometric Turbidity Units (NTU). Salinity in parts per thousand (ppt).

Table 4.24: Spearman pairwise correlation coefficients for water quality parameters, Surfside Beach. Days with rain in previous 24 hours

	Entero. dCT	Entero. ddCT	Bacter. dCT	Bacter. ddCT	Entero.CFU	Turb.	Salinity
Entero. dCT	1	0.94*	0.051	0.037	0.398*	0.017	-0.306*
Entero. ddCT	0.94*	1	0.008	0.142*	0.344*	0.038	-0.247*
Bacter. dCT	0.051	0.008	1	0.848*	0.219*	0.319*	-0.296*
Bacter. ddCT	0.037	0.142*	0.848*	1	0.175*	0.318*	-0.209*
Entero. CFU	0.398*	0.344*	0.219*	0.175*	1	0.103	-0.271*
Turb.	0.017	0.038	0.319*	0.318*	0.103	1	-0.07
Salinity	-0.306*	-0.247*	-0.296*	-0.209*	-0.271*	-0.07	1

*p<0.05

dCT=delta CT (qPCR Calibrator Cell Equivalents); ddCT=delta-delta CT (qPCR Calibrator Cell Equivalents)

CFU=Colony Forming Units; Entero.=*Enterococcus*; Bacter.=*Bacteroidales*

Turbidity measured in Nephelometric Turbidity Units (NTU). Salinity in parts per thousand (ppt).

Table 4.25: Spearman pairwise correlation coefficients for water quality parameters, Surfside Beach. Days with no rain in previous 24 hours

	Entero. dCT	Entero. ddCT	Bacter. dCT	Bacter. ddCT	Entero.CFU	Turb.	Salinity
Entero. dCT	1	0.811*	0.006	-0.006	0.17*	0.125*	-0.247*
Entero. ddCT	0.811*	1	-0.009	0.132*	0.159*	0.116	-0.372*
Bacter. dCT	0.006	-0.009	1	0.87*	-0.089	-0.064	-0.208*
Bacter. ddCT	-0.006	0.132*	0.87*	1	-0.054	-0.073	-0.127*
Entero. CFU	0.17*	0.159*	-0.089	-0.054	1	0.107	-0.019
Turb.	0.125*	0.116	-0.064	-0.073	0.107	1	0.085
Salinity	-0.247*	-0.372*	-0.208*	-0.127*	-0.019	0.085	1

*p<0.05

dCT=delta CT (qPCR Calibrator Cell Equivalents); ddCT=delta-delta CT (qPCR Calibrator Cell Equivalents)

CFU=Colony Forming Units;Entero.=*Enterococcus*; Bacter.=*Bacteroidales*

Turbidity measured in Nephelometric Turbidity Units (NTU). Salinity in parts per thousand (ppt).

and *Enterococcus* CCE and CFU, no associations were observed between rainfall and *Bacteroidales* CCE.

Table 4.26: Spearman pairwise correlation coefficients for *Enterococcus* qPCR CCE and environmental measures, Surfside Beach

	Ent. dCT	Ent. ddCT	Bathers	Wind dir	Wind speed	UV	Water temp	Wave ht	Tide stage
Ent. dCT	1	0.928*	-0.498*	-0.083	-0.063	-0.417*	-0.383*	0.1	-0.139
Ent. ddCT	0.928*	1	-0.492*	-0.055	-0.107	-0.377*	-0.376*	0.059	-0.137
Bathers	-0.498*	-0.492*	1	-0.117	0.247*	0.698*	0.634*	0.078	0.12
Wind dir	-0.083	-0.055	-0.117	1	-0.111	-0.095	0.09	0.002	0.118
Wind speed	-0.063	-0.107	0.247*	-0.111	1	0.212*	0.098	0.334*	-0.098
UV	-0.417*	-0.377*	0.698*	-0.095	0.212*	1	0.366*	-0.013	0.001
Water temp	-0.383*	-0.376*	0.634*	0.09	0.098	0.366*	1	-0.148	0.159
Wave ht	0.1	0.059	0.078	0.002	0.334*	-0.013	-0.148	1	-0.229*
Tide stage	-0.139	-0.137	0.12	0.118	-0.098	0.001	0.159	-0.229*	1

*p<0.05

dCT=delta CT (qPCR Calibrator Cell Equivalents); ddCT=delta-delta CT (qPCR Calibrator Cell Equivalents)

CFU=Colony Forming Units; Entero.=*Enterococcus*; Bacter.=*Bacteroidales*

UV: Ultraviolet radiation; Tide: Tide stage at sampling time; Bathers Water: Bathers in the water; Wave ht: Wave height

Additional information see: Table 3.1

Table 4.27: Spearman pairwise correlation coefficients for *Bacteroidales* qPCR CCE and environmental measures, Surfside Beach

	Bact. dCT	Bact. ddCT	Bathers	Wind dir	Wind speed	UV	Water temp	Wave ht	Tide stage
Bact. dCT	1	0.886*	0.377*	-0.19	0.239*	0.292*	0.317*	0.232*	-0.104
Bact. ddCT	0.886*	1	0.347*	-0.107	0.233*	0.264*	0.312*	0.203	-0.112
Bathers	0.377*	0.347*	1	-0.117	0.247*	0.698*	0.634*	0.078	0.12
Wind dir	-0.19	-0.107	-0.117	1	-0.111	-0.095	0.09	0.002	0.118
Wind speed	0.239*	0.233*	0.247*	-0.111	1	0.212*	0.098	0.334*	-0.098
UV	0.292*	0.264*	0.698*	-0.095	0.212*	1	0.366*	-0.013	0.001
Water temp	0.317*	0.312*	0.634*	0.09	0.098	0.366*	1	-0.148	0.159
Wave ht	0.232*	0.203	0.078	0.002	0.334*	-0.013	-0.148	1	-0.229*
Tide stage	-0.104	-0.112	0.12	0.118	-0.098	0.001	0.159	-0.229*	1

*p<0.05

dCT=delta CT (qPCR Calibrator Cell Equivalents); ddCT=delta-delta CT (qPCR Calibrator Cell Equivalents)

CFU=Colony Forming Units; Entero.=*Enterococcus*; Bacter.=*Bacteroidales*

UV: Ultraviolet radiation; Tide: Tide stage at sampling time; Bathers Water: Bathers in the water; Wave ht: Wave height

Additional information see: Table 3.1

Table 4.28: Spearman pairwise correlation coefficients for *Enterococcus* CFU and environmental measures, Surfside Beach

	Entero CFU	Bathers Water	Wind dir	Wind speed	UV	Water temp	Wave ht	Tide stage
Entero CFU	1	-0.531*	-0.042	-0.044	-0.451*	-0.455*	0.007	-0.146
Bathers Water	-0.531*	1	-0.117	0.247*	0.698*	0.634*	0.078	0.12
Wind dir	-0.042	-0.117	1	-0.111	-0.095	0.09	0.002	0.118
Wind speed	-0.044	0.247*	-0.111	1	0.212*	0.098	0.334*	-0.098
UV	-0.451*	0.698*	-0.095	0.212*	1	0.366*	-0.013	0.001
Water temp	-0.455*	0.634*	0.09	0.098	0.366*	1	-0.148	0.159
Wave ht	0.007	0.078	0.002	0.334*	-0.013	-0.148	1	-0.229*
Tide stage	-0.146	0.12	0.118	-0.098	0.001	0.159	-0.229*	1

*p<0.05

dCT=delta CT (qPCR Calibrator Cell Equivalents); ddCT=delta-delta CT (qPCR Calibrator Cell Equivalents)

CFU=Colony Forming Units; Entero.=*Enterococcus*; Bacter.=*Bacteroidales*

UV: Ultraviolet radiation; Tide: Tide stage at sampling time; Bathers Water: Bathers in the water; Wave ht: Wave height

Additional information see: Table 3.1

Table 4.29: Spearman pairwise correlation coefficients for *Enterococcus* qPCR CCE and rainfall, Surfside Beach

	Entero. dCT	Entero. ddCT	Rain (current)	Rain (Lag 1 day)	Rain (Lag 2 day)
Entero. dCT	1	0.928*	0.264*	0.289*	0.156
Entero. ddCT	0.928*	1	0.307*	0.333*	0.252*
Rain (current)	0.264*	0.307*	1	0.186	0.319*
Rain (Lag 1 day)	0.289*	0.333*	0.186	1	0.464*
Rain (Lag 2 day)	0.156	0.252*	0.319*	0.464*	1

*p<0.05

dCT=delta CT (qPCR Calibrator Cell Equivalents); ddCT=delta-delta CT (qPCR Calibrator Cell Equivalents)

CFU=Colony Forming Units; Entero.=*Enterococcus*; Bacter.=*Bacteroidales*

UV: Ultraviolet radiation; Tide: Tide stage at sampling time; Bathers Water: Bathers in the water; Wave ht: Wave height

Additional information see: Table 3.1

Table 4.30: Spearman pairwise correlation coefficients for *Bacteroidales* qPCR CCE and rainfall, Surfside Beach

	Bacter. dCT	Bacter. ddCT	Rain (current)	Rain (Lag 1 day)	Rain (Lag 2 day)
Bacter. dCT	1	0.886*	-0.093	-0.096	-0.127
Bacter. ddCT	0.886*	1	-0.056	-0.044	-0.042
Rain (current)	-0.093	-0.056	1	0.186	0.319*
Rain (Lag 1 day)	-0.096	-0.044	0.186	1	0.464*
Rain (Lag 2 day)	-0.127	-0.042	0.319*	0.464*	1

*p<0.05

dCT=delta CT (qPCR Calibrator Cell Equivalents); ddCT=delta-delta CT (qPCR Calibrator Cell Equivalents)

CFU=Colony Forming Units; Entero.=*Enterococcus*; Bacter.=*Bacteroidales*

UV: Ultraviolet radiation; Tide: Tide stage at sampling time; Bathers Water: Bathers in the water; Wave ht: Wave height

Additional information see: Table 3.1

Table 4.31: Spearman pairwise correlation coefficients for *Enterococcus* CFU and rainfall, Surfside Beach

	Entero CFU	Rain (current)	Rain (Lag 1 day)	Rain (Lag 2 day)
Entero CFU	1	0.259*	0.349*	0.257*
Rain (current)	0.259*	1	0.186	0.319*
Rain (Lag 1 day)	0.349*	0.186	1	0.464*
Rain (Lag 2 day)	0.257*	0.319*	0.464*	1

*p<0.05

dCT=delta CT (qPCR Calibrator Cell Equivalents); ddCT=delta-delta CT (qPCR Calibrator Cell Equivalents)

CFU=Colony Forming Units; Entero.=*Enterococcus*; Bacter.=*Bacteroidales*

UV: Ultraviolet radiation; Tide: Tide stage at sampling time; Bathers Water: Bathers in the water; Wave ht: Wave height

Additional information see: Table 3.1

4.1.8 Associations between water quality and illness

The unadjusted incidence of illness across tertiles of fecal indicator exposure among swimmers and among non-swimmers is shown in Table 4.32. A slight increase in the incidence of GI illness and diarrhea is evident across exposure categories. A similar table for the delta-CT calculation is not shown as results are highly similar.

Table 4.32: Number and percentage of respondents with incident illness for non-swimmers and among body immersion swimmers by tertiles of daily average of indicator exposures. Surfside Beach. qPCR CCE determined through delta-delta CT calculation.

	GI		URI		Rash		Earache		Eye		Diarrhea	
	N	%[1]	N	%[1]	N	%[1]	N	%[1]	N	%[1]	N	%[1]
Enterococcus CCE												
Non-Swimmer	79	4.67	73	4.36	38	2.23	24	1.39	27	1.56	50	2.96
1.33,1.64	159	5.92	112	4.23	155	5.79	70	2.58	43	1.57	106	3.95
1.64,1.97	181	6.55	137	5.08	105	3.82	79	2.85	50	1.78	122	4.42
1.97,3.86	168	6.98	124	5.21	94	3.90	52	2.13	53	2.15	117	4.86
Bacteroidales CCE												
Non-Swimmer	79	4.67	73	4.36	38	2.23	24	1.39	27	1.56	50	2.96
2.36,2.59	142	6.09	121	5.24	117	5.01	67	2.83	51	2.14	94	4.03
2.59,2.85	168	6.39	121	4.71	113	4.33	64	2.42	47	1.75	114	4.34
2.85,3.49	198	6.84	131	4.60	124	4.30	70	2.40	48	1.63	137	4.74
Enterococcus CFU												
Non-Swimmer	79	4.67	73	4.36	38	2.23	24	1.39	27	1.56	50	2.96
-0.0758,0.188	182	6.32	133	4.68	125	4.32	72	2.47	47	1.60	122	4.24
0.188,0.493	139	6.00	104	4.55	110	4.77	66	2.82	39	1.65	88	3.80
0.493,1.76	187	7.04	136	5.24	119	4.52	63	2.36	60	2.22	135	5.08

1: Percentage of those within exposure category with symptom (row percentage). Number and percent not ill not shown
CCE: ($\log_{10}$) qPCR Calibrator cell equivalents (delta-delta method). CFU: ($\log_{10}$) colony forming units
URI: Upper respiratory illness

The tables in the following sections show the adjusted odds ratio (AOR) as a measure of the association between indicator density exposure and illness. The AORs are interpreted as the increase in odds of illness associated with a 1-log increase in indicator exposure. For example, an AOR of 1.32, indicates a 32% increase in the odds of illness with every 1-log increase in indicator exposure. No association, or a flat slope, results in an AOR of 1, and AORs of less than 1 indicate an inverse association, or negative slope.

Enterococcus CFU (Method 1600)

The association between culturable *Enterococcus* exposure as measured by EPA Method 1600 and illness are shown in Tables 4.33-4.38. Generally, there were no consistent associations. Positive linear trends were observed between GI illness and diarrhea and *Enterococcus* CFU, but the associations were not of statistical significance. A slight inverse association was observed with respiratory illness (p=0.04) for head immersion exposure, but not body immersion.

Enterococcus CFU exceeded the EPA recommended criteria of 35 CFU per 100 ml on one day when the geometric mean of 18 samples was 57 CFU/100 ml. Evaluations of health effects for swimming exposure on this day are shown in Tables 4.40 and 4.41. There was some evidence of a trend for GI illnesses and rash which were elevated among body immersion swimmers exposed to *Enterococcus* CFU greater than 35 compared to non-swimmers as illnesses were most frequent among swimmers exposed and least frequent among non-swimmers. (Table 4.40). However, although swimmers exposed to CFU greater than 35 had a higher proportion of illnesses compared to swimmers below 35 CFU, these comparisons were not statistically significant.

Generally similar associations between *Enterococcus* CFU and illness were observed among children as were among all subjects. No statistically significant linear associations were observed between the incidence of illness and exposure to *Enterococcus* CFU among swimming children. Although there was some evidence of stronger associations, most notably for diarrhea and where adjusted odds ratios were 1.72 (p=0.14) and 1.76 (p=0.16) for the association between the daily average *Enterococcus* CFU and body immersion and head immersion swimming exposures, respectively (Table 4.39). There was some evidence of excess illness among children on the single day when *Enterococcus* CFU geometric mean exceeded the 35 CFU criterion, although the sample size was small. As shown in Tables 4.42 and 4.43, and illustrated in Figure 4.15, 17% of children immersing their body the day when *Enterococcus* exceeded 35 CFU reported GI illness compared to 7% of children immersing their body under 35 CFU and less than 4% of non-swimming children. However, this association was not as apparent for children immersing their head 4.43, and firm conclusions are hampered by the few numbers of exposed.

Table 4.33: Adjusted Odds Ratios *Enterococcus* CFU (Method 1600) and GI illness. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Surfside Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	1.18	0.25	0.89	1.55	7752
Waist depth	1.17	0.27	0.89	1.54	7752
Shin depth	1.16	0.27	0.89	1.52	7752
8:00 AM	1.06	0.56	0.87	1.30	7752
Swimming-location	1.10	0.48	0.84	1.44	7752
Head immersion					
Daily	1.17	0.30	0.86	1.60	6141
Waist depth	1.15	0.38	0.84	1.56	6141
Shin depth	1.19	0.27	0.88	1.61	6141
8:00 AM	1.05	0.64	0.84	1.32	6139
Swimming-location	1.09	0.59	0.80	1.49	6141

Table 4.34: Adjusted Odds Ratios *Enterococcus* CFU (Method 1600) and Diarrhea. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Surfside Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	1.32	0.10	0.95	1.83	7748
Waist depth	1.29	0.14	0.92	1.81	7748
Shin depth	1.33	0.08	0.97	1.83	7748
8:00 AM	1.19	0.16	0.94	1.52	7748
Swimming-location	1.31	0.10	0.95	1.81	7748
Head immersion					
Daily	1.34	0.13	0.92	1.94	6137
Waist depth	1.28	0.20	0.88	1.86	6137
Shin depth	1.35	0.09	0.95	1.94	6137
8:00 AM	1.19	0.21	0.90	1.58	6137
Swimming-location	1.27	0.22	0.87	1.85	6137

Table 4.35: Adjusted Odds Ratios *Enterococcus* CFU (Method 1600) and Respiratory illness. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Surfside Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	0.79	0.15	0.57	1.09	7725
Waist depth	0.79	0.15	0.57	1.09	7725
Shin depth	0.81	0.18	0.59	1.10	7725
8:00 AM	0.86	0.20	0.69	1.08	7725
Swimming-location	0.75	0.06	0.56	1.01	7725
Head immersion					
Daily	0.67	0.04	0.46	0.98	6122
Waist depth	0.69	0.05	0.48	1.00	6122
Shin depth	0.69	0.04	0.48	0.98	6122
8:00 AM	0.82	0.12	0.65	1.05	6122
Swimming-location	0.72	0.05	0.52	0.99	6122

Table 4.36: Adjusted Odds Ratios *Enterococcus* CFU (Method 1600) and Rash. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Surfside Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	1.11	0.55	0.78	1.60	7829
Waist depth	1.21	0.27	0.86	1.71	7833
Shin depth	1.00	0.99	0.67	1.51	7725
8:00 AM	1.01	0.93	0.77	1.34	7725
Swimming-location	1.11	0.55	0.78	1.58	7829
Head immersion					
Daily	1.00	0.99	0.65	1.55	6105
Waist depth	1.12	0.56	0.76	1.65	6178
Shin depth	0.88	0.56	0.59	1.33	6107
8:00 AM	0.93	0.62	0.70	1.24	6107
Swimming-location	1.00	0.99	0.67	1.51	6105

Table 4.37: Adjusted Odds Ratios *Enterococcus* CFU (Method 1600) and Earache. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Surfside Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	0.85	0.46	0.56	1.30	7927
Waist depth	0.86	0.46	0.57	1.29	7823
Shin depth	0.87	0.50	0.57	1.32	7925
8:00 AM	0.92	0.55	0.69	1.22	7821
Swimming-location	0.78	0.22	0.53	1.15	7927
Head immersion					
Daily	0.90	0.68	0.57	1.44	6285
Waist depth	0.90	0.64	0.57	1.41	6196
Shin depth	0.95	0.81	0.61	1.47	6285
8:00 AM	0.94	0.71	0.69	1.29	6196
Swimming-location	0.82	0.36	0.53	1.26	6287

Table 4.38: Adjusted Odds Ratios *Enterococcus* CFU (Method 1600) and Eye irritations. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Surfside Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	0.95	0.80	0.64	1.41	7901
Waist depth	0.94	0.74	0.63	1.38	7901
Shin depth	0.97	0.86	0.65	1.43	7901
8:00 AM	1.05	0.70	0.81	1.36	7903
Swimming-location	0.98	0.92	0.65	1.47	7903
Head immersion					
Daily	1.06	0.78	0.70	1.61	6261
Waist depth	0.97	0.88	0.63	1.50	6259
Shin depth	1.15	0.48	0.78	1.71	6264
8:00 AM	1.08	0.60	0.81	1.43	6264
Swimming-location	1.13	0.56	0.74	1.73	6264

Table 4.39: Adjusted Odds Ratios *Enterococcus* CFU (Method 1600) and Diarrhea. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Surfside Beach. Children age 10 and under.

Exposure	**AOR**	**P-value**	**Lower 95% CI**	**Upper 95% CI**	**N**
Body immersion					
Daily	1.72	0.14	0.84	3.51	1481
Waist depth	1.57	0.23	0.76	3.27	1481
Shin depth	1.79	0.10	0.90	3.55	1481
8:00 AM	1.46	0.14	0.88	2.42	1481
Swimming-location	1.76	0.09	0.91	3.41	1481
Head immersion					
Daily	1.76	0.16	0.80	3.89	1323
Waist depth	1.65	0.22	0.73	3.73	1323
Shin depth	1.78	0.14	0.83	3.80	1323
8:00 AM	1.49	0.15	0.86	2.57	1323
Swimming-location	1.83	0.11	0.88	3.82	1323

Table 4.40: Incident illness by exposure to *Enterococcus* CFU, above and below EPA criteria. Body immersion exposure, Surfside Beach

	Number ill	%[1]	aCIR[2] (p-value)	aCIR[3] (p-value)
GI				
Non-swimmer	79	4.67		
Swimmer-below 35 CFU	493	6.42		
Swimmer-above 35 CFU	15	8.62		
Total	587	6.15	1.8(0.0433)	1.42(0.196)
Upper respiratory				
Non-swimmer	73	4.36		
Swimmer-below 35 CFU	368	4.87		
Swimmer-above 35 CFU	5	2.91		
Total	446	4.74	0.66(0.3695)	0.54(0.1774)
Rash				
Non-swimmer	38	2.23		
Swimmer-below 35 CFU	343	4.48		
Swimmer-above 35 CFU	11	6.32		
Total	392	4.11	2.6(0.0318)	1.4(0.4242)
Earache				
Non-swimmer	24	1.39		
Swimmer-below 35 CFU	198	2.55		
Swimmer-above 35 CFU	3	1.7		
Total	225	2.33	1.23(0.7428)	0.65(0.4637)
Eye irritation				
Non-swimmer	27	1.56		
Swimmer-below 35 CFU	146	1.86		
Swimmer-above 35 CFU	0	0		
Total	173	1.78	()	()

1: Percentage of those within exposure category with symptom (row percentage). Number and percent not ill not shown
2: Adjusted Cumulative Incidence Ratio: Swimmers Above 35 CFU vs. non-swimmers
3: Adjusted Cumulative Incidence Ratio: Swimmers above 35 CFU vs. swimmers below 35 CFU

Table 4.41: Incident illness by exposure to *Enterococcus* CFU, above and below EPA criteria. Head immersion exposure, Surfside Beach

	Number ill	%[1]	aCIR[2] (p-value)	aCIR[3] (p-value)
GI				
Non-swimmer	79	4.67		
Swimmer-below 35 CFU	394	6.45		
Swimmer-above 35 CFU	11	9.09		
Total	484	6.11	1.84(0.0631)	1.45(0.2323)
Upper respiratory				
Non-swimmer	73	4.36		
Swimmer-below 35 CFU	293	4.88		
Swimmer-above 35 CFU	3	2.5		
Total	369	4.73	0.56(0.3225)	0.45(0.1683)
Rash				
Non-swimmer	38	2.23		
Swimmer-below 35 CFU	282	4.64		
Swimmer-above 35 CFU	6	4.92		
Total	326	4.12	1.86(0.1874)	1.11(0.816)
Earache				
Non-swimmer	24	1.39		
Swimmer-below 35 CFU	172	2.79		
Swimmer-above 35 CFU	3	2.44		
Total	199	2.48	1.4(0.596)	0.77(0.6729)
Eye irritation				
Non-swimmer	27	1.56		
Swimmer-below 35 CFU	113	1.81		
Swimmer-above 35 CFU	0	0		
Total	140	1.73	()	()

1: Percentage of those within exposure category with symptom (row percentage). Number and percent not ill not shown
2: Adjusted Cumulative Incidence Ratio: Swimmers Above 35 CFU vs. non-swimmers
3: Adjusted Cumulative Incidence Ratio: Swimmers above 35 CFU vs. swimmers below 35 CFU

Table 4.42: Incident illness by exposure to *Enterococcus* CFU, above and below EPA criteria. Children age 10 and under. Body immersion exposure, Surfside Beach

	Number ill	%[1]	aCIR[2] (p-value)	aCIR[3] (p-value)
GI				
Non-swimmer	3	3.45		
Swimmer-below 35 CFU	97	6.71		
Swimmer-above 35 CFU	6	16.67		
Total	106	6.76	5.94(0.0451)	3.01(0.018)
Upper respiratory				
Non-swimmer	6	7.32		
Swimmer-below 35 CFU	79	5.65		
Swimmer-above 35 CFU	3	8.33		
Total	88	5.81	1.24(0.7801)	1.54(0.4985)
Rash				
Non-swimmer	3	3.49		
Swimmer-below 35 CFU	78	5.48		
Swimmer-above 35 CFU	3	8.33		
Total	84	5.44	2.1(0.4621)	1.38(0.6985)
Earache				
Non-swimmer	2	2.27		
Swimmer-below 35 CFU	58	4.03		
Swimmer-above 35 CFU	1	2.78		
Total	61	3.9	0.9(0.9304)	0.57(0.5909)
Eye irritation				
Non-swimmer	1	1.12		
Swimmer-below 35 CFU	18	1.23		
Swimmer-above 35 CFU	0	0		
Total	19	1.2	()	()

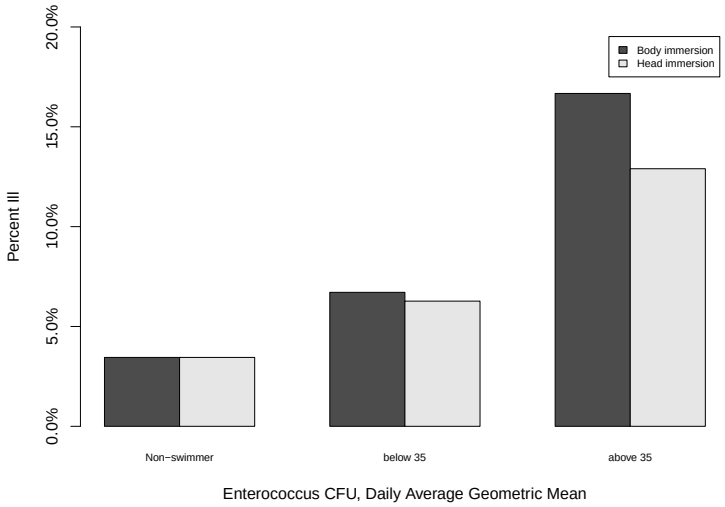
1: Percentage of those within exposure category with symptom (row percentage). Number and percent not ill not shown
2: Adjusted Cumulative Incidence Ratio: Swimmers Above 35 CFU vs. non-swimmers
3: Adjusted Cumulative Incidence Ratio: Swimmers above 35 CFU vs. swimmers below 35 CFU

Table 4.43: Incident illness by exposure to *Enterococcus* CFU, above and below EPA criteria. Children age 10 and under. Head immersion exposure, Surfside Beach

	Number ill	%[1]	aCIR[2] (p-value)	aCIR[3] (p-value)
GI				
Non-swimmer	3	3.45		
Swimmer-below 35 CFU	81	6.27		
Swimmer-above 35 CFU	4	12.9		
Total	88	6.24	4.11(0.1382)	2.34(0.1354)
Upper respiratory				
Non-swimmer	6	7.32		
Swimmer-below 35 CFU	69	5.52		
Swimmer-above 35 CFU	1	3.33		
Total	76	5.58	0.48(0.5037)	0.6(0.6181)
Rash				
Non-swimmer	3	3.49		
Swimmer-below 35 CFU	68	5.36		
Swimmer-above 35 CFU	1	3.23		
Total	72	5.19	0.96(0.9731)	0.65(0.6889)
Earache				
Non-swimmer	2	2.27		
Swimmer-below 35 CFU	53	4.12		
Swimmer-above 35 CFU	1	3.33		
Total	56	3.99	0.9(0.9331)	0.57(0.6004)
Eye irritation				
Non-swimmer	1	1.12		
Swimmer-below 35 CFU	14	1.07		
Swimmer-above 35 CFU	0	0		
Total	15	1.05	()	()

1: Percentage of those within exposure category with symptom (row percentage). Number and percent not ill not shown
2: Adjusted Cumulative Incidence Ratio: Swimmers Above 35 CFU vs. non-swimmers
3: Adjusted Cumulative Incidence Ratio: Swimmers above 35 CFU vs. swimmers below 35 CFU

Figure 4.15: GI illness among children age 10 and under and exposure to *Enterococcus* colony forming units above and below currently recommended EPA criteria for Method 1600. Surfside Beach



Enterococcus qPCR Calibrator Cell Equivalents

Associations between *Enterococcus* CCE and illness are shown in Tables 4.44-4.49 for the delta-delta CT method and Tables 4.50-4.55 for the delta CT method.

Non-significant trends were seen between GI illness and diarrhea and *Enterococcus* CCE. Associations for *Enterococcus* CCE calculated by the delta-delta CT method and diarrhea for body immersion exposure (AOR=1.27, p=0.08) are shown in Table 4.45. This association was slightly lessened for CCE calculated by the delta-CT method (AOR=1.24, p=0.20 for body immersion exposure, Table 4.51) No other positive associations were observed. An unexplained inverse association was observed between *Enterococcus* CCE and skin rash.

Non-significant trends were observed among children 10 and under with *Enterococcus* CCE and diarrhea (AOR=1.51, p=0.15 and AOR=1.36, p=0.27; AOR=1.24, p=0.53 for body immersion by the delta and delta-delta CT methods see Tables 4.56 and 4.57).

Table 4.44: Adjusted Odds Ratios *Enterococcus* qPCR CCE, Delta-delta CT calculation and GI illness. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Surfside Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	1.15	0.18	0.94	1.42	7752
Waist depth	1.16	0.16	0.94	1.41	7752
Shin depth	1.15	0.20	0.93	1.42	7752
8:00 AM	1.08	0.31	0.93	1.25	7750
Swimming-location	1.12	0.28	0.91	1.39	7752
Head immersion					
Daily	1.17	0.21	0.92	1.49	6141
Waist depth	1.14	0.26	0.90	1.44	6141
Shin depth	1.18	0.19	0.92	1.51	6141
8:00 AM	1.07	0.43	0.90	1.27	6141
Swimming-location	1.14	0.28	0.90	1.46	6141

Table 4.45: Adjusted Odds Ratios *Enterococcus* qPCR CCE, Delta-delta CT calculation and Diarrhea. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Surfside Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	1.27	0.08	0.97	1.64	7748
Waist depth	1.23	0.10	0.96	1.58	7748
Shin depth	1.26	0.08	0.97	1.64	7748
8:00 AM	1.16	0.10	0.97	1.40	7748
Swimming-location	1.25	0.10	0.96	1.62	7748
Head immersion					
Daily	1.23	0.18	0.91	1.66	6137
Waist depth	1.19	0.23	0.90	1.58	6137
Shin depth	1.24	0.17	0.92	1.68	6137
8:00 AM	1.14	0.24	0.92	1.40	6137
Swimming-location	1.22	0.20	0.90	1.64	6137

Table 4.46: Adjusted Odds Ratios *Enterococcus* qPCR CCE, Delta-delta CT calculation and Respiratory illness. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Surfside Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	1.05	0.68	0.82	1.34	7618
Waist depth	1.05	0.69	0.83	1.32	7721
Shin depth	1.05	0.70	0.83	1.32	7721
8:00 AM	0.99	0.89	0.84	1.17	7616
Swimming-location	1.05	0.69	0.82	1.34	7618
Head immersion					
Daily	1.09	0.47	0.86	1.40	6031
Waist depth	1.13	0.34	0.88	1.45	6122
Shin depth	1.04	0.76	0.82	1.30	6097
8:00 AM	1.03	0.74	0.86	1.23	6031
Swimming-location	1.10	0.46	0.86	1.41	6122

Table 4.47: Adjusted Odds Ratios *Enterococcus* qPCR CCE, Delta-delta CT calculation and Rash. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Surfside Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	0.66	0.01	0.47	0.91	7833
Waist depth	0.70	0.03	0.51	0.96	7833
Shin depth	0.65	0.01	0.47	0.88	7833
8:00 AM	0.74	0.01	0.60	0.92	7833
Swimming-location	0.67	0.01	0.50	0.91	7833
Head immersion					
Daily	0.62	0.02	0.42	0.91	6110
Waist depth	0.68	0.04	0.47	0.99	6181
Shin depth	0.59	0.00	0.41	0.85	6110
8:00 AM	0.72	0.01	0.56	0.92	6110
Swimming-location	0.66	0.02	0.46	0.92	6197

Table 4.48: Adjusted Odds Ratios *Enterococcus* qPCR CCE, Delta-delta CT calculation and Earache. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Surfside Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	0.85	0.31	0.62	1.16	7927
Waist depth	0.81	0.19	0.60	1.11	7927
Shin depth	0.91	0.53	0.68	1.22	7927
8:00 AM	0.87	0.18	0.71	1.07	7927
Swimming-location	0.79	0.14	0.58	1.08	7927
Head immersion					
Daily	0.84	0.30	0.61	1.17	6287
Waist depth	0.81	0.20	0.58	1.12	6287
Shin depth	0.90	0.49	0.66	1.22	6287
8:00 AM	0.86	0.17	0.69	1.07	6287
Swimming-location	0.76	0.10	0.54	1.05	6287

Table 4.49: Adjusted Odds Ratios *Enterococcus* qPCR CCE, Delta-delta CT calculation and Eye irritations. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Surfside Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	1.10	0.58	0.79	1.54	7983
Waist depth	1.07	0.66	0.78	1.48	7905
Shin depth	1.11	0.56	0.79	1.56	7983
8:00 AM	1.08	0.49	0.86	1.36	7983
Swimming-location	1.12	0.51	0.80	1.55	7983
Head immersion					
Daily	1.30	0.13	0.93	1.82	6353
Waist depth	1.23	0.22	0.88	1.73	6353
Shin depth	1.30	0.11	0.94	1.80	6353
8:00 AM	1.18	0.18	0.92	1.51	6350
Swimming-location	1.29	0.16	0.90	1.84	6328

Table 4.50: Adjusted Odds Ratios *Enterococcus* qPCR CCE, Delta CT calculation and GI illness. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Surfside Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	1.16	0.18	0.93	1.46	7752
Waist depth	1.17	0.16	0.94	1.46	7752
Shin depth	1.15	0.22	0.92	1.43	7752
8:00 AM	1.08	0.30	0.93	1.26	7750
Swimming-location	1.11	0.35	0.89	1.40	7752
Head immersion					
Daily	1.19	0.19	0.92	1.54	6141
Waist depth	1.16	0.26	0.90	1.48	6141
Shin depth	1.19	0.18	0.92	1.54	6141
8:00 AM	1.07	0.43	0.90	1.28	6141
Swimming-location	1.14	0.34	0.88	1.47	6141

Table 4.51: Adjusted Odds Ratios *Enterococcus* qPCR CCE, Delta CT calculation and Diarrhea. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Surfside Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	1.26	0.10	0.95	1.67	7748
Waist depth	1.25	0.11	0.95	1.63	7748
Shin depth	1.25	0.12	0.95	1.65	7748
8:00 AM	1.16	0.12	0.96	1.41	7748
Swimming-location	1.24	0.13	0.94	1.64	7748
Head immersion					
Daily	1.24	0.20	0.90	1.71	6137
Waist depth	1.21	0.23	0.89	1.64	6137
Shin depth	1.24	0.20	0.90	1.71	6137
8:00 AM	1.13	0.27	0.91	1.42	6137
Swimming-location	1.20	0.26	0.87	1.66	6137

Table 4.52: Adjusted Odds Ratios *Enterococcus* qPCR CCE, Delta CT calculation and Respiratory illness. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Surfside Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	1.03	0.81	0.80	1.34	7618
Waist depth	1.03	0.84	0.79	1.34	7616
Shin depth	1.03	0.79	0.81	1.32	7721
8:00 AM	0.97	0.76	0.82	1.15	7719
Swimming-location	1.02	0.88	0.78	1.33	7618
Head immersion					
Daily	1.05	0.73	0.79	1.39	6029
Waist depth	1.09	0.53	0.83	1.44	6031
Shin depth	1.01	0.96	0.77	1.32	6029
8:00 AM	1.01	0.93	0.83	1.22	6029
Swimming-location	1.04	0.77	0.79	1.36	6119

Table 4.53: Adjusted Odds Ratios *Enterococcus* qPCR CCE, Delta CT calculation and Rash. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Surfside Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	0.56	0.00	0.39	0.80	7731
Waist depth	0.61	0.00	0.44	0.86	7833
Shin depth	0.55	0.00	0.40	0.77	7833
8:00 AM	0.69	0.00	0.56	0.86	7731
Swimming-location	0.56	0.00	0.40	0.78	7731
Head immersion					
Daily	0.54	0.00	0.36	0.79	6197
Waist depth	0.58	0.01	0.40	0.86	6110
Shin depth	0.51	0.00	0.35	0.75	6197
8:00 AM	0.66	0.00	0.51	0.85	6110
Swimming-location	0.53	0.00	0.37	0.76	6197

Table 4.54: Adjusted Odds Ratios *Enterococcus* qPCR CCE, Delta CT calculation and Earache. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Surfside Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	0.83	0.28	0.60	1.16	7927
Waist depth	0.79	0.17	0.56	1.11	7927
Shin depth	0.89	0.48	0.66	1.22	7927
8:00 AM	0.85	0.15	0.69	1.06	7927
Swimming-location	0.75	0.10	0.54	1.05	7927
Head immersion					
Daily	0.85	0.35	0.60	1.20	6287
Waist depth	0.81	0.24	0.57	1.15	6287
Shin depth	0.90	0.55	0.65	1.25	6287
8:00 AM	0.85	0.18	0.68	1.07	6287
Swimming-location	0.74	0.09	0.52	1.05	6287

Table 4.55: Adjusted Odds Ratios *Enterococcus* qPCR CCE, Delta CT calculation and Eye irritations. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Surfside Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	1.07	0.72	0.75	1.51	8005
Waist depth	1.03	0.86	0.74	1.44	7905
Shin depth	1.09	0.63	0.76	1.56	7983
8:00 AM	1.05	0.69	0.83	1.32	7983
Swimming-location	1.09	0.63	0.77	1.55	7983
Head immersion					
Daily	1.26	0.22	0.87	1.83	6350
Waist depth	1.19	0.34	0.83	1.72	6350
Shin depth	1.30	0.16	0.90	1.87	6353
8:00 AM	1.14	0.34	0.87	1.48	6350
Swimming-location	1.28	0.21	0.87	1.89	6331

Table 4.56: Adjusted Odds Ratios *Enterococcus* qPCR CCE, Delta CT calculation and Diarrhea. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Surfside Beach. Children age 10 and under.

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	1.54	0.15	0.86	2.77	1481
Waist depth	1.40	0.24	0.80	2.43	1481
Shin depth	1.74	0.10	0.91	3.35	1481
8:00 AM	1.47	0.11	0.92	2.34	1481
Swimming-location	1.55	0.14	0.87	2.75	1481
Head immersion					
Daily	1.47	0.29	0.72	2.98	1323
Waist depth	1.37	0.35	0.71	2.63	1323
Shin depth	1.52	0.26	0.73	3.17	1323
8:00 AM	1.39	0.19	0.85	2.28	1323
Swimming-location	1.50	0.25	0.76	3.00	1323

Table 4.57: Adjusted Odds Ratios *Enterococcus* qPCR CCE, Delta-delta CT calculation and Diarrhea. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Surfside Beach. Children age 10 and under.

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	1.36	0.27	0.79	2.36	1481
Waist depth	1.23	0.44	0.73	2.05	1481
Shin depth	1.49	0.17	0.84	2.62	1481
8:00 AM	1.40	0.14	0.89	2.18	1481
Swimming-location	1.37	0.25	0.80	2.36	1481
Head immersion					
Daily	1.24	0.53	0.64	2.42	1323
Waist depth	1.16	0.64	0.63	2.13	1323
Shin depth	1.35	0.40	0.67	2.73	1323
8:00 AM	1.33	0.24	0.83	2.13	1323
Swimming-location	1.29	0.44	0.67	2.47	1323

Bacteroidales qPCR Calibrator Cell Equivalents

Associations between *Bacteroidales* CCE and illness are shown in Tables 4.58-4.63 for the delta-delta CT method and Tables 4.64-4.69 for the delta CT method.

No associations were observed between illness incidence and *Bacteroidales* CCE exposure (Tables 4.58 and 4.60). Similar patterns were observed for the delta-delta CT and the delta CT methods.

Statistically significant associations were observed for respiratory illness among children 10 and under with exposure to *Bacterdoidales* CCE (AOR=2.95, p=0.05, for delta CT method) for body immersion exposure but slightly less association among those with head immersion exposure (AOR=2.53, p=0.11). However, this finding should be interpreted with caution since as there was evidence of strong confounding which influenced this association. There was considerable difference between the unadjusted estimates (AOR=1.44, p=0.42) and the adjusted estimates shown above. The two principal components of environmental measures were the factors which seemed to strongly influence the adjusted results. Furthermore, the effect was restricted to comparisons among swimmers. Non-swimming children had a higher adjusted incidence of respiratory illness (6.4% among non-swimming children; compared to 6.8% among most highly exposed swimming children) complicating the risk interpretation. This is illustrated in Figure 4.16.

Table 4.58: Adjusted Odds Ratios *Bacteroidales* qPCR CCE, Delta-delta CT calculation and GI illness. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Surfside Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	1.19	0.35	0.83	1.70	7750
Waist depth	1.15	0.46	0.79	1.68	7853
Shin depth	1.22	0.24	0.87	1.71	7752
8:00 AM	1.21	0.35	0.81	1.81	7750
Swimming-location	1.18	0.32	0.85	1.63	7752
Head immersion					
Daily	1.14	0.52	0.76	1.72	6139
Waist depth	1.06	0.78	0.70	1.60	6139
Shin depth	1.20	0.34	0.82	1.75	6139
8:00 AM	1.15	0.55	0.72	1.83	6139
Swimming-location	1.18	0.38	0.82	1.70	6141

Table 4.59: Adjusted Odds Ratios *Bacteroidales* qPCR CCE, Delta-delta CT calculation and Diarrhea. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Surfside Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	1.19	0.43	0.77	1.86	7748
Waist depth	1.07	0.78	0.68	1.68	7746
Shin depth	1.25	0.28	0.83	1.88	7748
8:00 AM	1.35	0.23	0.82	2.23	7748
Swimming-location	1.12	0.60	0.74	1.68	7746
Head immersion					
Daily	1.16	0.56	0.70	1.92	6135
Waist depth	1.00	0.99	0.61	1.66	6135
Shin depth	1.28	0.29	0.81	2.03	6135
8:00 AM	1.26	0.43	0.71	2.22	6135
Swimming-location	1.10	0.69	0.69	1.75	6137

Table 4.60: Adjusted Odds Ratios *Bacteroidales* qPCR CCE, Delta-delta CT calculation and Respiratory illness. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Surfside Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	1.18	0.51	0.73	1.90	7725
Waist depth	1.09	0.71	0.68	1.75	7719
Shin depth	1.23	0.38	0.78	1.94	7725
8:00 AM	1.02	0.93	0.61	1.73	7616
Swimming-location	1.10	0.65	0.72	1.68	7622
Head immersion					
Daily	1.23	0.43	0.73	2.09	6120
Waist depth	1.13	0.64	0.68	1.89	6029
Shin depth	1.27	0.34	0.77	2.09	6122
8:00 AM	1.04	0.89	0.59	1.83	6032
Swimming-location	1.19	0.46	0.75	1.91	6120

Table 4.61: Adjusted Odds Ratios *Bacteroidales* qPCR CCE, Delta-delta CT calculation and Rash. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Surfside Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	0.92	0.69	0.60	1.41	7729
Waist depth	0.99	0.97	0.62	1.58	7725
Shin depth	0.86	0.44	0.58	1.27	7827
8:00 AM	1.42	0.19	0.84	2.42	7833
Swimming-location	1.06	0.78	0.71	1.57	7729
Head immersion					
Daily	0.81	0.41	0.50	1.32	6108
Waist depth	0.88	0.61	0.53	1.46	6108
Shin depth	0.78	0.27	0.49	1.22	6195
8:00 AM	1.32	0.36	0.73	2.36	6110
Swimming-location	0.97	0.91	0.61	1.56	6105

Table 4.62: Adjusted Odds Ratios *Bacteroidales* qPCR CCE, Delta-delta CT calculation and Earache. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Surfside Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	0.94	0.82	0.55	1.60	7908
Waist depth	0.95	0.83	0.57	1.57	7817
Shin depth	0.95	0.84	0.57	1.59	7895
8:00 AM	1.15	0.62	0.65	2.04	7927
Swimming-location	0.92	0.73	0.57	1.48	7921
Head immersion					
Daily	0.97	0.91	0.56	1.68	6263
Waist depth	0.95	0.85	0.55	1.65	6196
Shin depth	0.99	0.97	0.58	1.67	6193
8:00 AM	1.19	0.58	0.64	2.21	6287
Swimming-location	0.96	0.87	0.58	1.58	6285

Table 4.63: Adjusted Odds Ratios *Bacteroidales* qPCR CCE, Delta-delta CT calculation and Eye irritations. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Surfside Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	1.17	0.66	0.59	2.31	7905
Waist depth	1.05	0.89	0.54	2.02	8003
Shin depth	1.26	0.49	0.66	2.41	7905
8:00 AM	0.89	0.75	0.44	1.81	7905
Swimming-location	1.07	0.82	0.59	1.95	7981
Head immersion					
Daily	1.53	0.26	0.72	3.25	6353
Waist depth	1.27	0.53	0.60	2.68	6353
Shin depth	1.71	0.13	0.86	3.39	6353
8:00 AM	0.99	0.98	0.43	2.27	6259
Swimming-location	1.29	0.45	0.67	2.51	6353

Table 4.64: Adjusted Odds Ratios *Bacteroidales* qPCR CCE, Delta CT calculation and GI illness. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Surfside Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	1.14	0.53	0.75	1.73	7750
Waist depth	1.08	0.72	0.72	1.62	7853
Shin depth	1.19	0.37	0.81	1.73	7752
8:00 AM	1.13	0.57	0.74	1.75	7853
Swimming-location	1.11	0.58	0.77	1.60	7752
Head immersion					
Daily	1.13	0.61	0.71	1.79	6139
Waist depth	1.02	0.92	0.65	1.60	6139
Shin depth	1.21	0.39	0.78	1.86	6139
8:00 AM	1.10	0.71	0.66	1.82	6139
Swimming-location	1.13	0.57	0.74	1.71	6141

Table 4.65: Adjusted Odds Ratios *Bacteroidales* qPCR CCE, Delta CT calculation and Diarrhea. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Surfside Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	1.06	0.82	0.64	1.76	7746
Waist depth	0.94	0.81	0.57	1.54	7746
Shin depth	1.16	0.53	0.72	1.86	7746
8:00 AM	1.16	0.58	0.68	1.99	7746
Swimming-location	1.01	0.96	0.64	1.60	7746
Head immersion					
Daily	1.06	0.85	0.60	1.88	6135
Waist depth	0.89	0.69	0.50	1.58	6223
Shin depth	1.20	0.49	0.71	2.03	6135
8:00 AM	1.05	0.87	0.57	1.94	6135
Swimming-location	0.97	0.91	0.57	1.65	6135

Table 4.66: Adjusted Odds Ratios *Bacteroidales* qPCR CCE, Delta CT calculation and Respiratory illness. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Surfside Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	1.10	0.70	0.67	1.83	7702
Waist depth	0.99	0.95	0.62	1.56	7616
Shin depth	1.23	0.40	0.76	1.96	7725
8:00 AM	0.91	0.71	0.56	1.48	7706
Swimming-location	1.01	0.98	0.64	1.57	7616
Head immersion					
Daily	1.07	0.81	0.62	1.85	6029
Waist depth	0.88	0.63	0.54	1.45	6029
Shin depth	1.25	0.39	0.75	2.08	6120
8:00 AM	0.81	0.42	0.50	1.34	6122
Swimming-location	1.05	0.85	0.64	1.72	6029

Table 4.67: Adjusted Odds Ratios *Bacteroidales* qPCR CCE, Delta CT calculation and Rash. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Surfside Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	0.65	0.09	0.40	1.06	7831
Waist depth	0.76	0.27	0.46	1.25	7729
Shin depth	0.64	0.05	0.40	1.00	7831
8:00 AM	1.08	0.80	0.60	1.93	7810
Swimming-location	0.79	0.32	0.50	1.26	7831
Head immersion					
Daily	0.57	0.04	0.33	0.98	6197
Waist depth	0.66	0.14	0.38	1.14	6110
Shin depth	0.52	0.02	0.30	0.89	6197
8:00 AM	0.94	0.85	0.51	1.75	6176
Swimming-location	0.68	0.16	0.40	1.16	6197

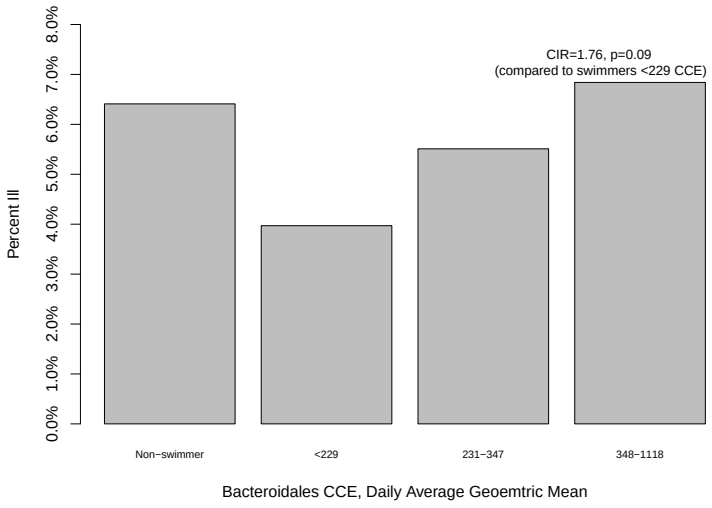
Table 4.68: Adjusted Odds Ratios *Bacteroidales* qPCR CCE, Delta CT calculation and Earache. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Surfside Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	0.94	0.84	0.51	1.72	7817
Waist depth	0.97	0.91	0.54	1.74	7817
Shin depth	0.92	0.80	0.51	1.67	7895
8:00 AM	1.17	0.60	0.66	2.06	7927
Swimming-location	0.89	0.67	0.51	1.53	7925
Head immersion					
Daily	1.04	0.90	0.54	2.00	6196
Waist depth	1.04	0.90	0.55	1.97	6196
Shin depth	1.04	0.90	0.57	1.91	6196
8:00 AM	1.35	0.35	0.72	2.52	6287
Swimming-location	0.98	0.95	0.54	1.78	6196

Table 4.69: Adjusted Odds Ratios *Bacteroidales* qPCR CCE, Delta CT calculation and Eye irritations. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Surfside Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	0.98	0.97	0.47	2.08	7899
Waist depth	0.83	0.58	0.42	1.61	8009
Shin depth	1.17	0.67	0.56	2.47	7899
8:00 AM	0.56	0.10	0.28	1.13	8009
Swimming-location	0.95	0.89	0.49	1.84	7977
Head immersion					
Daily	1.24	0.61	0.54	2.81	6353
Waist depth	0.93	0.84	0.43	1.99	6332
Shin depth	1.48	0.32	0.69	3.16	6353
8:00 AM	0.53	0.12	0.24	1.19	6353
Swimming-location	1.10	0.80	0.54	2.26	6351

Figure 4.16: Adjusted probabilities of respiratory illness among children age 10 and under and exposure to *Bacteroidales* CCE (delta CT). Surfside Beach



4.2 Boquerón Beach

4.2.1 Final site selection

Study investigators reviewed additional existing data and met with local and regional officials to make the final beach site selection. Based on the criteria described in Section 3.1.1 Boquerón Beach in the southwest of Puerto Rico was selected. High attendance at the beach was confirmed by the local beach manager as well as EPA's Caribbean Division staff.

Review of existing data confirmed a wide range in *Enterococcus* CFU densities. Over 58 samples taken from 2003-2007 *Enterococcus* CFU ranged from 0 to 605 CFU per 100 ml (mean=61, median=9 CFU per 100 ml). Twenty-four samples collected and tested by the EPA in the fall and winter of 2008 showed moderately low levels, but a range of 0-58 *Enterococcus* CFU per 100 ml (mean=10, median=5 CFU per 100 ml). A sewage treatment plant discharges into the bay less than 1 mile from the beach site. In addition, two smaller plants, which operate in times of high demand only discharge into the "mangrove swamp" which connects to the bay adjacent also under 1 mile away (Figure 4.18).

4.2.2 Site description

Boquerón Bay is a large horse-shoe shaped bay that is open to the Caribbean in the west. Boquerón Beach, which is approximately 1 mile long, is situated at the eastern side of the bay. It is gently sloping, shallow, with fine sand. There water is very calm with very little wave action and as a result wave sports or wave riding are not done here. The maximum approximate wave height observed during the study was about 0.5 feet

Information regarding the treatment plants was obtained through discussions with local EPA officials and through permit information. The Boquerón waste water treatment plant (WWTP) is a secondary wastewater treatment facility which is part of the Puerto Rico Aqueduct and Sewer Authority (NPDES Permit Number PR0023442). The WWTP is an activated sludge package plant with a capacity of 0.25 million gallons per day (MGD). Effluent is disinfected by chlorination/dechlorination. However, the WWTP is overloaded and discharges from 0.260 MGD to 0.60 MGD during high tourist season (April to August). The population served by the WWTP plant is 13,200 people.

The two smaller plants are privately owned by the the Recreational Development Company, and are covered by one NPDES permit (PR0021326) with 2 pipe outfalls, authorized to discharge up to 0.02 MGD each. These plants are used by cabins and rental facilities in the area. Treatment consists of aerobic digestions and disinfection by chlorination/dechlorination. The operator reported that during heavy rain and high tourism, the flow exceeds the plant capacity and causes overflows.

The beach site at Boquerón, locations of the outfalls and sampling locations are shown in Figure 4.17 and 4.18.

Figure 4.17: Boquerón Beach, Puerto Rico

Figure 4.18: Treatment plant discharges (POTW), beach site and contaminated sampling sites, Boquerón Beach, Puerto Rico

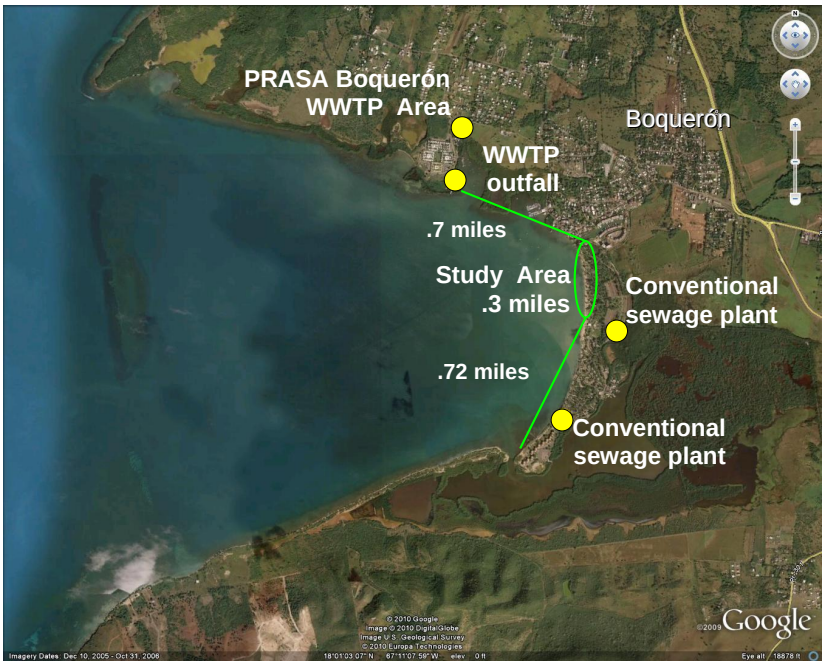
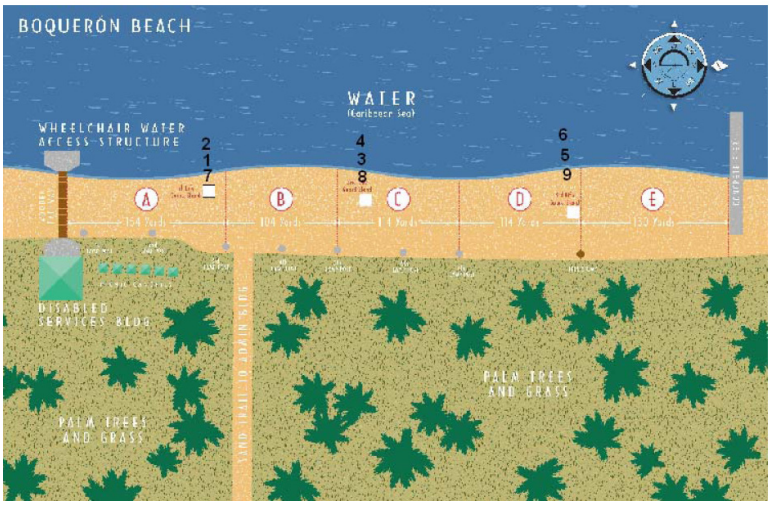


Figure 4.19: Boquerón Beach. Beach Site Sample Locations



Although tourists from outside of Puerto Rico do recreate at Boquerón Beach, it is highly popular with local residents, starting during school vacations and state holidays in April and extending throughout the summer season. Water temperatures remain relatively warm year round, but attendance and swimming drops off during the fall and winter seasons.

A schematic of Boquerón Beach and the location of water sampling sites are shown in Figure 4.19.

4.2.3 Health survey and respondent characteristics

Enrollment

The health surveys and interviews began in Boquerón Beach on May 16, 2009 and concluded on August 2, 2009. The study was conducted on 26 days. A total of 19,652 individuals from 8,748 households were offered enrollment. Of these, 581 households were ineligible because they either completed the study within the previous 28 days or there was no adult 18 years of age or older. Of those eligible, a total of 18,483 individuals from 7,724 households agreed to participate and completed the first interview. 16,505 individuals (90%) from 6,877 households (90%) returned to complete the second interview as they were leaving the beach for the day.

After accounting for probable duplicates, ineligible observations, and those who did not complete the final telephone interview, the final dataset consisted of a total of 15,726 individuals from 6,611 households. This represented 76% of those households initially approached and 96% of those completing the beach interview.

Note that in the following tables, deviations of the total from 15,726 are due to missing responses, except for tables for incident illness where respondents with baseline symptoms are also excluded.

Respondent characteristics and demographics

Basic demographic characteristics of the enrolled subjects are shown in Table 4.70. Nearly all participants identified themselves as "Hispanic" race/ethnicity >99%, likely reflecting the popularity of the beach with Puerto Rico residents. There were slightly more female respondents than males and children under 12 comprised 12% of the study population.

Baseline health conditions and illnesses are shown in Table 4.71. The most frequently reported chronic health condition was allergies, reported by 13% of subjects, followed by asthma, reported by 11%. Other health conditions and symptoms in the previous 24 hours were reported infrequently.

4.2.4 Swimming exposure

Swimming and related exposures are shown in Table 4.72. Compared to our previous studies at freshwater sites in the Great Lakes [2, 3] and similar to observations at Surfside Beach, a high proportion of subjects reported swimming exposure. Over 80% of subjects had at least some exposure to water, nearly 80% immersed their body and over 60% immersed their head.

Factors associated with body immersion and head immersion swimming exposure are shown in Table 4.73 and 4.74, respectively. Swimming exposure was associated with younger age, and was highest among those 5-10 years of age of whom 90% immersed their head and 95% immersed their body. The lowest frequency of water exposure was again among those age 55 and older of whom 68% immersed their head and 54% immersed their body. Male gender

Table 4.70: Basic demographics, Boquerón Beach

	N	%
Days of Study		
Total	26	100.00
Interviews		
Total	15726	100.00
Sex		
Male	7052	44.90
Female	8654	55.10
Total	15706	100.00
Age		
0-4	908	5.85
5-11	1791	11.55
12-19	2272	14.65
20-34	4407	28.41
35 and over	6134	39.54
Total	15512	100.00
Race		
White	65	0.41
Black	13	0.08
Asian	4	0.03
Am. Indian	2	0.01
Hispanic	15609	99.33
Multi-racial	1	0.01
Other	20	0.13
Total	15714	100.00
Annual Visits to Beach		
1-5	4592	29.20
6-10	7533	47.90
Over 10	3600	22.89
Total	15725	100.00

Table 4.71: Baseline illness and other health conditions, Boquerón Beach

	N	%
Chronic GI illness		
No	14969	95.19
Yes	756	4.81
Total	15725	100.00
Allergies		
No	13715	87.22
Yes	2010	12.78
Total	15725	100.00
Asthma		
No	14041	89.29
Yes	1684	10.71
Total	15725	100.00
Chronic skin condition		
No	15177	96.52
Yes	548	3.48
Total	15725	100.00
GI symptoms in past 3 days		
No	15416	98.03
Yes	309	1.97
Total	15725	100.00
Vomiting in past 3 days		
No	15595	99.17
Yes	130	0.83
Total	15725	100.00
Sore throat in past 3 days		
No	14462	91.97
Yes	1263	8.03
Total	15725	100.00
Skin rash in past 3 days		
No	15572	99.03
Yes	153	0.97
Total	15725	100.00
Earache in past 3 days		
No	15501	98.58
Yes	224	1.42
Total	15725	100.00
Eye infection in past 3 days		
No	15572	99.03
Yes	153	0.97
Total	15725	100.00

Table 4.72: Swimming and related exposures, Boquerón Beach

	N	%
Any contact with water		
No	2995	19.19
Yes	12615	80.81
Total	15610	100.00
Body immerison in water		
No	3499	22.42
Yes	12111	77.58
Total	15610	100.00
Head immersion in water		
No	5531	35.44
Yes	10074	64.56
Total	15605	100.00
Swallowed water		
No	12741	82.88
Yes	2632	17.12
Total	15373	100.00
Swam 1 week before beach visit		
No	11473	72.97
Yes	4249	27.03
Total	15722	100.00
Swam after beach visit		
No	10604	67.89
Yes	5015	32.11
Total	15619	100.00
Dug in sand		
No	11903	76.29
Yes	3699	23.71
Total	15602	100.00
Body buried in sand		
No	14962	95.90
Yes	640	4.10
Total	15602	100.00

was strongly associated with head immersion and to a lesser extent body immersion exposures. Head immersion exposure was also associated with absence of chronic GI illness, absence of chronic skin condition, presence of asthma, and no contact with other ill persons. Body immersion exposure was associated with similar factors with the exception of absence of chronic skin condition. Body immersion was also associated with unknown animal contact and more frequent beach visits.

Due to the large sample size, these statistically significant differences were often not necessarily meaningful and in many cases represent a small difference on an absolute scale. For example, 67% of those with asthma compared to 64% of those without asthma immersed their head, p=0.006, Table 4.74)

4.2.5 Health effects

Incident illness

Incident health effects are presented among subjects without reporting illness at baseline. The overall incidence of the health outcomes studied are shown in Table 4.75. Unlike other beach sites studied, respiratory illness was the most frequently reported illness at Boquerón Beach with nearly 7% of respondents reporting such an illness in the 10-12 day follow up period. Following respiratory illness, GI illness and skin rash were the next most commonly reported (4.7% and 4.4%, respectively). Eye irritations and earaches were reported by 3.6% and 1.9% of respondents, respectively.

Factors associated with incident illness

Non-swimming risk factors associated with the health outcomes studied are shown in Tables 4.76- 4.80.

GI illness GI illness was most frequent among young children (8.0% among those 0-4 years) and least frequent among those 11-18 and 55 and over (3.9% and 4.0%, respectively). Other factors associated with GI illness were female gender, less frequent visits to the beach, asthma, consumption of undercooked meat, chronic GI condition, unknown animal contact and contact with other ill people (Table 4.76).

Respiratory illness Respiratory illness was strongly associated with young age, with nearly 12% of those age 0-4 reporting an illness. It was reported least frequently among those 55 and over among whom 5% reported a respiratory illness. Respiratory illness was also associated with a chronic skin condition, asthma, consumption of raw or undercooked meat and raw fish, and contact with other ill people (Table 4.77).

Skin rash Skin rash was unassociated with age category. Female gender, chronic skin and GI condition, asthma, consumption of raw or undercooked

Table 4.73: Factors associated with swimming exposure (body immersion), Boquerón Beach

	Non-swimmer		Waders		Swimmer		P-value[1]
	N	%[2]	N	%[2]	N	%[2]	
Age category							
0-4	97	10.86	32	3.58	764	85.55	
5-10	71	4.00	24	1.35	1678	94.64	
11-19	277	12.30	35	1.55	1940	86.15	
20-54	799	18.24	160	3.65	3422	78.11	
55 and over	1709	27.98	248	4.06	4150	67.95	<0.001
Sex							
Male	1168	16.71	158	2.26	5664	81.03	
Female	1823	21.20	346	4.02	6431	74.78	<0.001
Race							
Non-white	2975	19.15	501	3.23	12058	77.62	
White	16	25.00	3	4.69	45	70.31	0.3683
Visits to this beach							
1 or less	920	20.12	146	3.19	3507	76.69	
2-5	1410	18.88	216	2.89	5841	78.22	
6 or more	665	18.63	142	3.98	2762	77.39	0.0127
Chronic GI illness							
No	2824	19.01	466	3.14	11565	77.85	
Yes	171	22.68	38	5.04	545	72.28	<0.001
Skin condition							
No	2871	19.06	485	3.22	11709	77.72	
Yes	124	22.79	19	3.49	401	73.71	0.0808
Asthma							
No	2720	19.52	455	3.27	10758	77.21	
Yes	275	16.41	49	2.92	1352	80.67	0.0055
Undercooked meat							
No	2879	19.30	471	3.16	11569	77.55	
Yes	116	16.79	33	4.78	542	78.44	0.0224
Unfamiliar animals							
No	2899	19.36	479	3.20	11593	77.44	
Yes	96	15.05	25	3.92	517	81.03	0.0188
Others with GI illness							
No	2744	18.98	463	3.20	11247	77.81	
Yes	251	21.71	41	3.55	864	74.74	0.0538

1: Pearson's Chi-square test of independence
2: Row percentages add to 100%

Table 4.74: Factors associated with swimming exposure (head immersion), Boquerón Beach

	Non-swimmer		Waders		Swimmer		P-value[1]
	N	%[2]	N	%[2]	N	%[2]	
Age category							
0-4	97	10.87	148	16.59	647	72.53	
5-10	71	4.01	114	6.43	1587	89.56	
11-19	277	12.31	199	8.84	1775	78.85	
20-54	799	18.24	938	21.42	2643	60.34	
55 and over	1709	27.99	1107	18.13	3290	53.88	<0.001
Sex							
Male	1168	16.72	715	10.23	5104	73.05	
Female	1823	21.20	1814	21.10	4961	57.70	<0.001
Race							
Non-white	2975	19.16	2523	16.25	10031	64.60	
White	16	25.00	10	15.62	38	59.38	0.4921
Visits to this beach							
1 or less	920	20.12	746	16.32	2906	63.56	
2-5	1410	18.89	1198	16.05	4855	65.05	
6 or more	665	18.63	591	16.56	2313	64.81	0.3526
Chronic GI illness							
No	2824	19.02	2359	15.89	9667	65.10	
Yes	171	22.68	176	23.34	407	53.98	<0.001
Skin condition							
No	2871	19.06	2428	16.12	9761	64.81	
Yes	124	22.79	107	19.67	313	57.54	0.0023
Asthma							
No	2720	19.53	2256	16.20	8952	64.27	
Yes	275	16.41	279	16.65	1122	66.95	0.0090
Undercooked meat							
No	2879	19.30	2409	16.15	9626	64.54	
Yes	116	16.79	127	18.38	448	64.83	0.1223
Unfamiliar animals							
No	2899	19.37	2426	16.21	9643	64.42	
Yes	96	15.09	110	17.30	430	67.61	0.0272
Others with GI illness							
No	2744	18.99	2296	15.89	9409	65.12	
Yes	251	21.71	240	20.76	665	57.53	<0.001

1: Pearson's Chi-square test of independence
2: Row percentages add to 100%

Table 4.75: Incident illness among all subjects (excluding those with baseline illness), Boquerón Beach

	N	%
GI illness		
Not ill	14537	95.29
Ill	719	4.71
Total	15256	100.00
Respiratory illness		
Not ill	13362	93.05
Ill	998	6.95
Total	14360	100.00
Rash		
Not ill	14596	95.58
Ill	675	4.42
Total	15271	100.00
Eye irritations/infections		
Not ill	14916	96.44
Ill	550	3.56
Total	15466	100.00
Earache		
Not ill	15104	98.13
Ill	288	1.87
Total	15392	100.00

meat and raw fish, contact with unknown animals, and contact with other ill people were associated with skin rash. Over twice of those who had contact with unknown animals reported skin rash than those who had no unknown animal contact (10% vs. 4%, Table 4.78).

Earache Earache also was not reported at a significantly higher frequency among young children and showed no association with age group. Incident earaches were associated with female gender, asthma, consumption of raw or undercooked meat and raw fish, contact with unknown animals, and contact with other ill people (Table 4.79).

Eye irritations Eye irritations were reported most frequently among those age 20-54 and least frequently among those 11-19. Eye irritations were associated with female gender, asthma, chronic skin condition, consumption of raw fish, contact with unknown animals, and contact with other ill people (Table 4.80)

Table 4.76: Factors associated with GI illness, Boquerón Beach

	Not Ill		Ill		P-value[1]
	N	%[2]	N	%[2]	N
Age category					
0-4	813	91.97	71	8.03	
5-10	1660	95.13	85	4.87	
11-19	2128	96.12	86	3.88	
20-54	4033	94.74	224	5.26	
55 and over	5702	95.91	243	4.09	<0.001
Sex					
Male	6558	95.77	290	4.23	
Female	7963	94.93	425	5.07	0.0175
Race					
Non-white	14477	95.31	712	4.69	
White	52	94.55	3	5.45	0.9594
Visits to this beach annually					
1 or less	4229	94.84	230	5.16	
2-5	6947	95.09	359	4.91	
6 or more	3360	96.28	130	3.72	0.0061
Chronic GI illness					
No	13902	95.51	653	4.49	
Yes	634	90.57	66	9.43	<0.001
Chronic skin condition					
No	14044	95.31	691	4.69	
Yes	492	94.62	28	5.38	0.5288
Asthma					
No	13037	95.52	612	4.48	
Yes	1499	93.34	107	6.66	<0.001
Raw or undercooked meat					
No	13922	95.40	672	4.60	
Yes	615	92.90	47	7.10	0.0041
Raw fish					
No	13676	95.36	665	4.64	
Yes	861	94.10	54	5.90	0.0950
Contact with unfamiliar animals					
No	13961	95.45	665	4.55	
Yes	575	91.41	54	8.59	<0.001
Others with GI illness					
No	13634	95.96	574	4.04	
Yes	903	86.16	145	13.84	<0.001

1: Pearson's Chi-square test of independence
2: Row percentages add to 100%

Table 4.77: Factors associated with respiratory illness, Boquerón Beach

	Not Ill		Ill		P-value[1]
	N	%[2]	N	%[2]	
Age category					
0-4	710	88.42	93	11.58	
5-10	1500	92.14	128	7.86	
11-19	2006	94.58	115	5.42	
20-54	3599	90.77	366	9.23	
55 and over	5345	94.85	290	5.15	<0.001
Sex					
Male	6062	93.23	440	6.77	
Female	7280	92.88	558	7.12	0.4285
Race					
Non-white	13303	93.04	995	6.96	
White	47	94.00	3	6.00	0.9902
Visits to this beach annually					
1 or less	3859	92.85	297	7.15	
2-5	6417	92.91	490	7.09	
6 or more	3085	93.60	211	6.40	0.3675
Chronic GI illness					
No	12781	93.11	946	6.89	
Yes	580	91.77	52	8.23	0.2257
Chronic skin condition					
No	12939	93.19	945	6.81	
Yes	422	88.84	53	11.16	<0.001
Asthma					
No	12101	93.55	835	6.45	
Yes	1260	88.55	163	11.45	<0.001
Raw or undercooked meat					
No	12814	93.19	937	6.81	
Yes	548	89.98	61	10.02	0.0031
Raw fish					
No	12589	93.22	916	6.78	
Yes	773	90.41	82	9.59	0.0022
Contact with unfamiliar animals					
No	12816	93.10	950	6.90	
Yes	545	91.91	48	8.09	0.3000
Others with GI illness					
No	12483	93.34	891	6.66	
Yes	879	89.15	107	10.85	<0.001

1: Pearson's Chi-square test of independence
2: Row percentages add to 100%

Table 4.78: Factors associated with rash, Boquerón Beach

	Not Ill		Ill		P-value[1]
	N	%[2]	N	%[2]	N
Age category					
0-4	840	95.89	36	4.11	
5-10	1662	95.24	83	4.76	
11-19	2125	95.89	91	4.11	
20-54	4050	95.27	201	4.73	
55 and over	5721	95.75	254	4.25	0.6366
Sex					
Male	6628	96.58	235	3.42	
Female	7950	94.78	438	5.22	<0.001
Race					
Non-white	14532	95.58	672	4.42	
White	52	94.55	3	5.45	0.9649
Visits to this beach annually					
1 or less	4184	94.55	241	5.45	
2-5	7021	95.76	311	4.24	
6 or more	3390	96.50	123	3.50	<0.001
Chronic GI illness					
No	13936	95.69	628	4.31	
Yes	659	93.34	47	6.66	0.0041
Chronic skin condition					
No	14155	95.68	639	4.32	
Yes	440	92.44	36	7.56	0.0011
Asthma					
No	13101	95.84	568	4.16	
Yes	1494	93.32	107	6.68	<0.001
Raw or undercooked meat					
No	13969	95.66	633	4.34	
Yes	627	93.72	42	6.28	0.0218
Raw fish					
No	13746	95.72	614	4.28	
Yes	850	93.30	61	6.70	<0.001
Contact with unfamiliar animals					
No	14024	95.81	614	4.19	
Yes	571	90.35	61	9.65	<0.001
Others with GI illness					
No	13588	95.86	587	4.14	
Yes	1008	91.97	88	8.03	<0.001

1: Pearson's Chi-square test of independence
2: Row percentages add to 100%

Table 4.79: Factors associated with earache, Boquerón Beach

	Not Ill		Ill		P-value[1]
	N	%[2]	N	%[2]	
Age category					
0-4	867	97.53	22	2.47	
5-10	1707	97.71	40	2.29	
11-19	2200	98.57	32	1.43	
20-54	4223	97.98	87	2.02	
55 and over	5897	98.25	105	1.75	0.1531
Sex					
Male	6802	98.41	110	1.59	
Female	8282	97.90	178	2.10	0.0231
Race					
Non-white	15037	98.12	288	1.88	
White	55	100.00	0	0.00	0.5975
Visits to this beach annually					
1 or less	4386	97.73	102	2.27	
2-5	7264	98.28	127	1.72	
6 or more	3453	98.32	59	1.68	0.0614
Chronic GI illness					
No	14398	98.17	268	1.83	
Yes	705	97.24	20	2.76	0.0957
Chronic skin condition					
No	14588	98.16	274	1.84	
Yes	515	97.35	14	2.65	0.2396
Asthma					
No	13531	98.29	235	1.71	
Yes	1572	96.74	53	3.26	<0.001
Raw or undercooked meat					
No	14448	98.20	265	1.80	
Yes	656	96.61	23	3.39	0.0045
Raw fish					
No	14209	98.22	257	1.78	
Yes	895	96.65	31	3.35	<0.001
Contact with unfamiliar animals					
No	14494	98.20	265	1.80	
Yes	609	96.36	23	3.64	0.0014
Others with GI illness					
No	14012	98.21	255	1.79	
Yes	1092	97.07	33	2.93	0.0089

1: Pearson's Chi-square test of independence
2: Row percentages add to 100%

Table 4.80: Factors associated with eye infection/irritation, Boquerón Beach

	Not Ill		Ill		P-value[1]
	N	%[2]	N	%[2]	
Age category					
0-4	862	96.42	32	3.58	
5-10	1710	96.77	57	3.23	
11-19	2184	97.46	57	2.54	
20-54	4153	95.91	177	4.09	
55 and over	5801	96.30	223	3.70	0.0253
Sex					
Male	6736	97.02	207	2.98	
Female	8160	95.97	343	4.03	<0.001
Race					
Non-white	14848	96.43	550	3.57	
White	56	100.00	0	0.00	0.2806
Visits to this beach annually					
1 or less	4348	96.37	164	3.63	
2-5	7136	96.34	271	3.66	
6 or more	3431	96.76	115	3.24	0.5164
Chronic GI illness					
No	14219	96.50	515	3.50	
Yes	696	95.21	35	4.79	0.0819
Chronic skin condition					
No	14417	96.53	519	3.47	
Yes	498	94.14	31	5.86	0.0052
Asthma					
No	13383	96.77	447	3.23	
Yes	1532	93.70	103	6.30	<0.001
Raw or undercooked meat					
No	14267	96.50	517	3.50	
Yes	649	95.16	33	4.84	0.0812
Raw fish					
No	14040	96.58	497	3.42	
Yes	876	94.29	53	5.71	<0.001
Contact with unfamiliar animals					
No	14324	96.61	503	3.39	
Yes	591	92.63	47	7.37	<0.001
Others with GI illness					
No	13856	96.67	478	3.33	
Yes	1060	93.64	72	6.36	<0.001

1: Pearson's Chi-square test of independence
2: Row percentages add to 100%

Swimming exposure and incident illness

Adjusted Cumulative Incidence Ratios (aCIRs) comparing the risk of illness among swimmers compared to non-swimmers for body immersion and head immersion swimming exposures are shown together with the crude (unadjusted) percentages of incident illness in Tables 4.81 and 4.82, respectively. Crude incident illness and the aCIRs for each illness group among swimmers with head immersion is also shown graphically in Figure 4.20. Skin rash was significantly elevated among swimmers who immersed their body, head or swallowed water. No other illnesses were significantly elevated among swimmers as compared to non-swimmers.

Although crude incidence was slightly higher among swimmers after adjustment for covariates there was no difference as indicated by the aCIR shown (see Table 4.81). This is likely due to confounding of the unadjusted association by age since young children were more likley to both swim and report respiratory illness.

Table 4.81: Incident illness by body immersion, Boquerón Beach

	Number ill	%[1]	aCIR[2] (p-value)
GI			
Non-swimmer	123	4.25	
Swimmer	563	4.78	
Total	686	4.68	1.04(0.7239)
Upper respiratory			
Non-swimmer	158	5.81	
Swimmer	786	7.08	
Total	944	6.83	0.99(0.9281)
Rash			
Non-swimmer	91	3.15	
Swimmer	561	4.76	
Total	652	4.44	1.51(0.0004)
Earache			
Non-swimmer	43	1.47	
Swimmer	229	1.93	
Total	272	1.84	1.17(0.3553)
Eye irritation			
Non-swimmer	111	3.8	
Swimmer	413	3.46	
Total	524	3.52	0.89(0.3403)

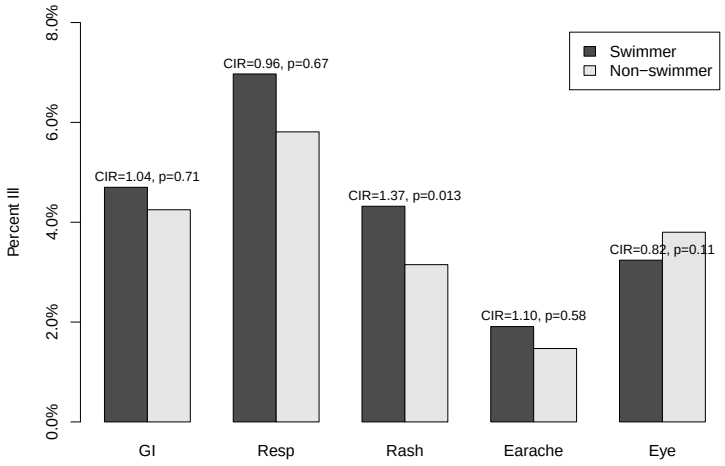
1: Percentage of those in row category with symptom (row percentage). Number and percent not ill not shown
2: Adjusted Cumulative Incidence Ratio

Table 4.82: Incident illness by head immersion, Boquerón Beach

	Number ill	%[1]	aCIR[2] (p-value)
GI			
Non-swimmer	123	4.25	
Swimmer	460	4.7	
Total	583	4.6	1.04(0.7055)
Upper respiratory			
Non-swimmer	158	5.81	
Swimmer	645	6.97	
Total	803	6.7	0.96(0.699)
Rash			
Non-swimmer	91	3.15	
Swimmer	424	4.32	
Total	515	4.05	1.37(0.0131)
Earache			
Non-swimmer	43	1.47	
Swimmer	189	1.91	
Total	232	1.81	1.1(0.5808)
Eye irritation			
Non-swimmer	111	3.8	
Swimmer	322	3.24	
Total	433	3.36	0.82(0.1092)

1: Percentage of those in row category with symptom (row percentage). Number and percent not ill not shown
2: Adjusted Cumulative Incidence Ratio

Figure 4.20: Incident illness by swimming status (head immersion), Boquerón Beach



CIR: Adjusted cumulative incidence ratio comparing proportion of illness among swimmers compared to non swimmers

4.2.6 Water quality

Turbidity, pH and water temperature measurements at Boquerón Beach are shown in Table 4.83. Mean turbidity was over twice as high as at Surfside and a strong increase in turbidity was observed over the course of the day, increasing from 5 NTU at 8:00 AM samples to 15 NTU at the 3:00 PM samples. Water temperature was constant across sample time. pH increased slightly over sampling time.

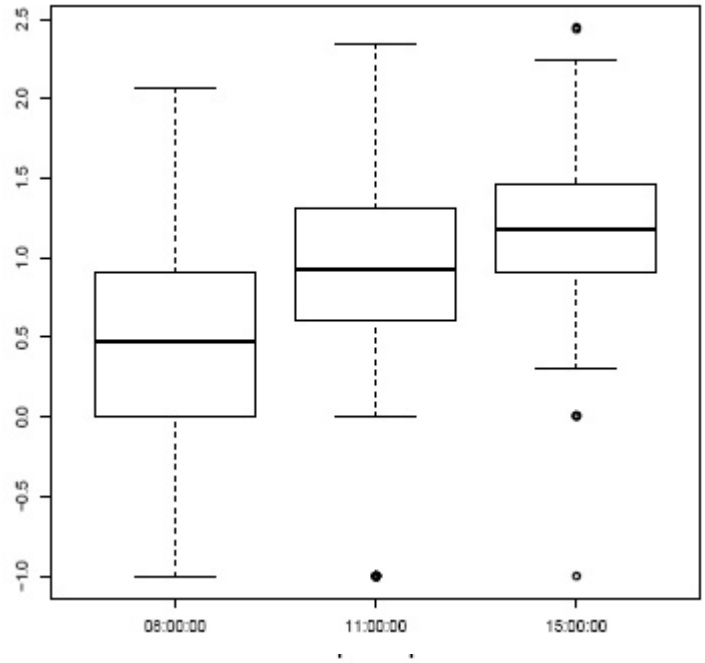
Enterococcus Method 1600

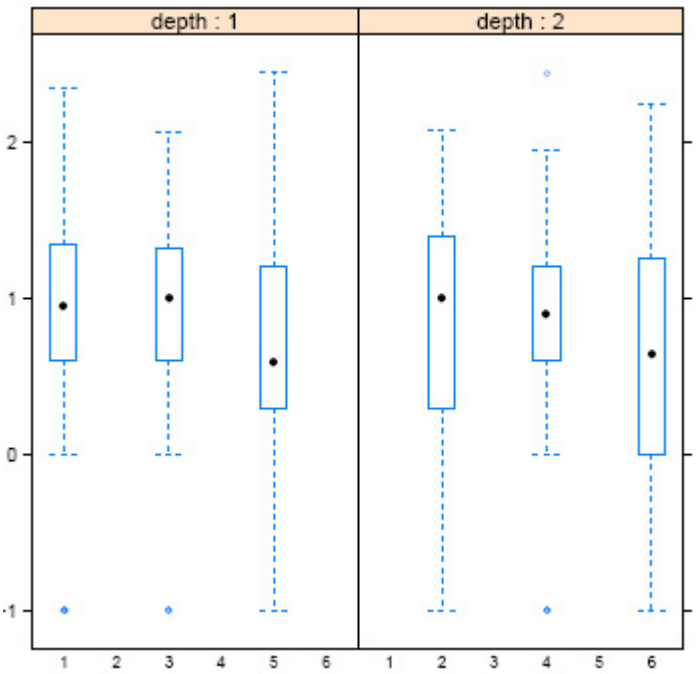
A total of 468 samples were collected and tested for *Enterococcus* CFU by Method 1600 at Boquerón Beach. As shown in Table 4.84 and Figure 4.21, levels of *Enterococcus* CFU were low at Boquerón Beach.

Unlike Surfside Beach, where *Enterococcus* CFU declined over time, at Boquerón Beach, *Enterococcus* CFU increased over time, with highest densities occurring at 3:00 PM (15 CFU/100 ml, Figure 4.21). Higher CFU also were observed at shin depth compared to waist depth. Sample location was also associated with levels of CFU with highest levels occurring along location 1 on the left side of the and lowest levels occurring along location 3 at the right side of the beach (see Figure 4.19).

Geometric means of the 18 samples collected were below the recommended

Figure 4.21: *Enterococcus* colony forming units ($\log_{10}$) per 100 ml, Boquerón Beach





See Fig 4.19 for sampling locations. Depth 1 refers to Shin depth, depth 2 to waist depth samples

Table 4.83: Turbidity pH and Water Temperature, Boquerón Beach

	N	Min	Median	Max	Mean	SD
Turbidity, NTU[1]						
All Samples	467	1.00	8.00	62.00	10.25	8.00
By Depth						
-Shin	233	1.00	8.00	62.00	10.65	8.04
-Waist	234	2.00	8.00	62.00	9.86	7.95
By Collection Time						
-08:00	156	1.00	5.00	13.00	5.05	2.15
-11:00	156	2.00	9.00	24.00	10.17	5.36
-15:00	155	2.00	12.00	62.00	15.58	10.19
pH						
All Samples	467	7.88	8.11	8.31	8.11	0.08
By Depth						
-Shin	233	7.89	8.10	8.31	8.11	0.08
-Waist	234	7.88	8.12	8.28	8.11	0.08
By Collection Time						
-08:00	156	7.89	8.05	8.26	8.05	0.05
-11:00	156	7.88	8.14	8.25	8.13	0.06
-15:00	155	7.92	8.18	8.31	8.16	0.07
Water Temperature (waist depth), ℃						
All Samples	78	28.00	29.90	31.40	29.90	0.81
By Collection Time						
-08:00	26	28.00	29.45	30.20	29.27	0.61
-11:00	26	28.60	30.20	31.10	30.02	0.68
-15:00	26	29.10	30.65	31.40	30.43	0.65

1: Nephelometric Turbidity Units

EPA criteria of 35 CFU per 100 ml for each of the 26 days. The highest daily geometric mean was 27 CFU per 100 ml. A cumulative frequency plot of daily average *Enterococcus* CFU is shown in Figure 4.22.

Enterococcus qPCR Calibrator Cell Equivalents

Low levels of *Enterococcus* qPCR CCE were also observed at Boquerón Beach. Interpretation of qPCR results for both *Enterococcus* and *Bacteroidales* was seriously hampered by three issues:

1. Non-detection, particularly for *Enterococcus*
2. Samples below the quantitation level
3. Samples failing the internal positive control assay criterion

Figure 4.22: Cumulative frequency plot. Daily average *Enterococcus* colony forming units ($\log_{10}$) per 100 ml, Boquerón Beach

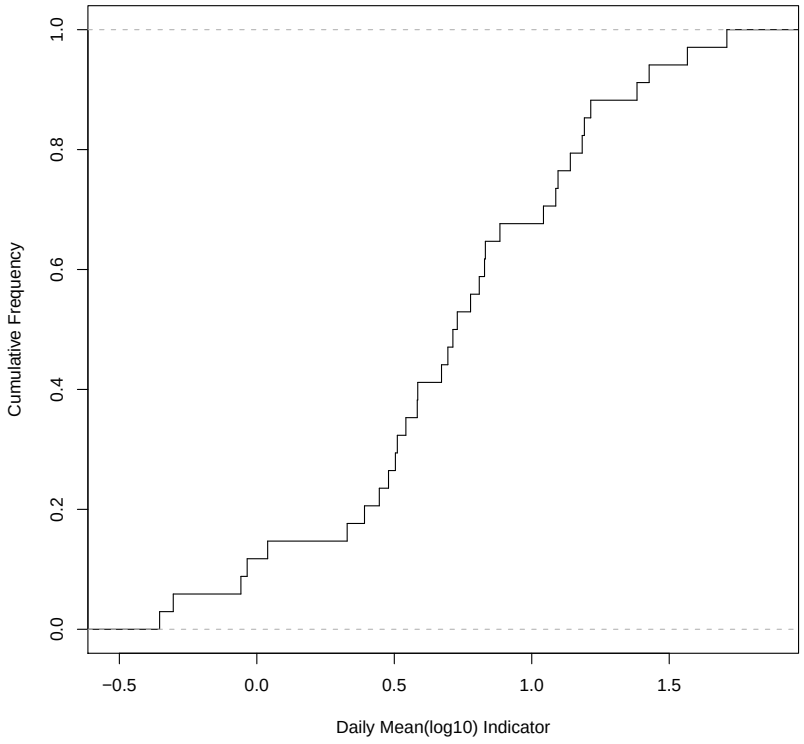


Table 4.84: *Enterococcus* CFU[1] ($\log_{10}$) per 100 ml at Boquerón Beach

	Min[2]	Median	Max	Mean	SD	N	Below Detect
Boqueron Beach							
Enterococcus CFU	-1.00	0.90	2.45	0.77	0.76	468	34
By Depth							
-Shin	-1.00	0.90	2.45	0.81	0.75	234	17
-Waist	-1.00	0.90	2.43	0.73	0.77	234	17
By Collection Time							
-08:00	-1.00	0.48	2.07	0.31	0.79	156	25
-11:00	-1.00	0.93	2.34	0.83	0.71	156	9
-15:00	-1.00	1.18	2.45	1.17	0.49	156	0
By Swim Location[3]							
-Location 1	-1.00	1.00	2.34	0.82	0.81	156	12
-Location 2	-1.00	0.95	2.43	0.86	0.67	156	7
-Location 3	-1.00	0.60	2.45	0.62	0.79	156	15

1: Colony Forming Units, Measured by EPA Method 1600

2: Minimum value set to 0.1 CFU per 100 ml, or -1 $\log_{10}$ CFU per 100 ml

3: See Figure 4.19. Location 1 is the left transect (samples 1 and 3), 2 center (2 and 4), 3 right (3 and 6)

Analyses at a higher dilution (1:25) only marginally improved the performance of the positive control salmon DNA assay and did not solve the issue satisfactorily. As a result, the quantification of many samples were questionable and impeded interpretation of both the results of the qPCR water quality analysis as well as the associations with health effects.

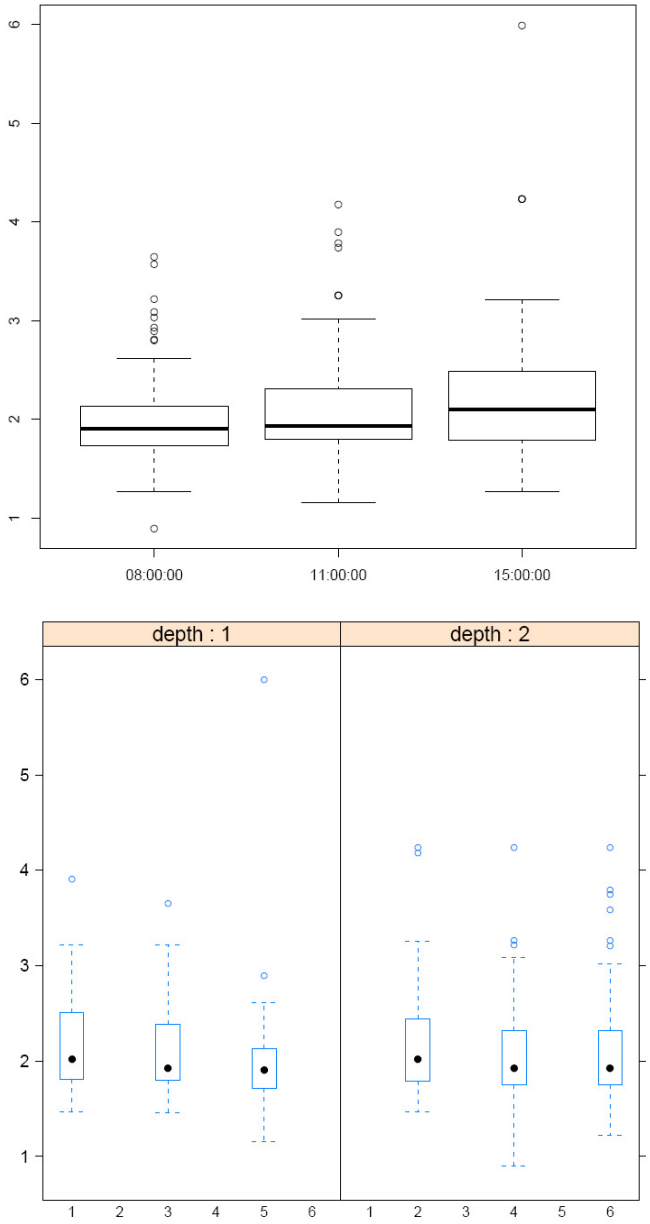
Of the 468 samples collected and tested on beach study days, 160 failed the positive control assay (34%), meaning that after spiking the test samples, much lower than expected levels of the control Salmon testes DNA was detected (see Table 4.85). Of the 308 samples that met the positive control assay criterion, an additional 239 (78%) showed no detection of *Enterococcus* qPCR CCE. Of the remaining 69 samples, 26 (38%) were below the quantitation limit (Cycle Threshold > 37.24), leaving 43 of the 468 tested samples (9%) which met the positive control criterion with quantifiable results.

Using criteria and methods described in Section 3.4.1 CCEs were estimated and health relationships derived despite the high uncertainty in the exposure measure. However, results and data presented should be interpreted with caution.

Enterococcus CCE calculated by the delta-delta CT approach are shown in Table 4.85 and Figure 4.23. *Enterococcus* CCE calculated by the Delta-CT approach are shown in Table 4.86 and Figure 4.24.

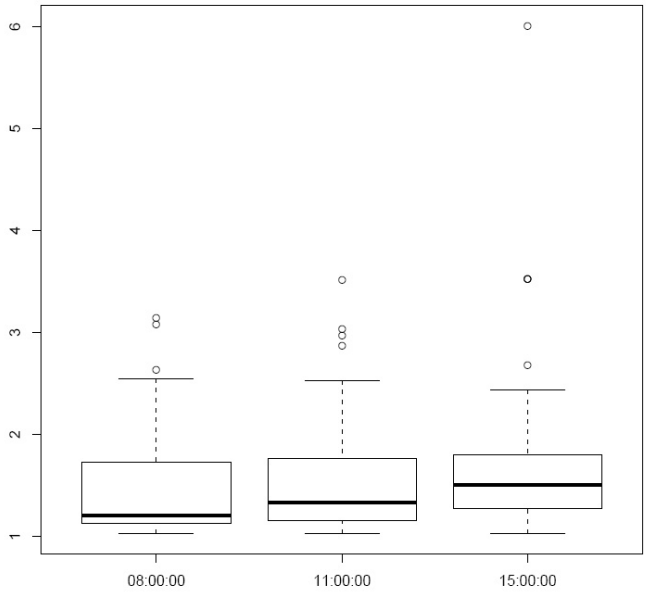
Overall levels of *Enterococcus* CCE were low but roughly comparable to the levels observed at Surfside Beach (geometric means of 32 and 126 for CCEs per 100 ml by the delta and delta-delta methods, respectively)

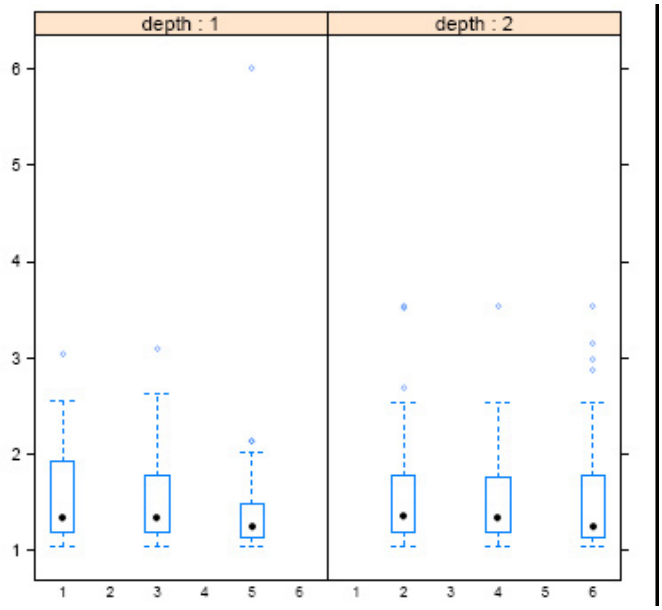
Figure 4.23: *Enterococcus* calibrator cell equivalents ($\log_{10}$) per 100 ml, delta-delta CT method, Boquerón Beach



See Fig 4.19 for sampling locations. Depth 1 refers to Shin depth, depth 2 to waist depth samples

Figure 4.24: *Enterococcus* calibrator cell equivalents ($\log_{10}$) per 100 ml, delta CT method, Boquerón Beach





See Fig 4.19 for sampling locations. Depth 1 refers to Shin depth, depth 2 to waist depth samples

Table 4.85: *Enterococcus* qPCR Calibrator Cell Equivalents (CCE), delta-delta CT method ($\log_{10}$), Boquerón Beach

	Min	Median	Max	Mean	SD	N	Below Detect[1]	Control Fail[2]
Boquerón Beach								
All samples	0.90	1.94	5.99	2.10	0.54	468	239(78%)	160(34%)
By Depth								
-Shin	1.16	1.94	5.99	2.08	0.51	234	113(77%)	87(37%)
-Waist	0.90	1.97	4.23	2.11	0.56	234	126(78%)	73(31%)
By Collection Time								
-08:00	0.90	1.91	3.65	2.01	0.44	156	86(79%)	47(30%)
-11:00	1.16	1.93	4.18	2.09	0.51	156	92(84%)	46(29%)
-15:00	1.27	2.10	5.99	2.20	0.63	156	61(62%)	57(37%)
By Swim Location[3]								
-Location 1	1.47	2.02	4.23	2.16	0.50	156	77(78%)	57(37%)
-Location 2	0.90	1.92	4.23	2.08	0.48	156	77(7%)	56(36%)
-Location 3	1.16	1.91	5.99	2.05	0.61	156	85(78%)	47(30%)

1: Number of samples passing salmon criteria with no detection after 45 cycles

2: Number of samples where salmon assay fails cycle threshold citerion (see Sections 3.4.1 and 3.4)

3: See Figure 4.19. Location 1 is the left transect (samples 1 and 3), 2 center (2 and 4), 3 right (3 and 6)

Consistent with observations for *Enterococcus* CFU, *Enterococcus* CCE for both the delta and delta-delta CT methods increased over collection time (p=0.005 and p=0.02, respectively). However, unlike *Enterococcus* CFU, no differences were observed by sample transect location or by sample depth.

Ratios of *Enterococcus* CFU to CCE are shown in Table 4.87 for qPCR samples which met the control criteria. CFU to CCE ratios were higher than for Surfside, with mean ratios of 0.22 and 0.58 for the delta-delta and delta-CT methods, respectively.

A cumulative distribution plot of the daily average *Enterococcus* CCE (delta-delta CT) is shown in Figure 4.25.

Figure 4.25: Cumulative frequency plot. Daily average *Enterococcus* CCE (delta-delta CT) ($\log_{10}$) per 100 ml, Boquerón Beach

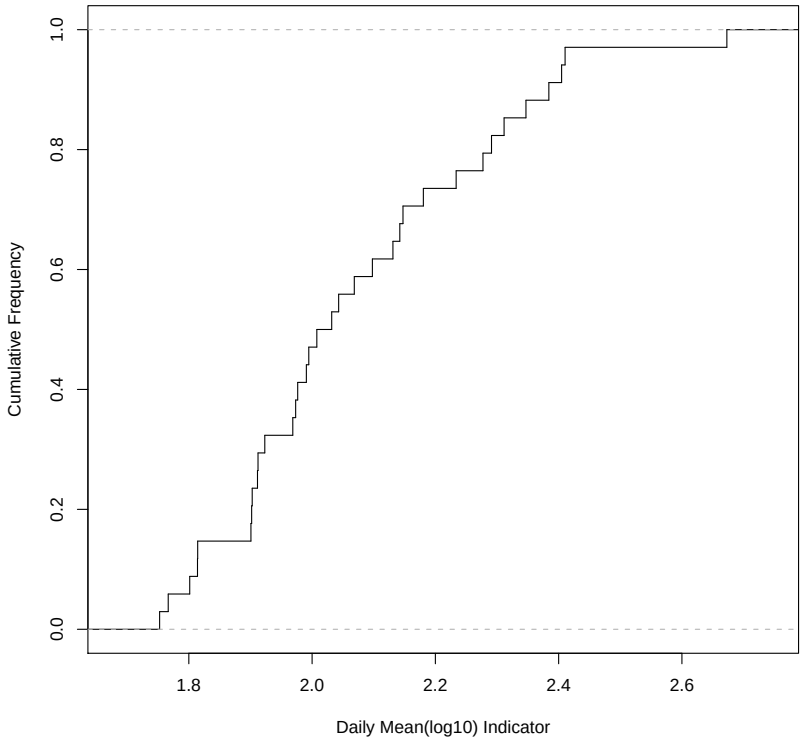


Table 4.86: *Enterococcus* qPCR Calibrator Cell Equivalents (CCE), delta CT method ($\log_{10}$), Boquerón Beach

	Min	Median	Max	Mean	SD	N	Below Detect[1]	Control Fail[2]
Boquerón Beach								
All samples	1.03	1.34	6.00	1.51	0.52	468	239(78%)	160(34%)
By Depth								
-Shin	1.03	1.34	6.00	1.49	0.53	234	113(77%)	87(37%)
-Waist	1.03	1.34	3.53	1.53	0.51	234	126(78%)	73(31%)
By Collection Time								
-08:00	1.03	1.20	3.15	1.42	0.46	156	86(79%)	47(30%)
-11:00	1.03	1.34	3.52	1.48	0.47	156	92(84%)	46(29%)
-15:00	1.03	1.50	6.00	1.64	0.61	156	61(62%)	57(37%)
By Swim Location[3]								
-Location 1	1.03	1.34	3.53	1.57	0.51	156	77(78%)	57(37%)
-Location 2	1.03	1.34	3.53	1.49	0.44	156	77(77%)	56(36%)
-Location 3	1.03	1.25	6.00	1.47	0.60	156	85(78%)	47(30%)

1: Number of samples passing salmon criteria with no detection after 45 cycles

2: Number of samples where salmon assay fails cycle threshold criterion (see Sections 3.4.1 and 3.4)

3: See Figure 4.19. Location 1 is left transect (samples 1 and 3), 2 center (2 and 4), 3 right (3 and 6)

Table 4.87: Ratio of Enterococcus CFU to *Enterococcus* CCE[1]. Boquerón Beach.

	Min	Median	Max	Mean	SD	N
delta-delta CT						
All Samples	0.0000	0.0588	5.8970	0.2191	0.5568	308
By Depth						
-Shin	0.0000	0.0665	2.2685	0.1855	0.3234	147
-Waist	0.0002	0.0493	5.8970	0.2497	0.7054	161
By Collection Time						
-08:00	0.0002	0.0322	4.7790	0.1386	0.4831	109
-11:00	0.0002	0.0716	1.5221	0.1644	0.2789	110
-15:00	0.0000	0.0988	5.8970	0.3853	0.8120	89
delta-CT						
All Samples	0.0000	0.2274	12.9659	0.5836	1.1862	308
By Depth						
-Shin	0.0000	0.2649	7.4848	0.6064	1.1009	147
-Waist	0.0007	0.1852	12.9659	0.5629	1.2621	161
By Collection Time						
-08:00	0.0007	0.1029	5.3093	0.2950	0.6240	109
-11:00	0.0010	0.2674	3.2600	0.5065	0.6591	110
-15:00	0.0000	0.3178	12.9659	1.0325	1.8916	89

CFU: Colony forming units, CCE: Calibrator cell equivalents

1: Sample to sample ratios. qPCR samples which failed QC excluded

Bacteroidales qPCR Calibrator Cell Equivalents

Since the same control assay was used for *Enterococcus* and *Bacteroidales* there were similar problems in the interpreting of the *Bacteroidales* CCE results. There were fewer non-detects for *Bacteroidales*, but the overall numbers of questionable or unusable samples remained high. Of the 308 *Bacteroidales* samples which passed the positive control criterion, 116 were below detection (38%). Twenty additional samples were below the quantitation limit for *Bacteroidales* (Cycle Threshold 36.48).

Bacteroidales CCE by the delta-delta CT approach are shown in Table 4.88 and Figure 4.26. *Enterococcus* CCE calculated by the delta CT approach are shown in Table 4.89 and Figure 4.27.

Overall geometric means for *Bacteroidales* CCE were 288 CCE per 100 ml for the delta-CT calculation and 1,097 for the delta-delta CCE calculation. Like *Enterococcus* CCE, *Bacteroidales* CCE increased over time ($p<0.0005$), with highest CCEs at the 3:00 PM sampling time, and lowest at 8:00 AM (Figure 4.26 and 4.27). As observed with *Enterococcus* CCE, *Bacteroidales* CCE were highest at swimming location 1 ($p=0.04$, for both delta and delta-delta CT).

A cumulative distribution plot of the daily average *Bacteroidales* CCE (delta-delta CT) is shown in Figure 4.28.

Table 4.88: *Bacteroidales* qPCR Calibrator Cell Equivalents (CCE), delta-delta CT method ($\log_{10}$), Boquerón Beach

	Min	Median	Max	Mean	SD	N	Below Detect[1]	Control Fail[2]
Boquerón Beach								
All samples	1.72	2.98	6.48	3.04	0.52	468	116(38%)	160(34%)
By Depth								
-Shin	1.98	2.95	6.18	3.02	0.51	234	57(39%)	87(37%)
-Waist	1.72	3.01	6.48	3.07	0.52	234	59(37%)	73(31%)
By Collection Time								
-08:00	1.72	2.81	6.48	2.89	0.55	156	54(50%)	47(30%)
-11:00	2.21	2.98	4.80	3.05	0.45	156	39(35%)	46(29%)
-15:00	2.03	3.23	4.32	3.21	0.49	156	23(26%)	67(43%)
By Swim Location[3]								
-Location 1	2.19	3.04	6.48	3.12	0.56	156	34(34%)	57(37%)
-Location 2	1.72	3.01	6.18	3.05	0.53	156	39(39%)	56(39%)
-Location 3	1.98	2.91	4.62	2.96	0.44	156	43(39%)	47(30%)

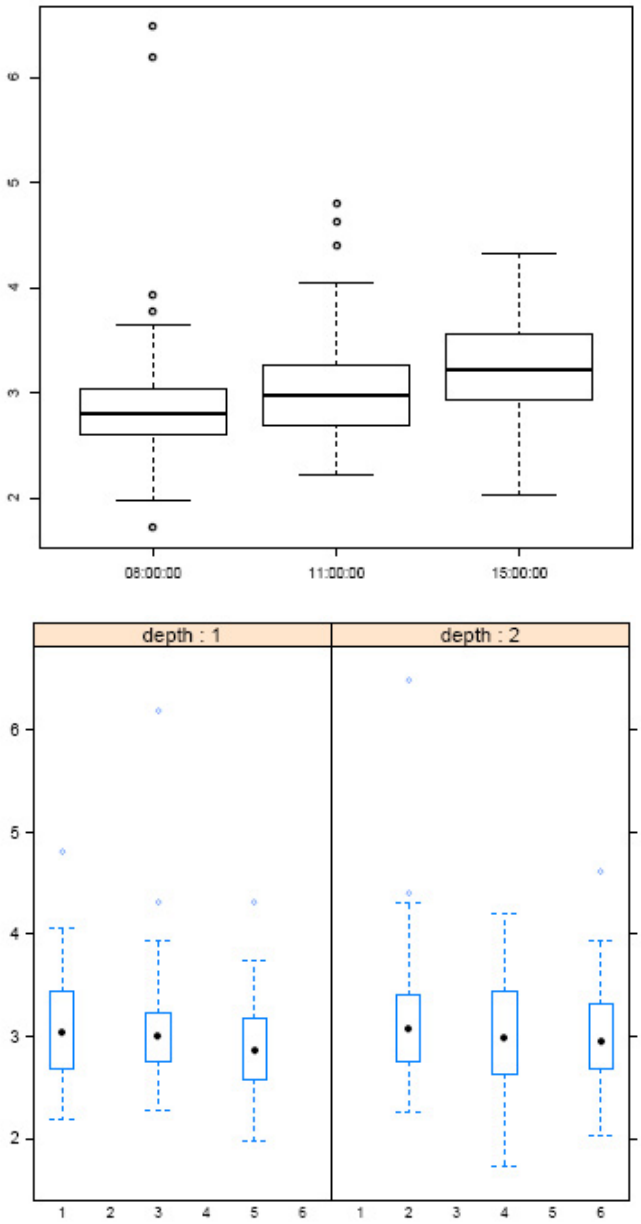
1: Number of samples passing salmon criteria with no detection after 45 cycles

2: Number of samples where salmon assay fails cycle threshold criterion (see Sections 3.4.1 and 3.4)

3: See Figure 4.19 swim location 1 is left transect (samples 1 and 3), 2 center (2 and 4), 3 right (3 and 6)

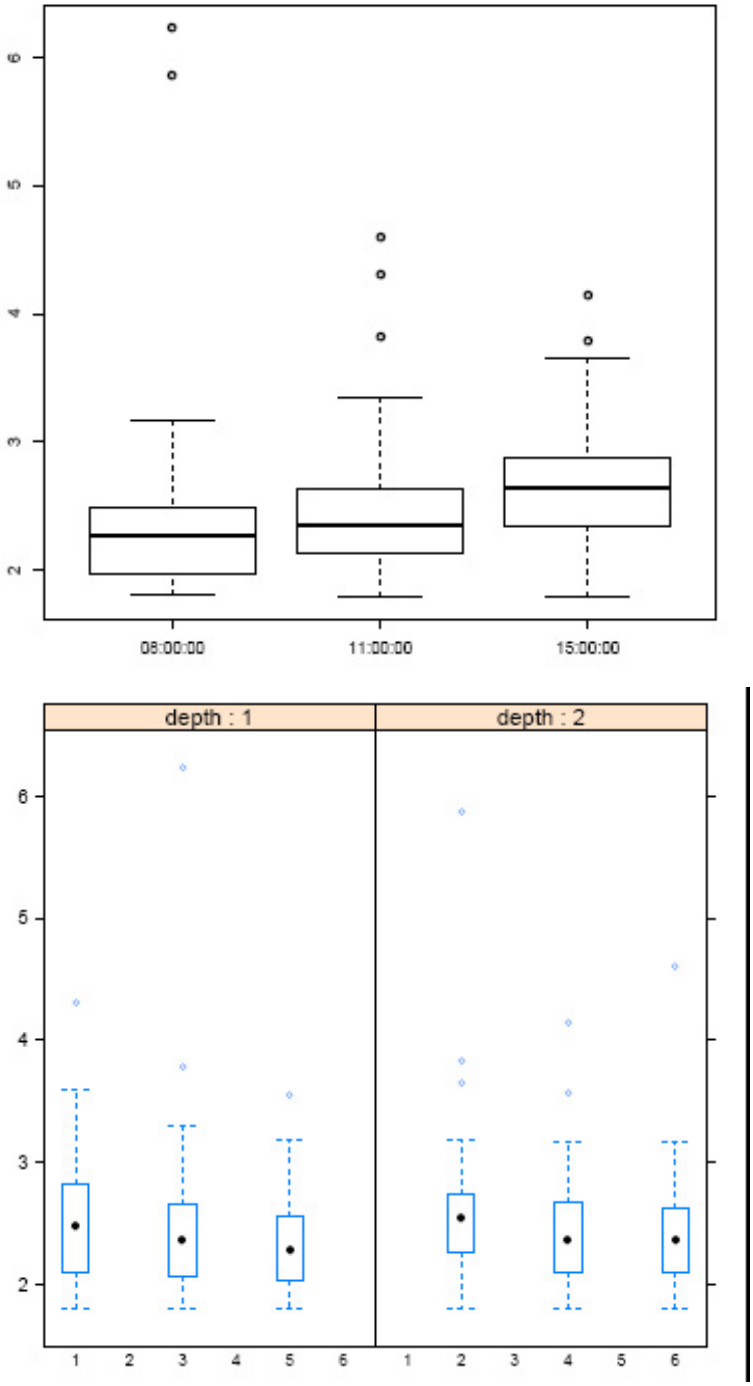
In addition to high water quality and low levels of fecal indicator bacteria at the beach, samples collected near the sewage discharge point and the mangrove

Figure 4.26: *Bacteroidales* calibrator cell equivalents ($\log_{10}$) per 100 ml, delta-delta CT method, Boquerón Beach



See Fig 4.19 for sampling locations. Depth 1 refers to Shin depth, depth 2 to waist depth samples

Figure 4.27: *Bacteroidales* calibrator cell equivalents ($\log_{10}$) per 100 ml, delta CT method, Boquerón Beach



See Fig 4.19 for sampling locations. Depth 1 refers to Shin depth, depth 2 to waist depth samples

Figure 4.28: Cumulative frequency plot. Daily average *Bacteroidales* CCE (delta-delta CT) ($\log_{10}$) per 100 ml, Boquerón Beach

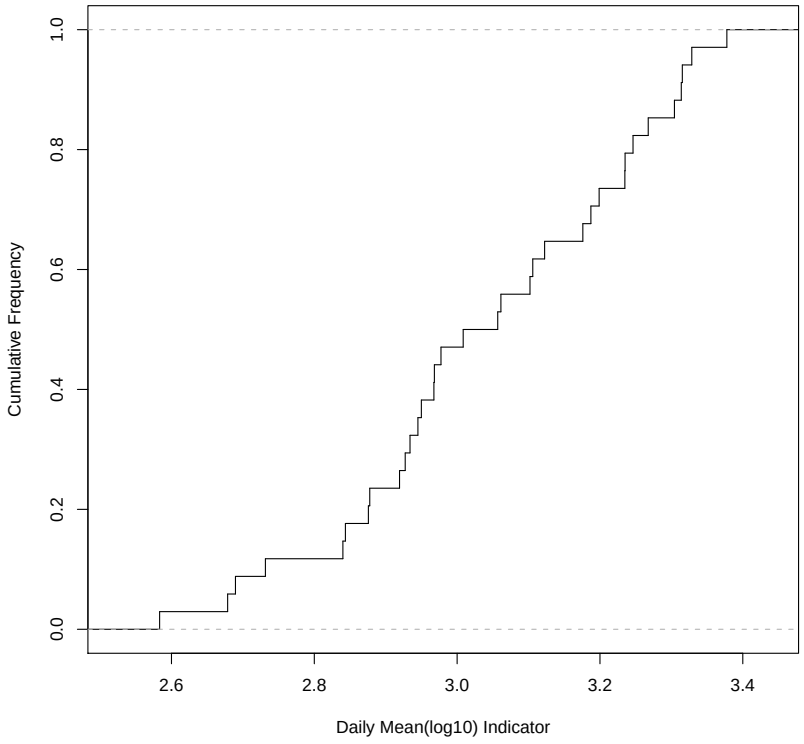


Table 4.89: *Bacteroidales* qPCR Calibrator Cell Equivalents (CCE), delta CT method ($\log_{10}$), Boquerón Beach

	Min	Median	Max	Mean	SD	N	Below Detect[1]	Control Fail[2]
Boqueron Beach								
All samples	1.80	2.37	6.23	2.46	0.50	468	116(38%)	160(34%)
By Depth								
-Depth 1	1.81	2.33	6.23	2.43	0.51	234	57(39%)	87(37%)
-Depth 2	1.80	2.37	5.86	2.48	0.49	234	59(37%)	73(31%)
By Collection Time								
-08:00	1.81	2.26	6.23	2.30	0.55	156	54(50%)	47(30%)
-11:00	1.80	2.36	4.60	2.44	0.46	156	39(35%)	46(29%)
-15:00	1.80	2.65	4.15	2.65	0.43	156	23(26%)	67(43%)
By Swim Location								
-Location 1	1.80	2.53	5.86	2.53	0.54	156	34(34%)	57(37%)
-Location 2	1.81	2.37	6.23	2.46	0.53	156	39(39%)	56(36%)
-Location 3	1.80	2.33	4.60	2.38	0.42	156	43(39%)	47(30%)

1: Number of samples passing salmon criteria with no detection after 45 cycles

2: Number of samples where salmon assay fails cycle threshold criterion (see Sections 3.4.1 and 3.4)

3: See Figure 4.19. Location 1 is left transect (samples 1 and 3), 2 center (2 and 4), 3 right (3 and 6)

lagoon also had very low levels of fecal indicators (see Fig 4.18). The geometric mean of Enterococcus CFU near the sewage treatment plant discharge was nearly the same as the beach sites (geometric mean=5 CFU per 100 ml). . Despite low levels of contamination at the mangrove swamp (Geometric mean=1 CFU per 100 ml), *Enterococcus* CFU measured at the swamp were associated with levels of *Enterococcus* CFU at the beach, with the strongest associations occurring at a lag of one day (see Figure 4.29). *Enterococcus* CFU at the discharge from the sewage treatment plant, however, were not associated with *Enterococcus* CFU at the beach (data not shown).

4.2.7 Associations among water quality and environmental measures

Sample to sample correlations for water quality measures, turbidity, pH and salinity are shown in Figure 4.30. Pairwise Spearman correlation coefficients are shown in Table 4.90.

Turbidity was positively correlated with all fecal indicator measures, but most strongly with *Enterococcus* CFU (Spearman's r=0.411). qPCR CCE for *Enterococcus* and *Bacteroidales* were correlated, with stronger correlations within the same calculation method. Interestingly, *Enterococcus* CCE by the delta-delta CT method and *Enterococcus* CFU were not correlated (r=0.037), possibly reflecting the high uncertainty in the CCE results and the influence of

Figure 4.29: Association between *Enterococcus* CFU at beach and mangrove swamp sampling sites (lagged by one day), Boquerón Beach.

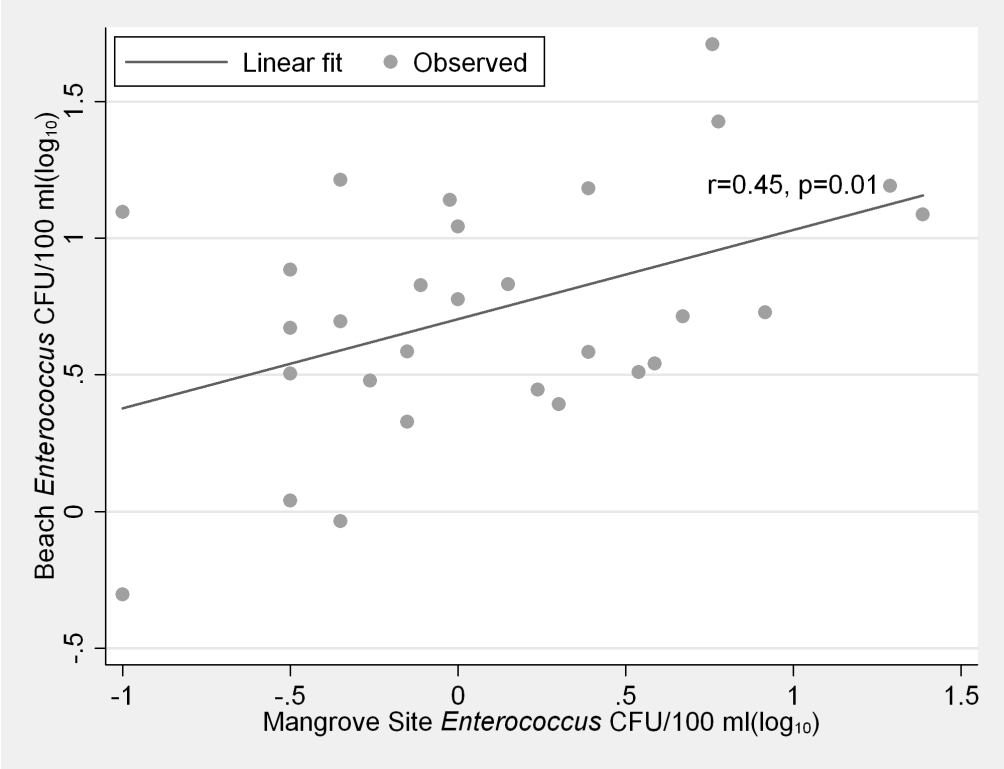
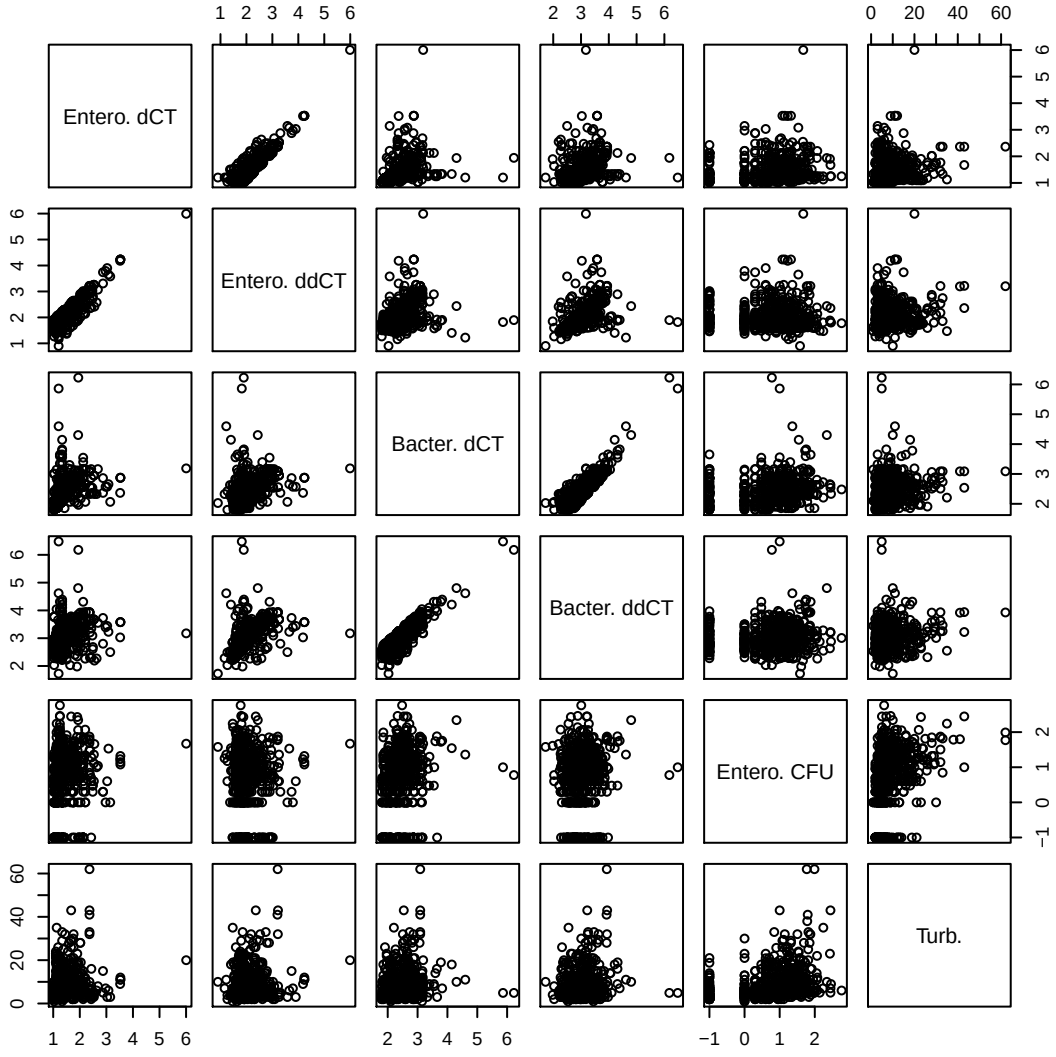


Figure 4.30: Multivariate plot of fecal indicator bacteria and water quality parameters, Boquerón Beach



dCT=delta CT (qPCR Calibrator Cell Equivalents); ddCT=delta-delta CT (qPCR Calibrator Cell Equivalents); CFU=Colony Forming Units
Entero.=*Enterococcus*; Bacter.=*Bacteroidales*
Turbidity measured in Nephelometric Turbidity Units (NTU)

Table 4.90: Spearman pairwise correlation coefficients for water quality parameters, Boquerón Beach

	Entero. dCT	Entero. ddCT	Bacter. dCT	Bacter. ddCT	Entero.CFU	Turb.
Entero. dCT	1	0.696*	0.597*	0.43*	0.164*	0.17*
Entero. ddCT	0.696*	1	0.392*	0.604*	0.037	0.182*
Bacter. dCT	0.597*	0.392*	1	0.839*	0.267*	0.204*
Bacter. ddCT	0.43*	0.604*	0.839*	1	0.159*	0.198*
Entero. CFU	0.164*	0.037	0.267*	0.159*	1	0.411*
Turb.	0.17*	0.182*	0.204*	0.198*	0.411*	1

*p<0.05

dCT=delta CT (qPCR Calibrator Cell Equivalents); ddCT=delta-delta CT (qPCR Calibrator Cell Equivalents)

CFU=Colony Forming Units; Entero.=*Enterococcus*; Bacter.=*Bacteroidales*

Turbidity measured in Nephelometric Turbidity Units (NTU)

the salmon assay in the calculation.

Additional associations between information collected at each sampling time and the average of the water quality measures from the same time period are shown in Tables 4.91- 4.93. Bathers in the water was the only measure consistently associated with all fecal indicators. such as water temperature and ultraviolet intensity were positively correlated with *Enterococcus* CCE. Measures such as wind direction, wind speed, water temperature and wave height were also positively correlations with some fecal indicators (see Tables 4.91- 4.93).

Associations between water quality measures and rainfall are shown in Tables 4.94. Due to the failure of the on site weather station to accurately record data for several weeks, rainfall information was collected from an on-site rain gauge, from the period 3:00 PM to 8:00 AM. Since this information was not collected during the week, except for Friday, two day or accurate current day lag is unavailable for all observations, and only a one day lag (since 3:00 PM the previous day) is reported. Precipitation was negatively correlated with *Enterococcus* delta-delta CT CCE, positively correlated with *Enterococcus* CFU and was not correlated with *Bacteroidales* CCE.

Table 4.91: Spearman pairwise correlation coefficients for *Enterococcus* qPCR CCE and environmental measures, Boquerón Beach

	Ent. dCT	Ent. ddCT	Bathers	Wind dir	Wind speed	UV	Water temp	Wave ht	Tide stage
Ent. dCT	1	0.731*	0.172	0.175	0.178	0.209*	0.06	0.137	0.033
Ent. ddCT	0.731*	1	0.246*	0.196	0.173	0.319*	0.332*	0.257*	0.031
Bathers	0.172	0.246*	1	0.232*	0.326*	0.434*	0.666*	0.124	-0.104
Wind dir	0.175	0.196	0.232*	1	0.149	0.25*	0.321*	0.157	0.209*
Wind speed	0.178	0.173	0.326*	0.149	1	0.363*	0.205*	0.288*	-0.09
UV	0.209*	0.319*	0.434*	0.25*	0.363*	1	0.255*	0.052	0.082
Water temp	0.06	0.332*	0.666*	0.321*	0.205*	0.255*	1	0.221*	-0.064
Wave ht	0.137	0.257*	0.124	0.157	0.288*	0.052	0.221*	1	-0.01
Tide stage	0.033	0.031	-0.104	0.209*	-0.09	0.082	-0.064	-0.01	1

*p<0.05

dCT=delta CT (qPCR Calibrator Cell Equivalents); ddCT=delta-delta CT (qPCR Calibrator Cell Equivalents)

CFU=Colony Forming Units; Entero.=*Enterococcus*; Bacter.=*Bacteroidales*

UV: Ultraviolet radiation; Tide: Tide stage at sampling time; Bathers Water: Bathers in the water; Wave ht: Wave height

Additional information see: Table 3.1

Table 4.92: Spearman pairwise correlation coefficients for *Bacteroidales* qPCR CCE and environmental measures, Boquerón Beach

	Bact. dCT	Bact. ddCT	Bathers	Wind dir	Wind speed	UV	Water temp	Wave ht	Tide stage
Bact. dCT	1	0.869*	0.245*	0.162	0.206*	0.083	0.006	0.102	-0.107
Bact. ddCT	0.869*	1	0.325*	0.258*	0.2	0.199	0.208*	0.213*	-0.04
Bathers	0.245*	0.325*	1	0.232*	0.326*	0.434*	0.666*	0.124	-0.104
Wind dir	0.162	0.258*	0.232*	1	0.149	0.25*	0.321*	0.157	0.209*
Wind speed	0.206*	0.2	0.326*	0.149	1	0.363*	0.205*	0.288*	-0.09
UV	0.083	0.199	0.434*	0.25*	0.363*	1	0.255*	0.052	0.082
Water temp	0.006	0.208*	0.666*	0.321*	0.205*	0.255*	1	0.221*	-0.064
Wave ht	0.102	0.213*	0.124	0.157	0.288*	0.052	0.221*	1	-0.01
Tide stage	-0.107	-0.04	-0.104	0.209*	-0.09	0.082	-0.064	-0.01	1

*p<0.05

dCT=delta CT (qPCR Calibrator Cell Equivalents); ddCT=delta-delta CT (qPCR Calibrator Cell Equivalents)

CFU=Colony Forming Units; Entero.=*Enterococcus*; Bacter.=*Bacteroidales*

UV: Ultraviolet radiation; Tide: Tide stage at sampling time; Bathers Water: Bathers in the water; Wave ht: Wave height

Additional information see: Table 3.1

Table 4.93: Spearman pairwise correlation coefficients for *Enterococcus* CFU and environmental measures, Boquerón Beach

	Entero CFU	Bathers Water	Wind dir	Wind speed	UV	Water temp	Wave ht	Tide stage
Entero CFU	1	0.354*	0.046	0.167	-0.06	0.142	0.228*	-0.043
Bathers Water	0.354*	1	0.232*	0.326*	0.434*	0.666*	0.124	-0.104
Wind dir	0.046	0.232*	1	0.149	0.25*	0.321*	0.157	0.209*
Wind speed	0.167	0.326*	0.149	1	0.363*	0.205*	0.288*	-0.09
UV	-0.06	0.434*	0.25*	0.363*	1	0.255*	0.052	0.082
Water temp	0.142	0.666*	0.321*	0.205*	0.255*	1	0.221*	-0.064
Wave ht	0.228*	0.124	0.157	0.288*	0.052	0.221*	1	-0.01
Tide stage	-0.043	-0.104	0.209*	-0.09	0.082	-0.064	-0.01	1

*p<0.05

dCT=delta CT (qPCR Calibrator Cell Equivalents); ddCT=delta-delta CT (qPCR Calibrator Cell Equivalents)

CFU=Colony Forming Units; Entero.=*Enterococcus*; Bacter.=*Bacteroidales*

UV: Ultraviolet radiation; Tide: Tide stage at sampling time; Bathers Water: Bathers in the water; Wave ht: Wave height

Additional information see: Table 3.1

Table 4.94: Spearman pairwise correlation coefficients for fecal indicator bacteria and rainfall, Boquerón Beach

	Rain (1-day lag)
Entero. dCT	-0.149
Entero. ddCT	-0.224*
Bacter. dCT	0.039
Bacter. ddCT	-0.031
Entero CFU	0.455*
Rain (1-day lag)	1

*p<0.05

dCT=delta CT (qPCR Calibrator Cell Equivalents); ddCT=delta-delta CT (qPCR Calibrator Cell Equivalents)

CFU=Colony Forming Units; Entero.=*Enterococcus*; Bacter.=*Bacteroidales*

UV: Ultraviolet radiation; Tide: Tide stage at sampling time; Bathers Water: Bathers in the water; Wave ht: Wave height

Additional information see: Table 3.1

4.2.8 Associations between water quality and illness

Crude (unadjusted) incidence of illness among body immersion swimmers by tertile of indicator exposure and among non-swimmers are shown for Boquerón Beach in Table 4.95. The crude incidence rates do not show any obvious trend or association with exposure category with the exception of a slight increase in respiratory illness across categories of *Enterococcus* CFU. Crude incidence of illness for indicators measured by qPCR CCE calculated by the delta CT method is also shown since generally there were considerable differences in qPCR CCE measured by these two calculations at Boquerón Beach (Table 4.96).

Table 4.95: Number and percentage of respondents with incident illness for non-swimmers and among body immersion swimmers by tertiles of daily average of indicator exposures. Boquerón Beach. qPCR CCE determined through delta-delta CT calculation.

	GI		URI		Rash		Earache		Eye		Diarrhea	
	N	%[1]	N	%[1]	N	%[1]	N	%[1]	N	%[1]	N	%[1]
Enterococcus CCE												
Non-Swimmer	123	4.25	158	5.81	91	3.15	43	1.47	111	3.80	84	2.91
1.8,1.98	170	4.90	221	6.70	156	4.47	72	2.05	97	2.74	118	3.40
1.98,2.15	184	4.88	273	7.74	164	4.35	72	1.90	152	3.98	118	3.13
2.15,2.67	209	4.61	292	6.84	241	5.32	85	1.86	164	3.57	128	2.83
Bacteroidales CCE												
Non-Swimmer	123	4.25	158	5.81	91	3.15	43	1.47	111	3.80	84	2.91
2.58,2.95	156	4.73	202	6.44	138	4.14	75	2.24	93	2.77	110	3.34
2.95,3.18	208	5.10	302	7.95	215	5.29	82	2.00	157	3.81	131	3.22
3.18,3.38	199	4.53	282	6.78	208	4.73	72	1.62	163	3.65	123	2.80
Enterococcus CFU												
Non-Swimmer	123	4.25	158	5.81	91	3.15	43	1.47	111	3.80	84	2.91
-0.0351,0.585	187	4.90	244	6.68	177	4.61	76	1.97	125	3.22	122	3.20
0.585,1.09	153	4.14	250	7.17	169	4.56	72	1.93	131	3.49	95	2.57
1.09,1.43	223	5.24	292	7.38	215	5.06	81	1.89	157	3.63	147	3.45

1: Percentage of those within exposure category with symptom (row percentage). Number and percent not ill not shown
CCE: $\log_{10}$ qPCR Calibrator cell equivalents (delta-delta method). CFU: $\log_{10}$ colony forming units
URI: Upper respiratory illness

Enterococcus CFU (Method 1600)

The association between culturable *Enterococcus* CFU exposure as measured by EPA Method 1600 and illness are shown in Tables 4.97-4.102. During study enrollment no single day exceeded the EPA geometric mean criteria of 35 CFU

Table 4.96: Number and percentage of respondents with incident illness for non-swimmers and among body immersion swimmers by tertiles of daily average of indicator exposures. Boquerón Beach. qPCR CCE determined through delta CT calculation.

	GI		URI		Rash		Earache		Eye		Diarrhea	
	N	%	N	%	N	%	N	%	N	%	N	%
Enterococcus CCE												
Non-Swimmer	123	4.25	158	5.81	91	3.15	43	1.47	111	3.80	84	2.91
1.13,1.44	192	5.35	236	6.98	166	4.60	87	2.39	122	3.34	135	3.76
1.44,1.54	172	4.29	270	7.17	203	5.08	68	1.69	147	3.62	104	2.59
1.54,1.95	199	4.77	280	7.09	192	4.59	74	1.76	144	3.40	125	3.00
Bacteroidales CCE												
Non-Swimmer	123	4.25	158	5.81	91	3.15	43	1.47	111	3.80	84	2.91
1.9,2.33	155	4.77	205	6.62	142	4.32	75	2.27	94	2.84	106	3.26
2.33,2.54	198	4.69	291	7.41	210	4.99	82	1.93	162	3.80	127	3.01
2.54,2.96	210	4.89	290	7.13	209	4.87	72	1.66	157	3.59	131	3.05
Enterococcus CFU												
Non-Swimmer	123	4.25	158	5.81	91	3.15	43	1.47	111	3.80	84	2.91
-0.0351,0.585	187	4.90	244	6.68	177	4.61	76	1.97	125	3.22	122	3.20
0.585,1.09	153	4.14	250	7.17	169	4.56	72	1.93	131	3.49	95	2.57
1.09,1.43	223	5.24	292	7.38	215	5.06	81	1.89	157	3.63	147	3.45

CCE: ($\log_{10}$) qPCR Calibrator cell equivalents (delta method). CFU: ($\log_{10}$) colony forming units
URI: Upper respiratory illness

per 100 ml making it not possible to compare illness incidence associated with swimming on days with geometric means above 35 CFU per 100 ml.

Despite the low CFU levels, there was some evidence of an association with *Enterococcus* CFU and respiratory illness (AOR=1.31, p=0.06 for the daily average CFU exposure and body immersion, Table 4.99). As shown in Table 4.95, the absolute increase in illness is modest, from about 5.8% in non-swimmers to 7.4% among swimmers exposed to greater than a geometric mean of 12 *Enterococcus* CFU. Following adjustment for covariates, the estimated incidence of illness was changed as follows: in non-swimmers, 6.7%, swimmers in the lowest exposure category, 6.5%, and swimmers above 12 CFU had an estimated incidence of 7.8%.

No consistent associations between *Enterococcus* CFU exposure and illness were observed among children 10 and under. Positive but non-significant trends were observed between rash and respiratory illness among children (Table 4.103 and 4.104).

Table 4.97: Adjusted Odds Ratios *Enterococcus* CFU (Method 1600) and GI illness. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Boquerón Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	0.95	0.80	0.67	1.35	10802
Waist depth	1.01	0.95	0.72	1.43	10795
Shin depth	0.91	0.57	0.66	1.26	10803
8:00 AM	0.99	0.90	0.79	1.23	10795
Swimming-location	0.97	0.83	0.73	1.28	10802
Head immersion					
Daily	0.90	0.57	0.62	1.30	9658
Waist depth	0.98	0.92	0.67	1.43	8979
Shin depth	0.84	0.36	0.59	1.21	8986
8:00 AM	0.95	0.71	0.75	1.22	8986
Swimming-location	0.95	0.73	0.71	1.27	9657

Table 4.98: Adjusted Odds Ratios *Enterococcus* CFU (Method 1600) and Diarrhea. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Boquerón Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	0.95	0.81	0.64	1.42	10801
Waist depth	0.97	0.89	0.66	1.44	10799
Shin depth	0.94	0.74	0.64	1.37	10802
8:00 AM	1.00	0.99	0.76	1.32	10794
Swimming-location	0.93	0.64	0.68	1.27	10802
Head immersion					
Daily	0.77	0.23	0.51	1.18	8986
Waist depth	0.80	0.30	0.53	1.22	8986
Shin depth	0.78	0.21	0.52	1.16	9095
8:00 AM	0.89	0.43	0.66	1.20	8986
Swimming-location	0.85	0.36	0.61	1.20	8986

Table 4.99: Adjusted Odds Ratios *Enterococcus* CFU (Method 1600) and Respiratory illness. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Boquerón Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	1.31	0.06	0.99	1.74	10184
Waist depth	1.28	0.08	0.97	1.69	10184
Shin depth	1.31	0.05	1.00	1.71	10184
8:00 AM	1.01	0.94	0.84	1.21	10179
Swimming-location	1.12	0.31	0.90	1.41	11096
Head immersion					
Daily	1.27	0.10	0.95	1.70	9129
Waist depth	1.24	0.15	0.92	1.66	9129
Shin depth	1.28	0.08	0.97	1.68	9129
8:00 AM	1.01	0.95	0.83	1.22	8484
Swimming-location	1.09	0.53	0.84	1.41	8489

Table 4.100: Adjusted Odds Ratios *Enterococcus* CFU (Method 1600) and Rash. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Boquerón Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	1.22	0.23	0.88	1.68	10820
Waist depth	1.20	0.26	0.88	1.64	10947
Shin depth	1.19	0.28	0.87	1.63	10821
8:00 AM	1.21	0.05	1.00	1.46	10821
Swimming-location	1.39	0.03	1.03	1.87	10947
Head immersion					
Daily	1.19	0.34	0.83	1.71	9011
Waist depth	1.21	0.28	0.85	1.72	9117
Shin depth	1.15	0.44	0.81	1.63	9011
8:00 AM	1.22	0.08	0.98	1.51	9117
Swimming-location	1.49	0.02	1.08	2.05	9117

Table 4.101: Adjusted Odds Ratios *Enterococcus* CFU (Method 1600) and Earache. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Boquerón Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	0.92	0.73	0.58	1.46	11872
Waist depth	0.89	0.64	0.56	1.43	11023
Shin depth	0.96	0.87	0.61	1.52	10893
8:00 AM	0.97	0.82	0.74	1.27	10893
Swimming-location	0.88	0.51	0.60	1.29	11878
Head immersion					
Daily	0.75	0.25	0.47	1.22	9173
Waist depth	0.70	0.12	0.46	1.09	9178
Shin depth	0.83	0.42	0.53	1.30	9888
8:00 AM	0.88	0.36	0.66	1.16	9758
Swimming-location	0.84	0.41	0.55	1.27	9178

Table 4.102: Adjusted Odds Ratios *Enterococcus* CFU (Method 1600) and Eye irritations. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Boquerón Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	1.23	0.21	0.89	1.69	11947
Waist depth	1.25	0.16	0.92	1.71	11947
Shin depth	1.18	0.33	0.85	1.64	11086
8:00 AM	1.06	0.58	0.85	1.33	10955
Swimming-location	1.08	0.59	0.80	1.47	10955
Head immersion					
Daily	1.17	0.42	0.80	1.70	9115
Waist depth	1.20	0.31	0.85	1.70	9944
Shin depth	1.13	0.51	0.79	1.63	9224
8:00 AM	1.05	0.72	0.81	1.34	9114
Swimming-location	1.08	0.65	0.77	1.52	9115

Table 4.103: Adjusted Odds Ratios *Enterococcus* CFU (Method 1600) and Rash. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Boquerón Beach. Children age 10 and under.

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	1.24	0.59	0.56	2.75	1944
Waist depth	1.22	0.58	0.61	2.43	2087
Shin depth	1.24	0.58	0.58	2.65	1944
8:00 AM	1.23	0.34	0.80	1.88	1944
Swimming-location	1.30	0.44	0.66	2.55	2087
Head immersion					
Daily	1.29	0.55	0.56	2.99	1895
Waist depth	1.38	0.39	0.67	2.85	1895
Shin depth	1.21	0.65	0.53	2.75	1757
8:00 AM	1.25	0.32	0.80	1.95	1757
Swimming-location	1.52	0.23	0.76	3.04	1895

Table 4.104: Adjusted Odds Ratios *Enterococcus* CFU (Method 1600) and Respiratory illness. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Boquerón Beach. Children age 10 and under.

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	1.19	0.50	0.72	1.97	1933
Waist depth	1.05	0.87	0.57	1.95	1810
Shin depth	1.29	0.36	0.75	2.22	1933
8:00 AM	0.87	0.45	0.61	1.25	1810
Swimming-location	1.04	0.85	0.67	1.63	1810
Head immersion					
Daily	1.15	0.67	0.60	2.23	1632
Waist depth	1.01	0.97	0.51	2.01	1627
Shin depth	1.31	0.37	0.72	2.37	1751
8:00 AM	0.85	0.40	0.60	1.23	1632
Swimming-location	1.06	0.82	0.62	1.84	1630

Enterococcus qPCR Calibrator Cell Equivalents

Associations between illness and exposure to *Enterococcus* qPCR CCE by the delta-delta CT method are shown in Tables 4.105-4.110 and for the delta-CT method in Tables 4.111-4.116.

Overall patterns and trends with illness were inconsistent, probably reflecting the variability in the exposure resulting from the few usable and quantifiable samples (see Section 4.2.6). Illness associations were not consistent across the delta-CT and delta-delta CT calculations. For example, an association was observed between skin rash and *Enterococcus* CE for head immersion exposure by the delta method (Table 4.114, AOR=1.76, p=0.04), but not by the delta-delta method (Table 4.108, AOR=1.13, p=0.72). Marked inconsistencies were also observed in health effects associations by sample depth and by sample time.

Examining children separately even presented greater difficulties in interpretation as the sample size was reduced in addition to the probable inaccuracy in the qPCR exposure measurement. As might be expected given these issues, associations with *Enterococcus* CCE exposure and illness among children were inconsistent. An inverse association was observed for diarrhea and CCE exposure among children as incidence declined with increasing levels of CCE. However, given the limitations of the exposure data, it is difficult to meaningfully interpret these results.

Table 4.105: Adjusted Odds Ratios *Enterococcus* qPCR CCE, Delta-delta CT calculation and GI illness. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Boquerón Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	0.69	0.18	0.41	1.19	10934
Waist depth	1.01	0.96	0.58	1.77	10173
Shin depth	0.82	0.17	0.62	1.09	10934
8:00 AM	0.75	0.20	0.48	1.17	10300
Swimming-location	0.73	0.13	0.48	1.10	10934
Head immersion					
Daily	0.71	0.25	0.39	1.27	9095
Waist depth	0.99	0.98	0.53	1.85	8463
Shin depth	0.84	0.29	0.61	1.16	9095
8:00 AM	0.77	0.30	0.47	1.26	8574
Swimming-location	0.67	0.08	0.43	1.04	9095

Table 4.106: Adjusted Odds Ratios *Enterococcus* qPCR CCE, Delta-delta CT calculation and Diarrhea. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Boquerón Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	0.49	0.04	0.25	0.96	10933
Waist depth	1.09	0.81	0.55	2.16	11092
Shin depth	0.68	0.06	0.46	1.01	10933
8:00 AM	0.75	0.30	0.43	1.30	10300
Swimming-location	0.84	0.50	0.52	1.38	10802
Head immersion					
Daily	0.59	0.18	0.27	1.27	9095
Waist depth	1.09	0.82	0.50	2.40	8469
Shin depth	0.74	0.17	0.49	1.14	9095
8:00 AM	0.80	0.46	0.44	1.45	8574
Swimming-location	0.79	0.40	0.46	1.36	8986

Table 4.107: Adjusted Odds Ratios *Enterococcus* qPCR CCE, Delta-delta CT calculation and Respiratory illness. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Boquerón Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	0.90	0.66	0.54	1.47	10184
Waist depth	0.61	0.04	0.37	0.99	10489
Shin depth	1.08	0.48	0.87	1.34	11096
8:00 AM	0.81	0.27	0.55	1.18	9617
Swimming-location	0.78	0.15	0.55	1.10	10310
Head immersion					
Daily	1.03	0.92	0.60	1.77	8481
Waist depth	0.67	0.15	0.38	1.16	8757
Shin depth	1.12	0.33	0.89	1.42	9129
8:00 AM	0.90	0.65	0.58	1.40	8019
Swimming-location	0.81	0.28	0.55	1.19	8595

Table 4.108: Adjusted Odds Ratios *Enterococcus* qPCR CCE, Delta-delta CT calculation and Rash. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Boquerón Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	0.92	0.79	0.50	1.70	10815
Waist depth	1.00	0.99	0.57	1.76	10190
Shin depth	0.98	0.91	0.71	1.36	10814
8:00 AM	0.78	0.30	0.49	1.25	10315
Swimming-location	0.60	0.03	0.38	0.95	11793
Head immersion					
Daily	1.13	0.72	0.57	2.24	9003
Waist depth	1.14	0.68	0.61	2.11	8598
Shin depth	1.07	0.70	0.75	1.54	9012
8:00 AM	0.95	0.86	0.55	1.65	8498
Swimming-location	0.73	0.24	0.44	1.23	9117

Table 4.109: Adjusted Odds Ratios *Enterococcus* qPCR CCE, Delta-delta CT calculation and Earache. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Boquerón Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	0.88	0.79	0.35	2.21	10888
Waist depth	0.39	0.01	0.19	0.82	11207
Shin depth	1.24	0.30	0.83	1.86	11028
8:00 AM	0.59	0.09	0.32	1.08	11207
Swimming-location	0.76	0.39	0.41	1.41	11878
Head immersion					
Daily	1.20	0.73	0.43	3.31	9065
Waist depth	0.50	0.10	0.22	1.15	9339
Shin depth	1.35	0.17	0.88	2.09	9178
8:00 AM	0.74	0.39	0.38	1.46	9338
Swimming-location	0.98	0.97	0.49	1.98	9030

Table 4.110: Adjusted Odds Ratios *Enterococcus* qPCR CCE, Delta-delta CT calculation and Eye irritations. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Boquerón Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	0.99	0.98	0.56	1.75	10943
Waist depth	1.08	0.80	0.58	2.02	10326
Shin depth	0.97	0.82	0.72	1.30	10946
8:00 AM	1.22	0.41	0.76	1.98	10450
Swimming-location	0.69	0.10	0.45	1.07	11947
Head immersion					
Daily	1.23	0.52	0.66	2.31	9219
Waist depth	1.48	0.19	0.82	2.65	8701
Shin depth	1.04	0.81	0.75	1.45	9111
8:00 AM	1.37	0.24	0.81	2.33	8701
Swimming-location	0.71	0.18	0.43	1.18	9944

Table 4.111: Adjusted Odds Ratios *Enterococcus* qPCR CCE, Delta CT calculation and GI illness. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Boquerón Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	0.75	0.28	0.44	1.26	10934
Waist depth	1.07	0.78	0.66	1.73	10298
Shin depth	0.83	0.22	0.62	1.12	10934
8:00 AM	0.69	0.11	0.44	1.08	10300
Swimming-location	0.77	0.23	0.50	1.18	10803
Head immersion					
Daily	0.79	0.43	0.44	1.42	9095
Waist depth	1.14	0.62	0.68	1.92	8469
Shin depth	0.85	0.32	0.61	1.18	9095
8:00 AM	0.72	0.16	0.45	1.14	9238
Swimming-location	0.73	0.17	0.47	1.14	9095

Table 4.112: Adjusted Odds Ratios *Enterococcus* qPCR CCE, Delta CT calculation and Diarrhea. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Boquerón Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	0.60	0.14	0.30	1.18	10933
Waist depth	1.11	0.73	0.62	1.99	10948
Shin depth	0.71	0.10	0.48	1.07	10802
8:00 AM	0.74	0.29	0.42	1.30	10300
Swimming-location	0.86	0.59	0.49	1.50	10802
Head immersion					
Daily	0.65	0.26	0.32	1.36	9095
Waist depth	1.13	0.72	0.59	2.17	8469
Shin depth	0.76	0.21	0.49	1.17	8986
8:00 AM	0.70	0.24	0.39	1.27	9237
Swimming-location	0.81	0.46	0.47	1.42	9095

Table 4.113: Adjusted Odds Ratios *Enterococcus* qPCR CCE, Delta CT calculation and Respiratory illness. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Boquerón Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	1.05	0.80	0.71	1.56	10944
Waist depth	0.76	0.15	0.52	1.11	10489
Shin depth	1.13	0.28	0.91	1.40	11096
8:00 AM	1.00	0.98	0.72	1.41	9605
Swimming-location	0.85	0.28	0.62	1.15	11096
Head immersion					
Daily	1.19	0.42	0.78	1.82	9129
Waist depth	0.78	0.27	0.51	1.21	8757
Shin depth	1.21	0.10	0.97	1.52	9129
8:00 AM	1.14	0.48	0.80	1.63	8757
Swimming-location	0.89	0.50	0.63	1.25	8595

Table 4.114: Adjusted Odds Ratios *Enterococcus* qPCR CCE, Delta CT calculation and Rash. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Boquerón Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	1.39	0.20	0.84	2.31	10947
Waist depth	1.15	0.52	0.75	1.77	10980
Shin depth	1.22	0.15	0.93	1.59	10947
8:00 AM	1.07	0.74	0.71	1.63	10195
Swimming-location	0.88	0.49	0.61	1.27	10816
Head immersion					
Daily	1.76	0.04	1.03	3.01	9117
Waist depth	1.20	0.49	0.72	1.99	8599
Shin depth	1.32	0.06	0.99	1.77	9117
8:00 AM	1.29	0.26	0.82	2.02	9283
Swimming-location	1.02	0.91	0.68	1.53	9001

Table 4.115: Adjusted Odds Ratios *Enterococcus* qPCR CCE, Delta CT calculation and Earache. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Boquerón Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	0.87	0.73	0.41	1.88	10899
Waist depth	0.47	0.03	0.24	0.92	10395
Shin depth	1.29	0.24	0.85	1.95	11028
8:00 AM	0.56	0.07	0.30	1.04	11204
Swimming-location	0.76	0.33	0.43	1.33	11878
Head immersion					
Daily	1.08	0.86	0.46	2.56	9069
Waist depth	0.56	0.12	0.27	1.16	8658
Shin depth	1.39	0.15	0.89	2.17	9178
8:00 AM	0.65	0.20	0.34	1.26	9338
Swimming-location	0.97	0.92	0.53	1.76	9059

Table 4.116: Adjusted Odds Ratios *Enterococcus* qPCR CCE, Delta CT calculation and Eye irritations. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Boquerón Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	1.24	0.40	0.75	2.05	11086
Waist depth	1.20	0.47	0.73	1.95	10450
Shin depth	1.08	0.59	0.82	1.42	10951
8:00 AM	1.36	0.13	0.92	2.02	11274
Swimming-location	0.88	0.49	0.60	1.27	11947
Head immersion					
Daily	1.39	0.27	0.78	2.48	9224
Waist depth	1.35	0.27	0.79	2.31	9392
Shin depth	1.13	0.38	0.85	1.50	9944
8:00 AM	1.47	0.10	0.93	2.31	9392
Swimming-location	0.88	0.56	0.57	1.36	9939

Bacteroidales qPCR Calibrator Cell Equivalents

Associations between illness and exposure to *Bacteroidales* qPCR CCE by the delta-delta CT method are shown in Tables 4.117-4.122 and for the delta-CT method in Tables 4.111-4.128.

As with *Enterococcus* CCE interpretation of the illness-*Bacteroidales* CCE association was hampered by the high proportion of samples which showed interference or were not detected. Positive trends with both eye irritations and skin rash were observed with *Bacteroidales* CCE exposure by both types of CT calculations (see Tables 4.120, 4.122, 4.126. The interpretation of the trend among swimmers for eye irritations is complicated by the finding that non-swimmers actually experienced higher rates of eye irritations even compared to the most highly exposed swimmer category (see Figure 4.4 and Table 4.95). . However, the association with skin rash was not evident for *Bacteroidales* CCE calculated by the delta-delta CT method (see Table 4.126) where statistically significant associations were not observed.

Positive associations between rash and *Bacteroidales* CCE exposure were observed among children 10 and under for the delta-CT calculation (AOR=2.53, p=0.04 for body immersion exposure, Table 4.129), but the association was considerably weaker for CCE using the delta-delta CT calculation (AOR=1.24, p=0.72 for body immersion exposure, Table 4.130). A similar inverse association was observed for GI illness and diarrhea for *Bacteroidales* CCE exposure among children.

Table 4.117: Adjusted Odds Ratios *Bacteroidales* qPCR CCE, Delta-delta CT calculation and GI illness. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Boquerón Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	0.77	0.34	0.45	1.32	10934
Waist depth	0.78	0.33	0.48	1.29	11094
Shin depth	0.82	0.41	0.52	1.30	10934
8:00 AM	0.80	0.30	0.52	1.22	11095
Swimming-location	0.73	0.16	0.48	1.12	11611
Head immersion					
Daily	0.77	0.41	0.42	1.42	8985
Waist depth	0.75	0.32	0.43	1.32	9238
Shin depth	0.85	0.54	0.51	1.43	8985
8:00 AM	0.80	0.36	0.50	1.29	9238
Swimming-location	0.78	0.32	0.48	1.27	9658

Table 4.118: Adjusted Odds Ratios *Bacteroidales* qPCR CCE, Delta-delta CT calculation and Diarrhea. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Boquerón Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	0.64	0.18	0.33	1.23	10933
Waist depth	0.75	0.27	0.44	1.26	11094
Shin depth	0.66	0.14	0.38	1.15	10933
8:00 AM	0.72	0.20	0.44	1.18	11094
Swimming-location	0.64	0.08	0.39	1.06	10933
Head immersion					
Daily	0.64	0.24	0.30	1.36	9095
Waist depth	0.76	0.43	0.38	1.51	8470
Shin depth	0.69	0.27	0.36	1.34	9095
8:00 AM	0.81	0.49	0.44	1.48	8573
Swimming-location	0.72	0.28	0.40	1.31	9095

Table 4.119: Adjusted Odds Ratios *Bacteroidales* qPCR CCE, Delta-delta CT calculation and Respiratory illness. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Boquerón Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	0.97	0.88	0.62	1.51	10179
Waist depth	0.77	0.21	0.51	1.16	10489
Shin depth	1.14	0.44	0.82	1.60	10944
8:00 AM	0.86	0.42	0.61	1.23	9737
Swimming-location	0.74	0.10	0.52	1.06	11096
Head immersion					
Daily	0.98	0.93	0.59	1.61	8484
Waist depth	0.80	0.34	0.51	1.27	8634
Shin depth	1.16	0.48	0.77	1.74	8489
8:00 AM	0.78	0.25	0.51	1.19	8019
Swimming-location	0.80	0.26	0.54	1.19	9257

Table 4.120: Adjusted Odds Ratios *Bacteroidales* qPCR CCE, Delta-delta CT calculation and Rash. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Boquerón Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	1.49	0.12	0.91	2.46	11793
Waist depth	1.51	0.07	0.97	2.37	11128
Shin depth	1.29	0.22	0.86	1.94	11793
8:00 AM	0.90	0.60	0.60	1.35	11127
Swimming-location	1.32	0.21	0.85	2.03	10947
Head immersion					
Daily	1.42	0.23	0.80	2.54	9117
Waist depth	1.36	0.23	0.82	2.25	9283
Shin depth	1.29	0.30	0.80	2.07	9117
8:00 AM	0.82	0.41	0.51	1.32	9283
Swimming-location	1.28	0.32	0.79	2.09	9117

Table 4.121: Adjusted Odds Ratios *Bacteroidales* qPCR CCE, Delta-delta CT calculation and Earache. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Boquerón Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	0.73	0.39	0.36	1.48	10899
Waist depth	0.58	0.11	0.30	1.13	10395
Shin depth	1.03	0.92	0.56	1.89	10887
8:00 AM	0.67	0.14	0.39	1.14	10395
Swimming-location	0.76	0.37	0.42	1.39	10899
Head immersion					
Daily	0.99	0.99	0.46	2.14	9030
Waist depth	0.87	0.71	0.42	1.81	8654
Shin depth	1.19	0.60	0.61	2.32	9065
8:00 AM	0.81	0.45	0.46	1.41	8653
Swimming-location	0.91	0.77	0.47	1.74	9060

Table 4.122: Adjusted Odds Ratios *Bacteroidales* qPCR CCE, Delta-delta CT calculation and Eye irritations. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Boquerón Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	1.81	0.03	1.06	3.08	11086
Waist depth	1.62	0.05	1.00	2.62	10450
Shin depth	1.56	0.05	1.01	2.43	11086
8:00 AM	1.18	0.48	0.75	1.84	10324
Swimming-location	0.98	0.93	0.61	1.56	10906
Head immersion					
Daily	1.73	0.07	0.95	3.13	9224
Waist depth	1.56	0.10	0.92	2.66	8701
Shin depth	1.52	0.10	0.92	2.53	9224
8:00 AM	1.08	0.78	0.64	1.82	8591
Swimming-location	0.99	0.97	0.59	1.66	9104

Table 4.123: Adjusted Odds Ratios *Bacteroidales* qPCR CCE, Delta CT calculation and GI illness. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Boquerón Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	0.81	0.40	0.49	1.33	10932
Waist depth	0.88	0.61	0.55	1.42	10174
Shin depth	0.84	0.45	0.54	1.31	10932
8:00 AM	0.75	0.15	0.50	1.11	11092
Swimming-location	0.77	0.21	0.51	1.16	11748
Head immersion					
Daily	0.84	0.53	0.49	1.44	9788
Waist depth	0.90	0.71	0.53	1.54	8469
Shin depth	0.87	0.57	0.54	1.41	8985
8:00 AM	0.75	0.22	0.48	1.19	9238
Swimming-location	0.83	0.43	0.53	1.31	9788

Table 4.124: Adjusted Odds Ratios *Bacteroidales* qPCR CCE, Delta CT calculation and Diarrhea. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Boquerón Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	0.70	0.27	0.38	1.31	10800
Waist depth	0.83	0.51	0.48	1.44	11092
Shin depth	0.74	0.28	0.42	1.28	10933
8:00 AM	0.72	0.24	0.42	1.24	11095
Swimming-location	0.65	0.11	0.38	1.10	10933
Head immersion					
Daily	0.71	0.33	0.35	1.42	8985
Waist depth	0.85	0.60	0.46	1.57	9113
Shin depth	0.74	0.34	0.40	1.37	9095
8:00 AM	0.73	0.32	0.40	1.35	9238
Swimming-location	0.75	0.32	0.43	1.32	9658

Table 4.125: Adjusted Odds Ratios *Bacteroidales* qPCR CCE, Delta CT calculation and Respiratory illness. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Boquerón Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	1.10	0.65	0.74	1.63	10939
Waist depth	0.88	0.49	0.60	1.27	10345
Shin depth	1.26	0.21	0.87	1.81	10184
8:00 AM	1.06	0.74	0.76	1.46	10339
Swimming-location	0.83	0.29	0.60	1.16	11096
Head immersion					
Daily	1.13	0.58	0.73	1.75	9129
Waist depth	0.87	0.51	0.57	1.32	8757
Shin depth	1.37	0.13	0.91	2.04	8489
8:00 AM	1.00	1.00	0.68	1.48	8009
Swimming-location	0.91	0.64	0.62	1.34	8595

Table 4.126: Adjusted Odds Ratios *Bacteroidales* qPCR CCE, Delta CT calculation and Rash. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Boquerón Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	2.11	0.00	1.35	3.31	11793
Waist depth	1.58	0.03	1.04	2.39	11128
Shin depth	1.94	0.00	1.31	2.87	11793
8:00 AM	1.20	0.30	0.85	1.71	10320
Swimming-location	1.78	0.00	1.24	2.57	11793
Head immersion					
Daily	1.88	0.01	1.14	3.09	9826
Waist depth	1.40	0.17	0.86	2.27	8603
Shin depth	1.99	0.00	1.27	3.12	9117
8:00 AM	1.12	0.56	0.76	1.66	9283
Swimming-location	1.83	0.01	1.19	2.82	9117

Table 4.127: Adjusted Odds Ratios *Bacteroidales* qPCR CCE, Delta CT calculation and Earache. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Boquerón Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	0.78	0.46	0.41	1.49	10899
Waist depth	0.64	0.14	0.36	1.16	10395
Shin depth	1.07	0.82	0.59	1.94	10892
8:00 AM	0.62	0.06	0.37	1.02	10390
Swimming-location	0.78	0.40	0.45	1.38	11028
Head immersion					
Daily	0.94	0.86	0.46	1.91	9060
Waist depth	0.78	0.46	0.41	1.49	8648
Shin depth	1.20	0.60	0.61	2.35	9070
8:00 AM	0.72	0.27	0.40	1.29	8657
Swimming-location	0.91	0.75	0.50	1.64	9065

Table 4.128: Adjusted Odds Ratios *Bacteroidales* qPCR CCE, Delta CT calculation and Eye irritations. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Boquerón Beach

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	1.95	0.01	1.19	3.22	11086
Waist depth	1.68	0.03	1.05	2.69	10450
Shin depth	1.78	0.01	1.16	2.72	11947
8:00 AM	1.34	0.13	0.92	1.95	11274
Swimming-location	1.25	0.29	0.82	1.88	10956
Head immersion					
Daily	1.73	0.06	0.98	3.04	9224
Waist depth	1.51	0.12	0.90	2.55	8701
Shin depth	1.69	0.04	1.02	2.82	9224
8:00 AM	1.18	0.48	0.75	1.86	8697
Swimming-location	1.20	0.45	0.75	1.92	9115

Table 4.129: Adjusted Odds Ratios *Bacteroidales* qPCR CCE, Delta CT calculation and Rash. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Boquerón Beach. Children age 10 and under.

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	2.53	0.04	1.06	6.04	2087
Waist depth	1.70	0.21	0.74	3.88	1955
Shin depth	2.63	0.02	1.14	6.05	1944
8:00 AM	0.82	0.62	0.38	1.79	1819
Swimming-location	1.34	0.44	0.64	2.82	2087
Head immersion					
Daily	2.72	0.04	1.07	6.90	1895
Waist depth	1.82	0.18	0.76	4.38	1782
Shin depth	2.53	0.03	1.07	5.96	1757
8:00 AM	0.98	0.97	0.43	2.23	1648
Swimming-location	1.40	0.43	0.60	3.28	1895

Table 4.130: Adjusted Odds Ratios *Bacteroidales* qPCR CCE, Delta-delta CT calculation and Rash. Daily, waist depth, shin depth, 8:00 AM and swimming-location indicator averages. Boquerón Beach. Children age 10 and under.

Exposure	AOR	P-value	Lower 95% CI	Upper 95% CI	N
Body immersion					
Daily	1.24	0.72	0.38	4.04	1944
Waist depth	1.34	0.62	0.42	4.27	1819
Shin depth	1.16	0.75	0.46	2.95	1944
8:00 AM	0.43	0.11	0.15	1.20	1821
Swimming-location	0.56	0.28	0.19	1.62	2087
Head immersion					
Daily	1.05	0.93	0.30	3.69	1755
Waist depth	1.20	0.78	0.34	4.29	1648
Shin depth	0.93	0.88	0.37	2.35	1757
8:00 AM	0.45	0.14	0.16	1.28	1650
Swimming-location	0.51	0.26	0.16	1.65	1895

4.3 Sensitivity analyses

Several variations of the analyses were conducted to assess the stability of the results. In the above analyses participants who reported swimming exposure in the 1-week prior to enrollment were included (but this exposure was controlled for in regression modeling). Below results are also presented for excluding respondents with recent swimming exposure and for various different approaches to calculating qPCR CCE below the limit of detection. The tables below only show results for body immersion swimming exposure and the daily indicator average.

4.3.1 Surfside Beach

Excluding those with swimming exposure in 1-week prior to enrollment

Results are shown below in Tables 4.131-4.133 for the daily averaged indicator exposures for *Enterococcus* CFU and qPCR CCEs using the delta CT calculation. Sample size is greatly reduced by excluding those with prior swimming exposures, but there are little differences in the effect estimates.

For comparison with original results, see Tables 4.33- 4.38 for *Enterococcus* CFU, Tables 4.50- 4.55 for *Enterococcus* qPCR CCE, and Tables 4.64- 4.69 for *Bacteroidales* qPCR CCE.

Table 4.131: Adjusted Odds Ratios *Enterococcus* CFU (Method 1600). Daily indicator averages. Excluding those with swimming exposure in past 1-week. Body immersion swimming exposure. Surfside Beach

Illness	AOR	P-value	Lower 95% CI	Upper 95% CI	N
GI illness	1.19	0.37	0.81	1.75	3815
Diarrhea	1.33	0.21	0.85	2.09	3813
Respiratory Illness	0.86	0.47	0.58	1.28	3792
Rash	0.82	0.45	0.49	1.37	3880
Eye irritations	0.96	0.88	0.53	1.72	3884
Earache	0.86	0.67	0.43	1.73	3913

Alternate approaches for results below limit of detection for qPCR

Because even at Surfside beach there were a fairly high proportion of *Enterococcus* qPCR CCE which were below the limit of detection (N=167), some variation in results was expected with different approaches to handle results below the detection limit. However, health effects associations with qPCR CCE using the maximum likelihood approach and the regression on order statistic approach showed no fundamental differences in interpretation as using one-half the detection limit (Tables 4.134 and 4.135).

Table 4.132: Adjusted Odds Ratios *Enterococcus* qPCR CCE, Delta CT calculation. Daily indicator averages. Excluding those with swimming exposure in past 1-week. Body immersion swimming exposure. Surfside Beach

Illness	**AOR**	**P-value**	**Lower 95% CI**	**Upper 95% CI**	**N**
GI illness	0.98	0.91	0.71	1.36	3692
Diarrhea	1.18	0.38	0.82	1.70	3690
Respiratory Illness	0.76	0.15	0.52	1.10	3612
Rash	0.50	0.01	0.29	0.85	3760
Eye irritations	0.81	0.44	0.47	1.39	3764
Earache	0.92	0.72	0.59	1.43	3787

Table 4.133: Adjusted Odds Ratios *Bacteroidales* qPCR CCE, Delta CT calculation. Daily indicator averages. Excluding those with swimming exposure in past 1-week. Body immersion swimming exposure. Surfside Beach

Illness	**AOR**	**P-value**	**Lower 95% CI**	**Upper 95% CI**	**N**
GI illness	0.97	0.93	0.53	1.77	3690
Diarrhea	0.92	0.81	0.44	1.90	3688
Respiratory Illness	1.00	0.99	0.50	2.00	3669
Rash	0.77	0.40	0.41	1.43	3760
Eye irritations	1.24	0.71	0.41	3.72	3758
Earache	0.67	0.39	0.28	1.65	3730

Table 4.134: Adjusted Odds Ratios *Enterococcus* qPCR CCE, Delta CT calculation. Maximum likelihood estimate for non-detects. Daily indicator averages. Body immersion swimming exposure. Surfside Beach

Illness	**AOR**	**P-value**	**Lower 95% CI**	**Upper 95% CI**	**N**
GI illness	1.14	0.14	0.96	1.35	7752
Diarrhea	1.16	0.18	0.94	1.43	7748
Respiratory Illness	1.04	0.73	0.85	1.26	7618
Rash	0.74	0.00	0.61	0.90	7833
Eye irritations	1.02	0.87	0.79	1.32	7901
Earache	0.90	0.41	0.72	1.14	7927

Table 4.135: Adjusted Odds Ratios *Enterococcus* qPCR CCE, Delta CT calculation. Regresson on order statistics estimate for non detects. Daily indicator averages. Body immersion swimming exposure. Surfside Beach

Illness	**AOR**	**P-value**	**Lower 95% CI**	**Upper 95% CI**	**N**
GI illness	1.16	0.14	0.95	1.40	7752
Diarrhea	1.25	0.07	0.98	1.60	7748
Respiratory Illness	1.12	0.28	0.92	1.36	7725
Rash	0.66	0.00	0.52	0.84	7731
Eye irritations	1.10	0.56	0.80	1.51	8009
Earache	0.89	0.38	0.68	1.16	7821

Health effects associations with *Bacteroidales*, which had considerably fewer non-detects results, showed essentially no differences as compared to using one-half the detection limit (Tables 4.136 and 4.137).

Table 4.136: Adjusted Odds Ratios *Bacteroidales* qPCR CCE, Delta CT calculation. Maximum likelihood estimate for non-detects. Daily indicator averages. Body immersion swimming exposure. Surfside Beach

Illness	**AOR**	**P-value**	**Lower 95% CI**	**Upper 95% CI**	**N**
GI illness	1.16	0.49	0.76	1.77	7750
Diarrhea	1.09	0.75	0.65	1.83	7746
Respiratory Illness	1.14	0.59	0.70	1.86	7725
Rash	0.68	0.12	0.41	1.11	7831
Eye irritations	0.99	0.98	0.46	2.15	7899
Earache	0.95	0.88	0.50	1.80	7817

Table 4.137: Adjusted Odds Ratios *Bacteroidales* qPCR CCE, Delta CT calculation. Regresson on order statistics estimate for non detects. Daily indicator averages. Body immersion swimming exposure. Surfside Beach

Illness	**AOR**	**P-value**	**Lower 95% CI**	**Upper 95% CI**	**N**
GI illness	1.22	0.31	0.83	1.78	7853
Diarrhea	1.14	0.62	0.68	1.89	7746
Respiratory Illness	1.18	0.49	0.73	1.91	7719
Rash	0.74	0.20	0.46	1.18	7729
Eye irritations	1.00	1.00	0.47	2.14	7899
Earache	0.91	0.76	0.49	1.68	7817

4.3.2 Boquerón Beach

Excluding those with swimming exposure in 1-week prior to enrollment

Results are shown in Tables 4.138-4.140. The association between respiratory illness and *Enterococcus* CFU was more pronounced (Table 4.138). Other associations were similar, although the same concerns regarding interpretation of the qPCR results described previously still apply.

For comparison with original results, see Tables 4.97- 4.102 for *Enterococcus* CFU, Tables 4.111- 4.116 for *Enterococcus* qPCR CCE, and Tables 4.123- 4.128 for *Bacteroidales* qPCR CCE.

Table 4.138: Adjusted Odds Ratios *Enterococcus* CFU (Method 1600). Daily indicator averages. Excluding those with swimming exposure in past 1-week. Body immersion swimming exposure. Boquerón Beach

Illness	AOR	P-value	Lower 95% CI	Upper 95% CI	N
GI illness	1.00	0.98	0.66	1.50	7651
Diarrhea	0.99	0.96	0.61	1.60	7651
Respiratory Illness	1.44	0.03	1.03	2.02	7237
Rash	1.18	0.42	0.79	1.74	7672
Eye irritations	1.25	0.28	0.83	1.89	8467
Earache	1.07	0.81	0.61	1.89	8421

Table 4.139: Adjusted Odds Ratios *Enterococcus* qPCR CCE, Delta CT calculation. Daily indicator averages. Excluding those with swimming exposure in past 1-week. Body immersion swimming exposure. Boquerón Beach

Illness	AOR	P-value	Lower 95% CI	Upper 95% CI	N
GI illness	1.34	0.36	0.72	2.52	8335
Diarrhea	1.40	0.40	0.64	3.09	7758
Respiratory Illness	1.01	0.97	0.62	1.65	7232
Rash	1.83	0.04	1.04	3.20	8362
Eye irritations	1.08	0.79	0.61	1.90	8467
Earache	0.51	0.19	0.19	1.39	8420

Alternate approaches for results below limit of detection for qPCR

As expected with the high proportion of results below detection, the results are affected by the method used to handle these results, especially for *Enterococcus* CCE. For *Bacteroidales* CCE, no fundamental differences in interpretation are

Table 4.140: Adjusted Odds Ratios *Bacteroidales* qPCR CCE, Delta CT calculation. Daily indicator averages. Excluding those with swimming exposure in past 1-week. Body immersion swimming exposure. Boquerón Beach

Illness	**AOR**	**P-value**	**Lower 95% CI**	**Upper 95% CI**	**N**
GI illness	1.18	0.51	0.72	1.94	7649
Diarrhea	1.26	0.44	0.71	2.23	8204
Respiratory Illness	1.07	0.75	0.71	1.60	7230
Rash	2.13	0.00	1.35	3.37	8367
Eye irritations	1.38	0.14	0.90	2.13	8467
Earache	0.61	0.22	0.28	1.34	7828

observed and swimming-associated rash and eye irritations are still evident for the delta-CT calculation. However, as discussed above, the association with rash and *Bacteroidales* CCE is still diminished for the delta-delta CT calculation for alternate detection limit calculations (AOR=1.38, p=0.19; and AOR=1.23, p=0.38 for the maximum likelihood and regression on order statistics estimates).

Table 4.141: Adjusted Odds Ratios *Enterococcus* qPCR CCE, Delta CT calculation. Maximum likelihood estimate for non-detects. Daily indicator averages. Body immersion swimming exposure. Boquerón Beach

Illness	**AOR**	**P-value**	**Lower 95% CI**	**Upper 95% CI**	**N**
GI illness	1.15	0.25	0.91	1.46	11095
Diarrhea	1.19	0.28	0.87	1.62	11095
Respiratory Illness	1.08	0.52	0.85	1.38	9733
Rash	1.18	0.23	0.90	1.54	10315
Eye irritations	1.06	0.70	0.79	1.42	10326
Earache	1.04	0.85	0.71	1.51	10379

Table 4.142: Adjusted Odds Ratios *Enterococcus* qPCR CCE, Delta CT calculation. Regresson on order statistics estimate for non detects. Daily indicator averages. Body immersion swimming exposure. Boquerón Beach

Illness	**AOR**	**P-value**	**Lower 95% CI**	**Upper 95% CI**	**N**
GI illness	0.89	0.45	0.66	1.20	9666
Diarrhea	0.87	0.46	0.59	1.27	9666
Respiratory Illness	1.21	0.07	0.98	1.50	9945
Rash	1.02	0.89	0.73	1.43	9682
Eye irritations	1.34	0.09	0.96	1.88	9928
Earache	1.04	0.89	0.62	1.74	9757

Table 4.143: Adjusted Odds Ratios *Bacteroidales* qPCR CCE, Delta CT calculation. Maximum likelihood estimate for non-detects. Daily indicator averages. Body immersion swimming exposure. Boquerón Beach

Illness	**AOR**	**P-value**	**Lower 95% CI**	**Upper 95% CI**	**N**
GI illness	0.88	0.63	0.52	1.50	10173
Diarrhea	0.83	0.58	0.42	1.63	10174
Respiratory Illness	1.03	0.90	0.69	1.54	10335
Rash	1.89	0.00	1.21	2.95	11128
Eye irritations	1.94	0.01	1.17	3.22	10450
Earache	0.78	0.46	0.40	1.52	10263

Table 4.144: Adjusted Odds Ratios *Bacteroidales* qPCR CCE, Delta CT calculation. Regresson on order statistics estimate for non detects. Daily indicator averages. Body immersion swimming exposure. Boquerón Beach

Illness	**AOR**	**P-value**	**Lower 95% CI**	**Upper 95% CI**	**N**
GI illness	0.83	0.47	0.49	1.38	10298
Diarrhea	0.79	0.49	0.41	1.53	10174
Respiratory Illness	1.03	0.88	0.69	1.54	9608
Rash	1.62	0.03	1.05	2.48	11128
Eye irritations	1.87	0.01	1.15	3.06	10450
Earache	0.80	0.49	0.41	1.54	10263

Chapter 5

Summary and discussion

5.1 Overview

This report provides a rigorous, detailed analysis and presentation of water quality and illnesses among swimmers at studies conducted by the EPA during the summer of 2009. The primary goal of this research and report was to describe associations between water quality indicators and health effects at a beach site impacted by urban runoff and a tropical beach site. The tropical beach site was selected with nearby discharges from sewage treatment so that results could be compared with previous studies conducted by EPA and others [3, 2, 54].

Enrollment and follow up in the epidemiology study was successful. A large number of subjects were enrolled with more than 10,000 usable responses from each beach site. Follow up was also very successful with more than 60% of those initially approached completing the study at Surfside Beach and nearly 80% at Boquerón Beach. Among those agreeing or eligible to be in the study, 5% or less were lost to follow up at both sites.

The overall incidence of symptoms appears to be consistent with what has been previously observed, at least for GI illness (the symptom most frequently associated with recreational water exposure [7, 8]). At Surfside and Boquerón beaches in 2009, the overall rates of GI illness were consistent with the rates reported in previous population based surveys and epidemiology studies. The yearly equivalent rates for GI illness (both swimmers and non-swimmers) were 1.56 and 2.04 per year at Boquerón and Surfside, respectively. If swimmers are excluded, the incidence is lower (1.44 for Surfside and 1.41 at Boquerón). CDC's Foodnet Survey reported an annual rate of diarrhea of 1.4 episodes per person-year [35]. In a review of of studies and surveys in developed countries, Roy et. al. [55] found a range of rates from 0.1-3.5 per person-year. One surprise, however, was the relatively low incidence of GI illness at Boquerón Beach, where it was anticiapted a higher endemic incidence may have been observed due to the tropical setting.

In interpreting the results, the following criteria were used to establish associations between water quality and swimming-associated illnesses.

- The water quality (exposure) indicator must be sufficiently sensitive and have demonstrated accuracy and reliability.
- The incidence of illness among swimmers should increase as exposure to the concentration of the indicator increases.
  - The above association should demonstrate consistent and predictable patterns, such as consistency of effect across approaches to calculating and averaging the exposure
- The adjusted incidence among the most highly exposed swimmers should generally (not necessarily always) be higher among swimmers as compared to non swimmers

The last item is technically not required to establish associations with water quality and illness, but the lack of a difference between swimmers and non-swimmers complicates a sensible description of risk associated with swimming and exposure.

Conclusions regarding relationships to health effects should hold for both approaches in calculating the Calibrator Cell Equivalents by qPCR and should be robust to different approaches in calculating results below the limit of detection.

Also in interpreting the results, care should be taken to not simply select a statistically significant result as evidence of an effect or an association. Given the large number of analyses conducted p-values should be interpreted with caution and meant as a guide to the relative strength of the associations presented.

5.2 General limitations

Water quality at both beaches was relatively good compared to previous beaches studied. Unfortunately a low range of exposure can impact the determination of health effects associations since statistical power and ability to detect an association are reduced. However due to the large number of subjects enrolled, the study likely had statistical power to observe effects of a reasonable size. For example, statistical simulations of statistical power [56] conducted using the actual exposure data and observed incidence of GI illness indicated that for *Enterococcus* qPCR CCE (delta-delta CT) there was a sufficient sample size to observe an AOR of about 1.4 for Boquerón Beach and 1.3 for Surfside Beach. These effect sizes are lower than those observed at other marine sites [57] but slightly higher than the effect observed at freshwater beaches [3].

As shown in Table 5.1 fecal indicators measured at Surfside and Boquerón were low compared to the other three marine beach sites studied in 2005-2007. With the exception of *Enterococcus* CFU at Goddard Beach in Rhode Island, which had an overall lower geometric mean than Boquerón Beach, geometric means for all the indicators were lower at the 2009 beach sites. The fecal

indicators measured by qPCR were considerably lower in 2009: less than half the estimated CCE at the other marine beach sites. There is also a variation in the CFU to CCE ratio across the beach sites. This could be partially explained by the detection of non-viable organisms by qPCR.

Table 5.1: Geometric mean of fecal indicator bacteria, marine beach sites, 2005-2009

	Enterococcus CFU[1]	*Enterococcus* CCE[2]	*Bacteroidales* CCE[2]
Edgewater Beach	8	368	2750
Fairhope Beach	21	258	1791
Goddard Beach	4	159	1092
Surfside Beach	3	55	295
Boquerón Beach	6	32	288

CCE=qPCR Calibrator Cell Equivalents per 100 ml (delta-delta CT

CFU: Colony forming units per 100 ml

The low levels of fecal indicators were also reflected in the relatively high number of samples where no target was detected, especially for *Enterococcus*. Even at Surfside Beach 32% of samples were not detected for *Enterococcus* by qPCR. Also at Surfside, 12% of samples were not detected for *Bacteroidales*. These were considerably higher than the numbers of non-detects observed at the three previously studied marine sites where for *Enterococcus* 10% of samples were not detected, and for *Bacteroidales* 5% were not detected. For Boquerón Beach, the problem with non-detected target sequences was greatly exacerbated, where only a small minority of samples were actually quantifiable (see Section 4.2.6 for discussion).

For some environmental measures, relying on few measures may not be problematic for establishing valid associations with health effects. However, it has been shown that fecal indicator bacteria can vary considerably over time, space and location [58, 59] and relying on few samples is likely to result less accurate exposure measurement and classification, further reducing the ability to determine valid associations with health effects.

An additional issue not seen in most previous beach studies was the low proportion of non-swimmers. At the three previous marine sites, 42% of respondents reported body immersion swimming exposure, whereas 72% reported body immersion exposure at Surfside and 77% at Boquerón Beach. While this provided more statistical power to examining associations of the effects among swimmers exposed to varying levels of water quality, it may have reduced the ability to accurately describe differences between swimmers and non-swimmers.

5.3 Surfside Beach

Indicator bacteria and health effects associations were originally derived by EPA [60, 61, 5, 6] and others [62, 54] at beaches impacted by human sewage. These original sites were selected for several reasons. It was expected the indicator bacteria and pathogen association would be more consistent at such sites (so long as point sources were not "small", or experiencing outbreak conditions, see [5] for discussion). Furthermore it was expected that human-feces exposures pose a larger risk to human health [63, 1].

An issue with sites impacted by runoff or non-point source pollution is that the fecal contamination may be from an inconsistent or variable sources which may present problems in understanding or deriving health effects from fecal indicators. It may be analogous to the "small point source" problem described by Cabelli [5]:

> The rationale for the use of guidelines and standards based on fecal indicator densities for indexing the health hazards in sewage polluted waters is that, under average conditions of illness in the discharging population, there is a reasonably constant indicator to pathogen ratio in the sewage and its receiving waters. Thereby, an acceptable probability of illness caused by the pathogen can be extrapolated to a given indicator density, which is then recommended as a guideline and promulgated as a standard. Such relationships appear to hold for waters receiving the discharges from relatively large municipal sewage treatment facilities. However, as the number of individuals who contribute to the source of the fecal wastes becomes smaller and smaller, the indicator-pathogen ratio will vary more and more from the average upon which the guideline or standard is based. [5]

Previous studies at runoff impacted sites in marine waters have had mixed results. For example, an increased risk of illness was found among swimmers near storm drains in Santa Monica beaches in California [14] but in Mission Bay, California, no associations were observed with levels of fecal indicator organisms and illness with the possible exception of male-specific coliphage, but this was based on few observations [18]. Recently a randomized trial found an association with skin symptoms at a runoff impacted site in Florida, but failed to find an association with GI or respiratory symptoms [64, 65]. *Enterococcus* CFU at this site during the study was higher than at Surfside Beach with a median of 19 CFU per 100 ml, though the sampling design was considerably different (see [65]).

Surfside Beach was selected as a "urban runoff" site and had no known point source of human fecal contamination which affected the site. Water quality was generally of high quality and only one day exceeded EPA's recommended geometric mean criterion of 35 CFU per 100 ml. Swimmers immersing their head had a higher incidence of skin rash, earache and GI illness compared to non-swimmers.

Positive but generally non-significant trends were observed between *Enterococcus* CFU measured by culture and GI illness. A slightly stronger, but still statistically insignificant association was observed with diarrhea. GI illness increased from 5% among non swimmers to 6% among those exposed in the lowest tertile to 7% among thosed exposed to the highest tertile of exposure of *Enterococcus* CFU. Those who were exposed on the single day when the geometric mean exceeded 35 CFU had a higher incidence of illness compared to non-swimmers (8.6% vs. 4.7%). Illness was also elevated compared to other swimmers but differences between swimmers were not stastically significant. Among children the incidence of GI illness was more pronounced on this day (17% of those with body immersion reported illness) but was based on few cases of illness. No other associations were obsered with other illnesses and *Enterococcus* CFU either on a continuous scale or above the criteria levels.

Enterococcus qPCR CCE also was positively associated with both GI illness and diarrhea, but again the trends were not statistically significant. The strongest association was between *Enterococcus* qPCR CCE by the delta-delta method and diarrhea for the daily averaged indicator values and body immersion swimming exposure (AOR=1.28, p=0.08, see Table 4.45). Among children, the trend between diarrhea and *Enterococcus* CCE was not statistically significant (AOR=1.36, p=0.27 for body immersion exposure by the delta-delta CT method). In contrast *Bacteroidales* exposures showed very few positive or even borderline associations with illness.

An unexplained inverse association was observed between *Enterococcus* CCE and incidence of skin rash was observed among swimmers. Examination of numerous other water quality parameters (turbidity, dissolved oxygen, conductivity, pH) and other risk factors for rash could not explain this inverse association. More puzzling was that the incidence was increased in swimmers compared to non-swimmers, but among swimmers declined as exposures to *Enterococcus* CCE increased. Since it is not entirely plausible that *Enterococcus* CCE would be causally associated with a decline in rash, it may be that *Enterococcus* CCE are inversely associated with other water quality factors which may cause or influence the risk of skin rash.

Respiratory symptoms were associated with *Bacteroidales* CCE among swimming children 10 under . The interpretation of this finding was complicated in that the adjusted estimates affected the association considerably, which was absent from the crude and unadjusted estimates. This is a concern because the relatively small sample sizes among children could be producing unstable estimates which are highly affected by adjustment. Because of the finding of a high incidence of illness among the non-swimming group and the few numbers of children in this group, the representativeness of it as a comparison group may be suspect for this illness outcome. When compared against non-swimmers, risks are not elevated even among the most highly exposed swimming children. However, previous studies have also observed increased respiratory illnesses in relation to fecal contamination [66, 14].

The results observed at Surfside Beach are consistent with what would be expected from lower illness risk at runoff impacted beach sites compared to

beach sites impacted by human sewage. However, no conclusion can be made on the basis of these data alone due to the high quality of water observed at Surfside Beach . .

5.4 Boquerón Beach

As described previously, interpretation of results at Boquerón Beach was seriously hampered by the interference shown in the qPCR assay. However, even had there been no interference there was surprisingly low levels of fecal indicator bacteria even as measured by the standard culture based method for *Enterococcus*.

Presently, the exact reason for the interference is not known. In samples collected during dry runs prior to the start of the study, this interference was not seen. Preliminary analyses were conducted to attempt to identify patterns with the samples which failed the Salmon assay criterion. Birds in the water, turbidity, density of bathers in the water, amount of debris in the water, conductivity, collection time, tide stage, total algal density, and water depth were associated with a failure of the Salmon assay criterion. In a multivariate model, water depth, tide stage at 8:00 AM, debris and total algal density were associated. A formal analysis of the causes and correlates of the interference was beyond the scope of this report, however it appears that factors associated with floating debris in the water could have influenced the interference. Although ignoring the results of the Salmon assay was considered, there would be concern regarding the validity of the target assay results. Some of the factors or biological processes which could be causing the failure would be the presence of nucleases in the water which degrade the Salmon DNA, or humic substances interfering with the assay.

As a result, the health associations with qPCR CCE at Boquerón Beach are very difficult to interpret and the attempt to draw conclusions regarding the data reported would be questionable. Although results were presented and described, until there is is more confidence in the exposure measure it is impossible to place much emphasis on any associations observed. It appears the Salmon assay was a strong determinant in the results presented. For example, if the delta-delta method is used, but failure of the ±3 CT Salmon assay criterion is ignored associations between *Enterococcus* CCE and GI illness are reversed from those shown in the report, and are statistically significant and consistent with those observed previously (AOR=1.22, p=0.008, data not shown). However this type of variation across calculation approaches indicates a lack of consistency and calls into question the confidence in the exposure measure.

The low levels of indicator bacteria at Boquerón Beach were unexpected. More surprising was the low levels seen at samples collected near the outfalls and discharge points for the sewage treatment plant and the mangrove swamp (see 4.18) where it was expected that levels of fecal indicator organisms would be high. It may have been that discharges from the sewage treatment plant and various package plants were actually not impacting the beach as suspected,

despite their proximity (less than 1-mile). For example, the bay has little wave and tidal action and as a result there could be little circulation from the discharge points to the beach. Or water could be transported away from the beach site into the open ocean.

Boquerón Beach had high densities of bathers who often stayed in the water for long periods of time. The numbers of bathers in the water were positively associated with each of the fecal indicator measures (see Tables 4.91, 4.93 4.91). Studies have shown that bathers shed large amounts of indicator bacteria [67] and bather load has been associated with pathogens in recreational waters [68, 69]. Bather density was considered in the principal components analysis of environmental measures which were then subsequently incorporated into the regression models for health effects (see Section 3.5.4). Although not a principal goal of this report, models using bather density as a direct predictor of illness showed no associations (data not shown). Furthermore the lack of differences in illnesses between swimmers and non-swimmers (with the exception of skin rash, see Table 4.82) do not support a high risk of illness resulting from other swimmers. However, bather density was rather crudely categorized (see Table 3.1) and may not have been adequately represented for the purposes of deriving health risks.

Skin rash was the only symptom significantly elevated among swimmers compared to non-swimmers. Although associations between rash and *Enterococcus* CFU among swimmers were positive, they were weak. . There was a statistically insignificant trend between *Enterococcus* CFU and respiratory illness (p=0.06). This association improved (p=0.03) when those with recent swimming exposure were excluded (Table 4.131). This association was present despite no overall differences between swimmers and non-swimmers. Two factors can account for this apparent contradiction: Few numbers of non-swimmers, and few swimmers exposed to poor quality water. Furthermore, the non-swimming group was quite different from the swimming group, complicating swimmer and non-swimmer comparisons. This can be seen in Table 4.81 where despite higher crude incidence of respiratory illness among swimmers compared to non-swimmers (7.0% vs. 5.8%) the adjusted risk ratio is slightly less than 1.

Incidence of GI illness was lower than respiratory illness at Boquerón Beach. At all previous sites studied, GI illness had the highest incidence. Furthermore, no positive trends were observed between GI illness and any of the indicators. One potential explanation could be immunity in the local population to pathogens causing GI illness. Furthermore, it was noted that there were cases of the H1N1 flu in Puerto Rico at this time. While transmission through water is unlikely, there may have been an actual increased respiratory symptoms which may have been exacerbated by the large crowds on the beach. In addition, concern regarding H1N1 could have resulted in an increased attention to and reporting of respiratory symptoms.

5.5 Future work and next steps

Despite completion of this effort, EPA will continue with further studies and analyses including:

- Evaluate the possible reasons and potential remedies for the interference with the PCR assay observed at Boquerón Beach. Replicate filters have been preserved from the both beach sites and can be reanalyzed if the problem can be resolved.
- Evaluate health effects associations with other fecal indicator organisms measured by qPCR when results are available for both beach sites.
- Evaluate additional ancillary exposures collected such as sand samples, composite samples and cyanbocateria samples. Compare these with health effects if warranted.
- Complete testing and analyze results of saliva samples collected at Boquerón Beach. 1,209 households were enrolled in the saliva sampling protocol and over 5,000 samples were collected. Assays are being developed to detect salivary antibodies to waterborne pathogens.
- Continue to evaluate the nature of the source of fecal contamination at the two beaches

5.6 Conclusions

- Surfside Beach
  - Enrollment, follow up and completion of health surveys was successful with over 11,159 completed interviews.
  - Compared with previous marine and freshwater sites [3, 2, 57], a high proportion of beach-goers had water exposure, immersing their bodies and heads in the water (73% and 58%, respectively).
  - Swimmers had a higher incidence of GI illness, rash and earache compared to non swimmers.
  - The swash impacting Surfside Beach was affected by runoff and had poor water quality. The swash also had lower salinity than the beach, suggesting it was affected by runoff.
  - Water quality measured by *Enterococcus* CFU, *Enterococcus* CCE, and *Bacteroidales* CCE was of high quality. Only one day exceeded current EPA recommended criteria for *Enterococcus*(35 CFU per 100 ml). This limited the ability to demonstrate associations with health effects.
  -

  - Body immersion swimmers on the single day when the geometric mean of *Enterococcus* CFU exceeded 35 CFU per 100 ml had a higher incidence of GI illness compared to non-swimmers, but not compared to swimmers exposed on days when the geometric mean was below 35 CFU per 100 ml.
  - Children 10 years of age and under who immersed their body on the single day when the geometric mean of *Enterococcus* CFU exceeded 35 CFU per 100 ml had a higher incidence of GI illness compared to non-swimmers and swimmers exposed on days when the geometric mean was below 35 CFU per 100 ml.
  - Upper respiratory symptoms were associated with *Bacteroidales* CCE among swimming children 10 years of age and under. There was some concern regarding the robustness and stability of this finding as adjusted estimates differed considerably from unadjusted estimates.

- Boquerón Beach
  - Enrollment, follow up and completion of the health survey was successful with 15,726 completed interviews.
  - Despite proximity to a sewage treatment plant discharge (less than 1 mile from the beach), low levels of fecal indicator bacteria were present at the beach sites.
  - Interpretation of water quality measures and health effects associations using fecal indicator bacteria measured by qPCR was complicated by poor recovery of the salmon DNA added as an exogenous positive control, indicating potential interference or inhibition.
  - Interpretation of qPCR results was further complicated by the high proportion of results which showed no detection for the fecal indicator bacteria target sequences.
  - As a result, no firm conclusions can be made regarding the associations between health effects and water quality indicators measured by qPCR .
  - No single day exceeded the *Enterococcus* geometric mean criteria of 35 CFU per 100 ml.
  -
  - There was some evidence of an association between *Enterococcus* CFU exposure and increased risk for respiratory illness among those without recent swimming exposure.

Chapter 6

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Appendix A

Questionnaires

BEACH INTERVIEW

OMB Control No.: 2080-0068

Expiration Date: xx/xx/xxxx

Site ID _____

Friday _____ Saturday _____ Sunday_____ Monday _____
Date:__-__-____
Time: _____

1. Have you been interviewed by the National Beaches Survey in the last 28 days?

2. Would you be willing to participate in a study on illnesses associated with recreation at the beach?

   Yes (give brochure with consent form, inform about 2 follow-up calls)
   No (Terminate Interview).

2a. Our survey is primarily for households of one or more persons that live together at the same address; Do you all live at the same address?

3. How many members in your party are at the beach today including yourself?

4. What time did you and your household arrive at the beach today?

5. We are interested in asking about the health of your household during the few weeks following your beach visit. Could you please give me your telephone number so we can get in touch with you in 10-12 days from now?

5a. If "NO" Is it fro one of the following reasons? Too busy, no longer interested, will not be available, specify, other reason?

5b. 10-12 days from now which phone number(s) should we call?

5c. Is this your home, vacation, or cell phone number?

5d. Additional phone numbers?

6. What are the best times to reach you during week days?

6a. Can I please have your mailing address so that we can send you your $25 *Thank you* check? We will destroy your identifying information after we mail the check.

7. Please tell me the first name of the members of your household at the beach today, their birth dates, gender race ethnicity, and whether they are in diapers.

8. Will (you/all these people with you at the beach today) be living (with you) at the same address (es) during the next two weeks?

9. Have any of these household members at the beach today been ill in the past 3 days with:
   Diarrhea or loose bowels
   Urinary tract infection or burning sensation
   Throwing-up or vomiting
   Sore throat or cough
   Earache, ear infection or runny ears
   Eye infection
   Rash or itchy skin
   Sunburn

10 Are there any household members NOT present at the beach today?

10a. Have any household members NOT present at the beach today been ill in the past 3 days with:
   Diarrhea or loose bowels
   Urinary tract infection or burning sensation
   Throwing-up or vomiting
   Sore throat or cough
   Earache, ear infection or runny ears
   Eye infection
   Rash or itchy skin
   Sunburn

11. Do you or any household members at the beach today, not including anyone who stayed at home, suffer from any of the following chronic long-term conditions:
   Gastrointestinal problems such as Crohn's disease or irritable bowel syndrome
   Chronic respiratory diseases such as asthma or emphysema
   Allergies, other than drug allergies
   Skin problems such as psoriasis or eczema

12. How many times do you usually come to this beach each summer (Memorial Day to Labor Day)?

13. How many miles did you travel to the beach today?

14. During the past two weeks, did you (anyone in your household at the beach today) go bathing or swimming anywhere - at this or some other beach, pool or lake?

14a. Did you go bathing or swimming anywhere in the past one week (Monday through Friday) at this or some other beach, pool or lake?

14b. Did you actually get your head or face wet?

14c. During the past 2 weeks, did you get a sunburn that lasted more that 12 hours?

PART B– Exit Beach Interview

15. Were you the person that we interview on the beach or earlier today?

16a. Did you or anyone in your household wade, swim, or play in the water today?

16a1. Did you immerse your body, not necessarily you head, in the water today?

16a2. Did you put your face in the water or submerge head in the water today?

16a3. Did you get water in the mouth today?

16a4. Did you swallow the water?

16b. Were you in the water at the following times today? {time charts given}

16b1. If "YES" what part of the beach did you swim in? {include all beach areas}

16b2. If "YES" what part of the beach did you swim in most of the time?

16c. What total time did you stay in the water? We are only interested in time actually in the water, not the total time at the beach?

16d. Did you engage in any of the following water-related activities while at the beach today? {Dropdown list of water activities}

17. What would you estimate your total time in direct sunlight was? This does not include being indoors or under umbrellas, etc.?

18. Did you engage in any of the following activities while at the beach today?
    a. Collecting sea shells, rocks, feathers, etc?
    b. Digging in sand or building sand castles?
    c. Had their body buried in the sand?

18c1. Did person get sand in their mouth
    After digging in sand, or building sand castles…did person eat or drink anywhere (not necessarily at the beach)?
    After digging in sand, or building sand castles…did person wash their hands before eating? Washing of hands may include the use of personal waterfree hand sanitizer?

18c1b. Was the sand the person dug in or played with dry or wet?

18d. Did you engage in any of the following activities while at the beach today?
    Playing with algae or seaweed

18d1. Did you get any seaweed in their mouth?

18d1a. After playing with algae or seaweed…did you eat or drink anywhere (not necessarily at the beach)?

18d1b. After playing with algae or seaweed… did person wash their hands before eating? Washing of hands may include the use of personal water free hand sanitizer?

19. Did you cut yourself today or have an open cut when you came to beach today?

20. Did you wear sunscreen/sunblock today?

21. What was the SPF rating of the sunscreen/sunblock you used most often today?

21a. When you used sunscreen/sunblock today, how did you apply it? Only to certain areas of my body? All exposed skin?

22. Did you reapply at least once today?

23. Did you wear a hat today?

23a. Did the hat have a wide brim or another way to shade face, ears, and back of the neck from the sun?

23a1. Did you use protective equipment such as a canopy, umbrella or other type of sunshade today?

23b. Did you wear protective clothing, such as a long-sleeved shirt or cover-up?

24. During the summer, if you go out in the sun repeatedly without sunscreen or protective clothing, which one of these things most usually happens to your skin?
   A dark tan
   Some tanning
   No tan, maybe some freckles
   Repeated sunburns
   Other (specify)
   Never go out in the sun

25. Did you wear insect repellant today?

26. Did your or any member of your household consume food while at the beach today?

26a. Was the food brought from home?

26b. Was the food purchased from vending machines or a vendor at the beach?

26c. Was the food purchased from a vendor outside the beach?

27. Did your or any member of your household consume drinks while at the beach today?

27a. Were the drinks brought from home?
27b. Were the drinks purchased from vending machines or a vendor at the beach?

27c. Were the drinks purchased from a vendor outside the beach?

28. In the past 48 hours has anyone who is at the beach today done the following…

a. Have you come in contact with any unknown animals?
b. Come in contact with someone who has complained of diarrhea, vomiting, or stomach illness?
c. Consumed raw shellfish?
d. Consumed rare/raw meat?
e. Consumed runny or raw eggs?

Telephone Interview - follow-up (10-12 days)

Is this person (primary respondent from beach interview)?

Yes (continue)

No (reschedule or continue)

Were you at the beach on (give date) with (primary respondent)?

Yes - Continue

I'm going to ask questions about any swimming you've done and illnesses you've experienced in the last week for the following people:

A1. May I have your first name please?

During your beach visit where you enrolled in this study on _____

A2. Did you wear ear plugs while in the water? Ask for all household members at the beach.

A3. Did you wear nose plugs while in the water? Ask for all household members at the beach.

A4. Did you wear eye goggles while in the water? Ask for all household members at the beach.

A5. During the beach interview, did you have contact with an animal? Ask for all household members at the beach.

A6. Between your beach visit on _____ date and today were you menstruating or pregnant? Ask for all household members at the beach.

We are now going to switch and ask you questions about activities that have occurred since the Beach Interview.

B1. Have you, or any of the people I just mentioned, gone bathing or swimming anywhere since we talked to you at the beach interview on _____? Please include any bathing or swimming such as at a beach, waterpark, public pool, private pool, or wading pool.

B2. Who was it that went bathing or swimming? Ask for all household members at the beach.

B3a. Did you go bathing or swimming at the beach (where the interview was taken) since the beach interview on this date {BEACH INTERVIEW DATE}.

B3b. Did you go bathing or swimming at any other beach since the beach interview on this date {BEACH INTERVIEW DATE}.

B3c. Was this beach at a:

Lake
River
Ocean
Other, specify

B3d. Did you go bathing or swimming at a waterpark?

B3e. Did you go bathing or swimming at a public pool?

B3f. Did you go bathing or swimming at a private pool?

B3g. Did you go bathing or swimming in a wading pool?

B3h. Did you go bathing or swimming any other place?

B3i. Swim location of any other place?

B4. Did you actually get your face wet while bathing or swimming? Ask for all household members at the beach.

B5. On which days did you go bathing or swimming? Ask for all household members at the beach.

B6. Have you or anyone else had a stomachache or abdominal cramping since the interview at {STUDY BEACH} ON {BEACH INTERVIEW DATE}? Ask for all household members at the beach.

B6a. Who had a stomachache or abdominal cramping since the interview at {STUDY BEACH} ON {BEACH INTERVIEW DATE}? Ask for all household members at the beach.

B7. Have you or anyone else had diarrhea or loose bowels since the interview at {STUDY BEACH} ON {BEACH INTERVIEW DATE}? Ask for all household members at the beach.

B7a. Who had diarrhea or loose bowels since the interview at {STUDY BEACH} ON {BEACH INTERVIEW DATE}? Ask for all household members at the beach.

B8. Have you or anyone else had nausea since the interview at {STUDY BEACH} ON {BEACH INTERVIEW DATE}? Ask for all household members at the beach.

B8a. Who had nausea since the interview at {STUDY BEACH} ON {BEACH INTERVIEW DATE}? Ask for all household members at the beach.

B9. Have you or anyone else had throwing-up or vomiting since the interview at {STUDY BEACH} ON {BEACH INTERVIEW DATE}? Ask for all household members at the beach.

B9a. Who had throwing-up or vomiting since the interview at {STUDY BEACH} ON {BEACH INTERVIEW DATE}? Ask for all household members at the beach.

B10. Have you or anyone else had urinary tract infection or burning sensation when urinating since the interview at {STUDY BEACH} ON {BEACH INTERVIEW DATE}? Ask for all household members at the beach.

B10a. Who had urinary tract infection or burning sensation when urinating since the interview at {STUDY BEACH} ON {BEACH INTERVIEW DATE}? Ask for all household members at the beach.

B11. Have you or anyone else had fever since the interview at {STUDY BEACH} ON {BEACH INTERVIEW DATE}? Ask for all household members at the beach.

B11a. Who had fever since the interview at {STUDY BEACH} ON {BEACH INTERVIEW DATE}? Ask for all household members at the beach.

B12. Have you or anyone else had headache lasting more than a few hours since the interview at {STUDY BEACH} ON {BEACH INTERVIEW DATE}? Ask for all household members at the beach.

B12a. Who had headache lasting more than a few hours since the interview at {STUDY BEACH} ON {BEACH INTERVIEW DATE}? Ask for all household members at the beach.

B13. Have you or anyone else had sore throat since the interview at {STUDY BEACH} ON {BEACH INTERVIEW DATE}? Ask for all household members at the beach.

B13a. Who had sore throat since the interview at {STUDY BEACH} ON {BEACH INTERVIEW DATE}? Ask for all household members at the beach.

B14. Have you or anyone else had a bad cough since the interview at {STUDY BEACH} ON {BEACH INTERVIEW DATE}? Ask for all household members at the beach.

B14a. Who had a bad cough since the interview at {STUDY BEACH} ON {BEACH INTERVIEW DATE}? Ask for all household members at the beach.

B15. Have you or anyone else had a cold since the interview at {STUDY BEACH} ON {BEACH INTERVIEW DATE}? Ask for all household members at the beach.

B15a. Who had a cold since the interview at {STUDY BEACH} ON {BEACH INTERVIEW DATE}? Ask for all household members at the beach.

B16. Have you or anyone else had a runny or stuffy nose since the interview at {STUDY BEACH} ON {BEACH INTERVIEW DATE}? Ask for all household members at the beach.

B16a. Who had a runny or stuffy nose since the interview at {STUDY BEACH} ON {BEACH INTERVIEW DATE}? Ask for all household members at the beach.

B17. Have you or anyone else had an earache, ear infection, or runny ears since the interview at {STUDY BEACH} ON {BEACH INTERVIEW DATE}? Ask for all household members at the beach.

B17a. Who had an earache, ear infection, or runny ears since the interview at {STUDY BEACH} ON {BEACH INTERVIEW DATE}? Ask for all household members at the beach.

B18. Have you or anyone else had watery eyes since the interview at {STUDY BEACH} ON {BEACH INTERVIEW DATE}? Ask for all household members at the beach.

B18a. Who had watery eyes since the interview at {STUDY BEACH} ON {BEACH INTERVIEW DATE}? Ask for all household members at the beach.

B19. Have you or anyone else had an eye infection since the interview at {STUDY BEACH} ON {BEACH INTERVIEW DATE}? Ask for all household members at the beach.

B19a. Who had an eye infection since the interview at {STUDY BEACH} ON {BEACH INTERVIEW DATE}? Ask for all household members at the beach.

B20. Have you or anyone else had an infected cut since the interview at {STUDY BEACH} ON {BEACH INTERVIEW DATE}? Ask for all household members at the beach.

B20a. Who had an infected cut since the interview at {STUDY BEACH} ON {BEACH INTERVIEW DATE}? Ask for all household members at the beach.

B21. Have you or anyone else had a rash or itchy skin since the interview at {STUDY BEACH} ON {BEACH INTERVIEW DATE}? Ask for all household members at the beach.

B21a. Who had a rash or itchy skin since the interview at {STUDY BEACH} ON {BEACH INTERVIEW DATE}? Ask for all household members at the beach.

B22. Have you or anyone else had a sunburn since the interview at {STUDY BEACH} ON {BEACH INTERVIEW DATE}? Ask for all household members at the beach.

B22a. Who had a sunburn since the interview at {STUDY BEACH} ON {BEACH INTERVIEW DATE}? Ask for all household members at the beach.

We will now ask about some activities people may have done since the day of the beach interview on the {BEACH INTERVIEW DATE}

B23a. Since the day of the beach interview, have you or anyone else come in contact with any animals? Ask for all household members at the beach.

B23b. Who came into contact with animals since {BEACH INTERVIEW DATE}? Ask for all household members at the beach.

B23c. Was this animal or any of these animals unfamiliar to you?

B23d. What kind of animals were they?

B24a. Since the day of the beach interview has anyone come into contact with someone who has complained of diarrhea, vomiting, or stomach illness?

B24b. Who had contact with someone complaining of diarrhea, vomiting, or stomach illness since {BEACH INTERVIEW DATE}?

B25a. Since the day of the beach interview has anyone eaten raw shell fish, such as oysters, clams, mussels, crabs?

B25b. Who has eaten raw shell fish, such as oysters, clams, mussels, crabs since {BEACH INTERVIEW DATE}?

B26a. Since the day of the beach interview has anyone rare or raw meat?

B26b. Who has eaten rare or raw meat since {BEACH INTERVIEW DATE}?

B27a. Since the day of the beach interview has anyone eaten raw or runny eggs?

B27b. Who has eaten raw or runny eggs since {BEACH INTERVIEW DATE}?

SECTION C

This section is for all persons that experience symptoms.

C1. On what day did your stomachache or abdominal cramping start?

C1a. Do you still have a stomachache or abdominal cramping?

C1b. (For persons that still have symptom) For how many days did you have a stomachache or abdominal cramping?

C2. On what day did your diarrhea or loose bowels start?

C2a. Do you still have a diarrhea or loose bowels cramping?

C2b. For how many days did you have a diarrhea or loose bowels cramping?

C2c. What was the maximum number of bouts or episodes of diarrhea experienced in a 24-hour period? Ask for each person with symptom.

C3. On what day did your nausea start?

C3a. Do you still have nausea?

C3b. For how many days did you have nausea?

C4. On what day did your throwing-up or vomiting start?

C4a. Do you still have throwing-up or vomiting?

C4b. For how many days did you have throwing-up or vomiting?

C4c. What was the maximum number of bouts or episodes of throwing-up or vomiting experienced in a 24-hour period? Ask for each person with symptom.

C5. On what day did your urinary tract infection or burning sensation start?

C5a. Do you still have a urinary tract infection or burning sensation?

C5b. For how many days did you have urinary tract infection or burning sensation?

C6. On what day did your fever start?

C6a. Do you still have a fever?

C6b. For how many days did you have a fever?

C6c. Was your temperature taken using a thermometer?

C6d. What is the highest temperature that you had since your beach interview on {BEACH INTERVIEW DATE}?

C7. On what day did your headache start?

C7a. Do you still have a headache?

C7b. For how many days did you have headache?

C8. On what day did your sore throat start?

C8a. Do you still have a sore throat?

C8b. (For persons that still have symptom) For how many days did you have sore throat?

C8c. (For persons that still have symptom) Was this sore throat related to allergies?

C9. On what day did your bad cough start?

C9a. Do you still have a bad cough?

C9b. For how many days did you have a bad cough?

C9c. Was this bad cough related to allergies?

C10. On what day did your cold start?

C10a. Do you still have a cold?

C10b. For how many days did you have a cold?

C10c. Was this cold related to allergies?

C11. On what day did your runny or stuffy nose start?

C11a. Do you still have a runny or stuffy nose?

C11b. For how many days did you have a runny or stuffy nose?

C11c. Was this runny or stuffy nose related to allergies?

C12. On what day did your earache, ear infection or runny ears start?

C12a. Do you still have an earache, ear infection or runny ears?

C12b. For how many days did you have an earache, ear infection or runny ears?

C12c. Was this earache, ear infection or runny ears related to allergies?

C13. On what day did your watery eyes start?

C13a. Do you still have watery eyes?

C13b. For how many days did you have watery eyes?

C13c. Was this watery eyes related to allergies?

C14. On what day did your eye infection start?

C14a. Do you still have eye infection?

C14b. For how many days did you have eye infection?

C15. On what day did your cut first get infected?

C15a. Do you still have an infected cut?

C15b. For how many days did you have an infected cut?

C15c. Where were you cut? Mark all that apply

C16. On what day did your rash, itchy skin, or skin infection start?

C16a. Do you still have a rash, itchy skin, or skin infection?

C16b. For how many days did you have a rash, itchy skin, or skin infection?

C16c. Where did you have a rash, itchy skin, or skin infection? Mark all that apply

C17. On which parts of the body were you sunburned? Mark all that apply
Drop down list

SECTION D

Ask only once for each person reporting symptoms

D1. When your condition began, were you working for pay either inside or outside the home? Please include jobs for which you were self-employed.

D2. During your illness, did you miss any time from work, for example because you called in sick or took time off to see a doctor?

D3. How many days?

D4. Did this illness prevent you from performing daily activities such as school, recreation, or vacation activities, or work around the home?

D5. How many days?

D6. Did this illness cause other household members to lose time at work?

D7. How many days?

D8a. Did you consult a healthcare provider over the phone about this illness/condition?

D8b. Did you visit a healthcare provider?

D8c. How many times?

D8d. What illness did the healthcare provider say you had?

D8e. Did you visit an emergency room?

D8f How many times?

D8g. Were you admitted to a hospital?

D8h. How many days were you hospitalized?

D8i. Were you given intravenous fluids?

D9a. Did you receive a prescription for an antibiotic or other drug for this illness/condition?

D9b. About how much of your own or your household's money was spent altogether for these prescription medicines? Amount to nearest dollar.

D10a. Did you use any over-the-counter medications, including things like special drinks, only because of this illness/condition?

D10b. About how much of your own or your household's money was spent altogether for these over-the-counter medications? Amount to nearest dollar.

SECTION E

E1. Before today, were you aware that people could become ill by swimming at the beach?

E2. After today, will you change the way you use the water at the beach?

SECTION Q

These are questions from beach interview to ensure data collection for important exposures. Ask for all households.

Q1. Did you or anyone in your household wade, swim, or play in the water on {BEACH INTERVIEW DATE}?

Q1a.1. Did you immerse your body, not necessarily your head in the water {BEACH INTERVIEW DATE}?

Q1a.2. Did you put your face in the water or submerge head in the water on {BEACH INTERVIEW DATE}?

Q1a.3. Did you get water in your mouth on {BEACH INTERVIEW DATE}?

Q1a.4. Did you gag or cough after getting water in your mouth on {BEACH INTERVIEW DATE}?

Q1a.5. Did you swallow the water on {BEACH INTERVIEW DATE}?

Appendix B

Quality Assurance Project Plan: Survey Data Collection

EPA Contract No. EPD-09-040
Work Assignment 0-01
WESTAT

WORK PLAN

For

The National Epidemiological and Environmental Assessment of Recreational Water Study for Beaches Program

Boquerón Beach, Puerto Rico
And
Surfside Beach, South Carolina

U.S. Environmental Protection Agency (EPA)
EPA Contracting Officer Representative: Elizabeth Sams

Submitted by:
Westat Work Assignment and Project Director: Karen Della Torre

April 27, 2009

BACKGROUND

In order to meet some of the requirements of the Clean Water Action Plan, the Beach Action Plan and the Beach Act of 2000, this beach study was initiated in 2003 to assist the Office of Water in formulating new health and risk guidelines for recreational water.

This study is being conducted jointly by the National Exposure Research Laboratory, Microbiological and Chemical Exposure Assessment Research Division (NERL/MCEARD), the National Health and Environmental Research Laboratory (NHERL) and the Centers for Disease Control and Prevention (CDC).

This information is being collected as part of a research program consistent with the Sec. 3(a)(v)(1) of the Beaches Environmental Assessment and Coastal Health Act of 2000 and the strategic plan for EPA's Office of Research and Development (ORD) and the Office of Water entitled "Action Plan for Beaches and Recreational Water." The Beaches Act and ORD's strategic plan has identified research on effects of microbial pathogens in recreational waters as a high-priority research area with particular emphasis on developing new water quality indicator guidelines for recreational waters. This data collection is for a series of epidemiological studies to evaluate exposure to and effects of microbial pathogens in marine and fresh recreational waters as part of the EPA's research program on exposure and health effects of microbial pathogens in recreational waters. The information collected by this study program will be used to estimate the relationship between water quality indicators and health effects. The questionnaire health data will be compared with routinely collected water quality measurements. The analysis will focus on determining whether any water quality parameters are associated with increased prevalence of swimming-related health effects.

Study Beach Site

The study period from May 15, 2009 through August 2, 2009 shall take place at Boquerón, Puerto Rico. The study period from June 6, 2009 through September 7, 2009 shall take place at Surfside Beach, South Carolina. This work assignment implements field procedures for the data collection (water quality and human health) at a beach study site and the follow-up telephone interviews. Westat will travel to this site.

WORK PLAN

Westat is submitting this Work Plan detailing procedures by which support will be provided to implement a study for the NEEAR (National Epidemiological Environmental Assessment of Recreational) Water Study. Support will include the collection of epidemiologic data, the collection and analysis of water and sand sample extracts, and collection of ancillary data.

TASK 1

Computer Assisted Interviews:

Westat will load existing electronic beach and telephone interviews modified under EPA Contract EP-D-04-064, Work Assignment 2-04 onto CAPI (computer-assisted personal interviews) and CATI (computer-assisted telephone interviews) devices needed to accomplish a complete sampling of beach goers at the designated beach area. The appropriate number of devices shall be based on the number of expected household interviews done on individual days at the designated beach area.

Westat will use Blaise® software and appropriate equipment to load the electronic questionnaires onto tablet handheld computers and telephone interviewing computers. Westat will provide interviewers to conduct the CAPI and CATI questionnaires by direct data entry utilizing the Blaise® software program. These questionnaires shall be conducted in both English and Spanish.

Implementation of Computer-Assisted Interviewer Training:
Westat will implement training programs modified in Work Assignment 2-04 for beach and telephone interviewers. All interviewers shall undergo training using training manuals modified in Work Assignment 2-04 as part of the training program and undergo Human Subjects Ethics Training in accordance to the required ethics training required by the UNC biomedical Institutional Review Board. Westat will provide documentation of training for all interviewers and produce a report including contractor comments. For Boqueron Beach, the training programs shall be completed by Friday, May 15, 2009 for beach interviewers and Sunday, May 24, 2009 for telephone interviewers. For Surfside Beach, the training programs shall be completed by Friday, June 5, 2009 for interviewers and Sunday, June 14, 2009 for telephone interviewers. EPA may be present at any or all of the interviewing training programs and will have access to CAPI/CATI electronic devices throughout training sessions.

Paper questionnaires shall be used only in the event that there is a technical failure that prohibits use of electronic CAPI or CATI devices. This study is to be almost paperless per Office of Management and Budget's initiative to use technology and decrease the amount of paper consumed.

TASK 2
Data Collection:

Beach Interviewing:
The beach interview is to be conducted in two parts with the first part, Part A, containing history, demographic information of household members and contact information for follow-up. Westat will collect Geographic Positioning System (GPS) coordinates and transect information (**See Figure 1**) at the family location on the beach, concurrent with collection of Part A of questionnaire data. Westat will ALSO COLLECT GPS COORDINATES FOR FAMILY LOCATION ON THE BEACH FOR NONPARTICIPATING FAMILIES. The second part, Part B (exposure date), shall be conducted at the study station(s) located in or near the beach entrance/exit points designated by the WACOR or COR. This data shall be included in weekly production reports and a comprehensive database described under Task 3.

Westat will provide trained beach interviewers (both English and Spanish bilingual) to administer questionnaires to household units (families or individuals) beginning Friday, May 15, 2009 through Sunday, August 2, 2009 at the Puerto Rico beach site. Westat will provide trained interviewers for the Surfside Beach site to administer questionnaires to household units. Spanish Bilingual interviewers shall be scheduled each data collection day and the number of Spanish Bilingual interviewers shall be proportionate to the mix of Spanish-speaking households that are expected to be enrolled in the study. The questionnaires have been reviewed by an Institutional Review Board (IRB), approved by EPA's Human Ethics Official and have received Office of Management and Budget (OMB) clearance under the Paper Reduction Act.

Westat will complete beach interviews for twelve (12) designated weekends and three (3) weekday holiday from Saturday, May 15, 2009 through Sunday, August 2, 2009 at the beach site in Boquerón,

Puerto Rico. Data collection weekdays include Memorial Day and two additional Puerto Rican holidays (July 20 and 27).

In Puerto Rico, Westat will then end beach data collection at designated beach areas Sunday, August 2, 2009 or upon technical direction from the WACOR or COR. Westat will work all designated non-holiday and holiday weekends and holiday weekdays except in the case of inclement weather conditions, beach closure or technical direction by the WACOR or COR. The status of inclement weather conditions will be determined by the WACOR or COR.

In South Carolina, Westat will complete beach interviews for thirteen (13) designated weekends and two weekday holidays from Saturday, June 6, 2009 through Sunday, September 6. Weekday holidays are Friday, July 3 and Monday, September 7, 2009.

Westat will set up study stations at appropriate exits at beach areas to be determined by the WACOR or COR. Westat will provide required furniture and computer equipment to complete this work assignment. Westat will also provide temporary "on beach" stations for beach interviewers. These beach stations shall display the USEPA seal and comply with park regulations concerning location and safety.

For each of the designated data collection days at the beach site, a sampling of households will be undertaken between 11:00 AM and 5:00 PM local time. Westat will terminate Part A Interview collection at 5:00 PM and terminate Part B Interview collection at 6:30 PM. These times will be flexible depending on beachgoer attendance at various times of day. Westat will set up a system of counting refusals and those who do not complete the interview processes. Westat will require interviewers to obtain verbal consent from participants by distributing and discussing the consent pamphlet.

Telephone Interviewing:

CATI telephone interviewer(s) shall implement trial interviews with at least three (3) members of the NEEAR Water Study project team designated by the WACOR or COR prior to Monday, June 1, 2009. This exercise allows the US EPA NEEAR Water Study project team to verify that the telephone interviews are being properly administered to study participants.

Westat will complete telephone surveys during a designated window of 10-12 days following the beach interviews. Telephone interviewers shall be available beginning on Monday, May 25, 2009 through Saturday, September 19, 2009 for beach data collection days May 15, 2009 through September 7, 2009. The telephone surveys will collect follow-up information from all households interviewed at the beach. This data shall be included in weekly production reports and comprehensive database described under Task 3.

Westat will train telephone interviewers using all data elements to be entered directly into a database utilizing CATI instruments during the course of the telephone interview. Spanish Bilingual interviewers shall be scheduled each data collection day and the number of Spanish Bilingual interviewers shall be proportionate to the mix of Spanish-speaking households that enrolled in the study and are expected to complete the telephone survey. The questionnaires have been reviewed by an Institutional Review Board (IRB), approved by EPA's Human Ethics Official and have received Office of Management and Budget (OMB) clearance under the Paper Reduction Act.

Field Implementation Plan:
Westat will utilize the 2007 Field Implementation Plan (FIP) describing data collection protocols for the beach and the telephone interviews and the collection of the environmental data including sources completed in Work Assignment 2-04 under Contract EP-D-04-064.

Electronic Data Matching and Back-up Files:
Westat will utilize the electronic method for data matching of Part A and Part B Interviews described in the 2007 FIP (This also included transfer of Part A data collection information for integration in Part B questionnaires). This electronic data matching is paperless and allows the USEPA Field Study Monitors to ascertain whether or not households are completing Part B Interviews that match Part A Interviews. It also allows the interviewers to see previous information (such as, but not limited to, participant name, household member names, date of interview, and time of arrival) collected from the participants in the Part A Interview so they will be able to coherently collect information for Part B Interviews.

As described in the 2007 FIP, Westat will include a back-up plan for this electronic method that duplicates data as it is collected and each individual participant record shall be stored in two separate physical locations. These two back-ups shall be designated as collection records only and shall not be utilized for access. A separate log of data shall be used to complete data linkage of Part A and Part B records.

Information Distribution:
Westat will provide electronic and written questionnaires, consent forms, and flyers modified in Work Assignment 2-04. Westat will provide these informational materials to potential participants in both English and Spanish.

Incentive Distribution:
Westat will provide nonmonetary incentives to households upon completion of Beach Interview Part A and Part B. Incentives shall be beach related, include the USEPA seal, and be distributed one (1) per household. The USEPA seal will be provided by the WACOR or COR.

Westat will distribute $25 incentives to each household upon completion of their participation (completion of both beach interviews and telephone interview). Westat will not distribute more than one check per household. Westat will distribute letters of participation status to all households enrolled in either the beach interview or telephone interview. This letter shall indicate completion of the study or termination of participation status.

Environmental Information:
Westat will collect the following environmental information: air and water temperature, wind direction, water current direction, UV radiation cloud cover and other information to be identified by the WACOR or COR. This information shall be collected from existing sources as well as field instrument collection. Westat will document their sources for this information. Westat will submit a report evaluating the field collection and environmental data procedures. Existing sources of environmental information must have approval of the WACOR or COR.

Project Final Report:

Westat will provide a final work assignment report that includes overview of field procedures, field implementation analysis, training and recommendations for improvement, and assessment of technology.

TASK 3

Data Management:

Westat will implement a data management plan modified in Work Assignment of 2-04 t include, but not be limited to, the following; 1) processing of beach and telephone interview data in the field (including back-up), 2) daily count of complete and incomplete interviews, and 3) downloading of beach data for field review by USEPA WACOR or COR.

Production Reports:

Westat will provide weekly production reports that summarize the daily completion rates to the USEPA by the close of business on Wednesday after a data collection weekend. For weekends that include a holiday the production report shall be due on the Friday following data collection weekend. On holidays not included in the weekend, the production reports are due by the close of business on Wednesday of the following week. Westat will submit the production reports (beach and phone) for the weekends for Friday, May 15, 2009 through Saturday, September 19, 2009. These reports shall be in Excel 2000 format.

Comprehensive Database:

Westat will develop a comprehensive database that includes all data collected from beach interviews, telephone interviews, environmental data, and water quality data. Westat will submit this database on a weekly basis outlined in Deliverable 7. This database shall be in SAS software program language. Westat will submit this database with a beach identifier number for all data. Westat will submit the database information for the data collection for Friday, May 15, 2009 through Saturday, September 19, 2009. The comprehensive database is due for each data collection weekend at close of business 10 calendar days after the final collection day. Westat will provide a draft of the comprehensive database prior to data collection weekends and obtain approval of the WACOR or COR. The USEPA will provide an example of a previous database. Westat will submit a report for this comprehensive database including, but not limited to, detailed explanation of database use, documentation of changes made over the course of the work assignment, and detailed definitions of variable names.

Transfer of Ownership of Project-Generated Materials:

Westat will transfer all project-generated products to the USEPA at the end of the work assignment. Products such as, but not limited to, data, computer-assisted interviews, programs, databases, reports and materials created under this work assignment belong to the USEPA. These products shall be transferred electronically, when appropriate. Otherwise, products will be shipped to the Human Studies Facility in Chapel Hill, NC.

TASK 4

Quality Assurance and Quality Control:

For all tasks except Task 5 (Sand and Water Sampling and Analysis), this project shall utilize a project Quality Assurance Project Plan (QAPP) modified in Work Assignment 2-04. For Task 5, this work plan will serve as the Quality Assurance Project Plan. The QAPP shall be approved by USEPA and implemented as written. In addition, any data forms developed by the contractors must be approved by USEPA for quality assurance purposes. The QAPP will be considered draft and

updated as required during the course of this work assignment. A final QAPP shall be required at the end of the work assignment.

TASK 5

Sand Sampling and Analysis:

Westat will collect 3 beach sand samples per day on Saturdays and Sundays, and on 3 holiday weekdays, Monday May 25th, 2009 (1 day), Monday July 20th, 2009 (1 day) and Monday July 27th, 2009 (1 day) from May 15th, 2009 through August 2nd, 2009 (See Table 1), at Boquerón Beach, Puerto Rico for microbiological analysis by two methods [the current approved membrane filter (MF) *Enterococci* method (mEI Agar) and Rapid Quantitative Polymerase Chain Reaction (QPCR)]. Westat will collect 3 beach sand samples per day on Saturdays and Sundays, and on 2 holiday weekdays, (Friday, July 3, 2009 Monday September 7, 2009) from June 6, 2009 through September 7, 2009 (See Table 2), at Surfside Beach, South Carolina for microbiological analysis. Additional samples may need to be collected if the weather interferes with the attached schedule. The dates for these samples will be arranged between USEPA and Westat. These samples will be taken in the designated area of the beach associated with the water quality samples. Westat will employ a "scoop" method to collect the samples.

Water Quality Sampling and Analysis:

Westat will collect 18 beach water samples per day on Saturdays and Sundays, and on 3 holiday weekdays, Monday May 25th, 2009 (1 day), Monday July 20th, 2009 (1 day) and Monday July 27th, 2009 (1 day) from May 15th, 2009 through August 2nd, 2009 (See Table 1), at Boquerón Beach, Puerto Rico for microbiological analysis by two methods [the current approved membrane filter (MF) *Enterococci* method (mEI Agar) and Rapid Quantitative Polymerase Chain Reaction (QPCR)]. Additional samples may need to be collected if the weather interferes with the attached schedule. Westat will collect 18 beach water samples per day on Saturdays and Sundays, and on 2 holiday weekdays, (Friday, July 3, 2009 Monday September 7, 2009) from June 6, 2009 through September 7, 2009 (See Table 2), at Surfside Beach, South Carolina for microbiological analysis. The dates for these samples will be arranged between USEPA and Westat. Westat will ensure that analysts at laboratories are proficient in each method and that they consult with USEPA personnel, the technical advisors for the methods, and/or the manufacturers of the instruments to ensure proper knowledge and use of the analytical methods. Westat will use the Global Positioning System (GPS) to identify the location of the beach on land and the individual sample sites in the water. Westat will transport the samples to the local analytical laboratory for analysis (MF) or processing (QPCR) within 6 hours of collection. Westat will ship the processed QPCR filters on dry ice to the QPCR laboratory for analysis. Westat will maintain a Tracking System for all samples and analyses. Westat will take additional measurements (pH, turbidity, conductivity and salinity) and collect other ancillary data (air and water temperature, cloud cover, rainfall, wind speed and direction, current direction, wave height, bather density in the water and on the beach, boats, animals, debris) at each sampling visit. Westat will take photographs of the beach and the water during the sampling at least once a day from an elevated vantage point, if possible, to aid researchers in determining the conditions at the beach.

In addition, Westat will collect additional water samples for shipment to the U.S. Geological Survey (USGS) for chemical analyses. Westat will provide a copy of the chemical sample airbills/shipping forms from each shipment to the WACOR or COR.

Table 1. Boquerón Beach Sampling Schedule

"Weekend"	Day	Date	Day	Water samples	Sand samples	Total samples	Notes
0	1	May 6, 2009	Wednesday	18	3	21	Dry Run
1	2	May 15, 2009	Friday	18	3	21	
	3	May 16, 2009	Saturday	18	3	21	
	4	May 17, 2009	Sunday	18	3	21	
2	5	May 22, 2009	Friday	18	3	21	
	6	May 23, 2009	Saturday	18	2	21	
	7	May 24, 2009	Sunday	18	3	21	
	8	May 25, 2009	Monday	18	3	21	
3	9	May 29, 2009	Friday	18	3	21	
	10	May 30, 2009	Saturday	18	3	21	
	11	May 31, 2009	Sunday	18	3	21	
4	12	June 5, 2009	Friday	18	3	21	
	13	June 6, 2009	Saturday	18	3	21	
	14	June 7, 2009	Sunday	18	3	21	
5	15	June 12, 2009	Friday	18	3	21	
	16	June 13, 2009	Saturday	18	3	21	
	17	June 14, 2009	Sunday	18	3	21	
6	18	June 19, 2009	Friday	18	3	21	
	19	June 20, 2009	Saturday	18	3	21	
	20	June 21, 2009	Sunday	18	3	21	
7	21	June 26, 2009	Friday	18	3	21	
	22	June 27, 2009	Saturday	18	3	21	
	23	June 28, 2009	Sunday	18	3	21	
8	24	July 3, 2009	Friday	18	3	21	
	25	July 4, 2009	Saturday	18	3	21	4th of July
	26	July 5, 2009	Sunday	18	3	21	
9	27	July 10, 2009	Friday	18	3	21	
	28	July 11, 2009	Saturday	18	3	21	
	29	July 12, 2009	Sunday	18	3	21	
10	30	July 17, 2009	Friday	18	3	21	
	31	July 18, 2009	Saturday	18	3	21	
	32	July 19, 2009	Sunday	18	3	21	
	33	July 20, 2009	Monday	18	3	21	Luis Muñoz Rivera's Birthday
11	34	July 24, 2009	Friday	18	3	21	
	35	July 25, 2009	Saturday	18	3	21	
	36	July 26, 2009	Sunday	18	3	21	
	37	July 27, 2009	Monday	18	3	21	José Celso Barbosa Birthday
12	38	July 31, 2009	Friday	18	3	21	
	39	Aug. 1, 2009	Saturday	18	3	21	
	40	Aug. 2, 2009	Sunday	18	3	21	
Totals				**720**	**120**	**840**	

Table 2. Surfside Beach Sampling Schedule

	Date Samples Collected	Day of the Week	No. Water Samples	No. Sand Samples	No. of Composite Samples
1	6/1/2009 Dry Run	Mon	18	3	9
2	6/6/2009	Sat	18	3	9
3	6/7/2009	Sun	18	3	9
4	6/13/2009	Sat	18	3	9
5	6/14/2009	Sun	18	3	9
6	6/20/2009	Sat	18	3	9
7	6/21/2009	Sun	18	3	9
8	6/27/2009	Sat	18	3	9
9	6/28/2009	Sun	18	3	9
10	*7/3/2009	Fri	18	3	9
11	7/4/2009	Sat	18	3	9
12	7/5/2009	Sun	18	3	9
13	7/11/2009	Sat	18	3	9
14	7/12/2009	Sun	18	3	9
15	7/18/2009	Sat	18	3	9
16	7/19/2009	Sun	18	3	9
17	7/25/2009	Sat	18	3	9
18	7/26/2009	Sun	18	3	9
19	8/1/2009	Sat	18	3	9
20	8/2/2009	Sun	18	3	9
21	8/8/2009	Sat	18	3	9
22	8/9/2009	Sun	18	3	9
23	8/15/2009	Sat	18	3	9
24	8/16/2009	Sun	18	3	9
25	8/22/2009	Sat	18	3	9
26	8/23/2009	Sun	18	3	9
27	8/29/2009	Sat	18	3	9
28	8/30/2009	Sun	18	3	9
29	9/5/2009	Sat	18	3	9
30	9/6/2009	Sun	18	3	9
31	*9/7/2009	Mon	18	3	9

Sample Analysis: Westat will analyze all water samples collected at the beach by three microbiological methods during the period of May 15, 2009 through September 7, 2009. Additional samples, if needed, will also be analyzed by the same methods. Westat will verify 5 colonies/sample for all samples analyzed by the MF method (Method 1600) on one day (either Saturday or Sunday) of the first weekend. Westat and laboratories will participate in a dry run to determine and correct any problems in the procedures at least one week before the study begins. USEPA personnel may attend. This dry run will consist of a full day's event of water quality sampling, water quality analysis, and form documentation on the beach and in the laboratory as it would occur during the actual study collection.

Data Collection and Handling: Westat will use sample collection/custody forms, approved by USEPA. Westat will maintain sample collection/custody sheets in a binder in the laboratory where the analysis is being performed. Westat will record and save all data from each method (counts, estimated counts, C_T values, setup values, complete run files, plots, growth curves, graphs, bit maps, other computer files, notes, QC data, statistical parameters and analyses, etc.) and submit them to USEPA in a hard copy and in electronic form. Westat will provide a hard copy of hand-entered sample collection and custody sheets, data sheets, and all other forms of data for each sampling event to the WACOR or COR (*i.e.*, within 24 hours) during the 2-day weekend period of sampling each week at the beach site. Westat will enter the data electronically into database forms/spreadsheets, and the electronic file(s) will be delivered to the WACOR or COR weekly and after the analyses have been completed at the beach site, along with all other forms of data. Westat will send a cover memo with the electronic data outlining the delivery contents by method. Westat will maintain original copies of the sampling and data worksheets and deliver them to USEPA at the end of the study. Additions or changes to data worksheets must be approved by USEPA.

Proficiency/Certification

There is no specific training anticipated by USEPA for the current approved membrane filter *Enterococci* method (mEI Agar; see Attachments 1 and 2). Westat will ensure that analysts are proficient in the above method and each of the rapid methods [Rapid Quantitative Polymerase Chain Reaction (QPCR) and Human Specific QPCR (See Attachment 6).]. The laboratory analysts who will be performing the assays will be proficient in each method and will consult with USEPA personnel, the technical advisors, and/or the manufacturers of the instruments to ensure proper knowledge and use of the analytical methods. Westat and laboratories will be required to process one or two performance evaluation samples (unknowns) for all methods during the study. General field and lab safety protocols will also be the responsibility of the laboratory.

Safety Protocol

Westat will utilize a safety plan specific to Task 5 modified in Work Assignment 2-04 contract EP-D-04-064.

Documents and Records

Data Deliverables

Westat will initially record membrane filter count data and lot number on field or laboratory worksheets, then enter and save data electronically in a database (Microsoft Access) or a spreadsheet

(Lotus 123 or Excel). Corel Word Perfect or Microsoft Word will be used for the text in reports. The worksheets, database format, data sheets, or spreadsheets for this method and all other methods in this study must be approved by the USEPA. If spreadsheets are employed, a separate spreadsheet file will be employed for each day of sample collection. The file name will incorporate the dates or range of dates of collection. Separate worksheets within the spreadsheet or different database forms will be used to record the following information:

- Field measurement data (See below),
- Sample collection time/analysis start time/incubation start and end time (See Microbiological Method Section), and
- Microbiological data for each sample/volume and associated quality control (QC) samples.

A hard copy format of hand-entered sample collection and custody sheets, data sheets, and all other forms of data (graphs, bit maps, C_T values, setup values, plots, growth curves, notes, QC data, statistical parameters and analyses, etc.) for each sampling event will be provided to the COR and WACOR daily *(i.e.*, within 24 hours) during the 2-day weekend period of sampling each week at the beach site. Westat will also enter the data electronically into the database forms/spreadsheets, and, after the analyses have been completed at the beach site, the electronic file(s) will be delivered to the COR and WACOR along with all other forms of data (graphs, bit maps, plots, curves, setup values, notes, QC data, statistical parameters and analysis, etc). The delivery will be accompanied by a brief cover memo outlining the delivery contents by method. The memo will also indicate if there are any unusual circumstances or known problems surrounding the deliverable, such as QC problems in any of the methods.

Critical data to be reported are the bacterial counts from the membrane filter tests; the complete run files for the QPCR method, including C_T and setup values, positive and negative controls, etc; the complete run files. All membrane filter plates will be examined and counted [If possible, plates with up to 200 colony forming units (CFUs) are to be considered countable, although the ideal number of CFUs is 20-80.], and the results for all plates will be reported, including zeros and those "too-numerous-to-count" (TNTC). An estimation procedure for TNTC plates will be provided to the laboratory by USEPA (**Attachment 5**). The estimation data from the TNTC plates (*i.e.*, the five counts from five squares on each filter) will be submitted to USEPA along with the count data for the other samples. QC data will be reported with the sample data for **all** methods.

In addition to the water samples to be collected, a number of ancillary data will be collected for each sampling visit. These are shown in Table 3, along with descriptions of their measurement. Field data will be entered with permanent non-running ink. In addition to the items detailed in Table 3, any items/activities specific to the beach will be added, for example, at Edgewater Beach in Biloxi (2005), data on the number of jet skis in and out of the water was recorded.

Any QC results associated with the collection of ancillary data will also be reported (QC samples are to be specified in Methods/SOPs describing ancillary data collection methods).

Table 3. Measurements to be Recorded at/for Each Sampling Visit

Measurement	Description	Units/Format	MQOs
Date and Time	Date and Time of day	Mm/dd/yy; hh:mm	∀5 minutes
Air temperature	Measured by thermometer at a fixed location every visit	°C	∀1°
Water temperature	Measured by thermometer at a fixed sampling location at appropriate depth for thermometer on every visit	°C	∀1°
Cloud Cover	Sunny, Mostly Sunny (20-50% cloud cover), Cloudy (50-70% cover) Mostly Cloudy (70-99% cover), Overcast	S, MS, C, MC, O	Field Person or Team Consensus
Rainfall	Measured by rain gauge near sampling area; collected each day at time of sampling and any time rain is known to have occurred at the beach since the last measurement was taken. Current conditions such as rain, lightning, hail, etc. noted	Rain in inches; other observations noted in comments field	∀ 0.25 Inches
Wind speed	Sustained speed measured by wind gauge; gusts indicated in comments fields	Miles per hour	∀ 5 mph
Wind direction	Compass direction to nearest semi-quadrant leeward measured on wind gauge	N, NE,E, SE, S, SW, W, or NW	Recorders judgement
Current Direction	Described in relation to shoreline facing out	Descriptive (onshore, right, etc.)	Field Person or Team Consensus
Wave height, if applicable	Meter stick measurement at central sampling point. This is the distance from the low point (trough) to the high point (peak) of the wave	Meters	∀ 0.2 M
Bather density	Number of bathers in the water, in the sampling area, and number of "bathers" on beach, within outer transects to edge of beach on land side	Categorical; <20, 20-100, 100-200, >200	Field Person or Team Consensus
Boats	Number/approximate number of boats in the water, within approximately 500 M of sampling area	Categorical; None, 1-5, 5-10, 10-20, 20-30, etc., etc.	Field Person or Team Consensus
Animals/Birds	Animals and birds potentially affecting the water (within approximately 20M of the sampling area in the water or laterally within 20M of the outer transects on the beach); also includes number of fowl or other birds in the air near the sampling area	Types of Animals, Numbers of Animal Types on beach and in water	Field Person or Team Consensus
Debris	Description of any debris floating in the water or washed on shore within the	Categorical; "None," "Very	Field Person or Team

	bathing area	Little," "Little," "Lots," describe types	Consensus

Table 3. Measurements to be Recorded at/for Each Sampling Visit (continued)

Measurement	Description	Units/Format	MQOs
pH	Each sample measured after microbiological analysis processing, per "Standard Methods" (3) or equivalent. *Equipment utilized for this measurement must be preapproved by WACOR or COR	pH units	∀ 0.2 units
Turbidity	Each sample measured by nephlometer after microbiological analysis processing, per Standard Methods (3) or equivalent *Equipment utilized for this measurement must be preapproved by WACOR or COR	Nephlometric Turbidity Units (NTUs)	Range dependent; see Standard Methods 2130B
Salinity	Each sample measured after microbiological analysis processing, per "Standard Methods" (3) or equivalent and Measured on site concurrently with temperature and air current	Parts per thousand	Field Person
Conductivity	Each sample measured after microbiological analysis processing, per "Standard Method" (3) or equivalent	microSiemens or milliSiemens as appropriate	Field Person
UV Reading	Measured by UV device	Units/Format	MQOs
Geographical Position	GPS Unit Coordinates will be taken in 3 places for each of the 3 transects. Total of 9 positions for each sample run (8:00 Am, 11:00 AM, 3:00 PM)	Lat/Long	Field Person or Team Consensus
Swim Advisory Flags	Flags put on beach by lifeguards or other official to indicate if swimming is advised, cautioned against, or unallowed for bacteria levels, weather, or roughness of water. Usually Green for Safe, Yellow for Advisory, and Red for Unsafe/Not Allowed	Indicate if advisory is due to bacteria, weather, or roughness of water.	Field Person

Photographic Data

To aid researchers in determining conditions at the beach that may not be readily apparent from the ancillary data recorded, photographs of the sample locations at the beach area will be taken for the record at least once a day during the sampling period at the beach site. While the work assignment requires taking photographs only once a day, Westat proposes to continue taking them at every sample collection, as in past years, because the conditions on the beach can change substantially over the course of a day from 8:00 am to 3:00 pm. The photographs at the beach will be taken from an elevated vantage point, if possible, on one side or the other of the study area. Photographs will be of sufficient quality to estimate the number of bathers on the beach and in the water. Photos should be labeled with the beach name, date, target sample collection time, and actual time of photograph. The camera will be configured to have the date (and time, if possible) displayed on the image. The beach name, target sample time, and actual time of photograph (if unable to configure camera to display on image) will be part of the image name. Submission of photographs to the WACOR or COR will occur at the end of the sampling period for the beach via appropriate means, expected to be delivery of a CD-ROM with the digital photographs.

General Laboratory Quality Control Records

Laboratories are expected to maintain records of general laboratory quality control activities, such as are described in this work assignment, the attachments, and some of the references (1,3,5) found at the end of this work assignment. Such records may become deliverables upon an amendment to the work assignment.

Data Formats

The exact format of all data fields will be approved by USEPA prior to data collection. Formats will be based on those specified for this project in the work assignment, in the attachments, or in the forms recommended by the manufacturers of the instruments. Where possible, database/spreadsheet templates will have fields preformatted.

Quality Assurance Plan and Revisions

All project personnel will receive copies of the most current version of the Quality Assurance Project Plan (QAPP) prior to dry run.

Other Records

Various other documents and records (*e.g.*, SOPs, reports, method validation records, laboratory QC, and maintenance records) are discussed in this document in appropriate sections. The USEPA reserves the right to request copies of any documents and records from Westats that could affect this project. Any records that are received, and any records generated by Westat will become part of the overall project file.

DATA GENERATION AND ACQUISTION

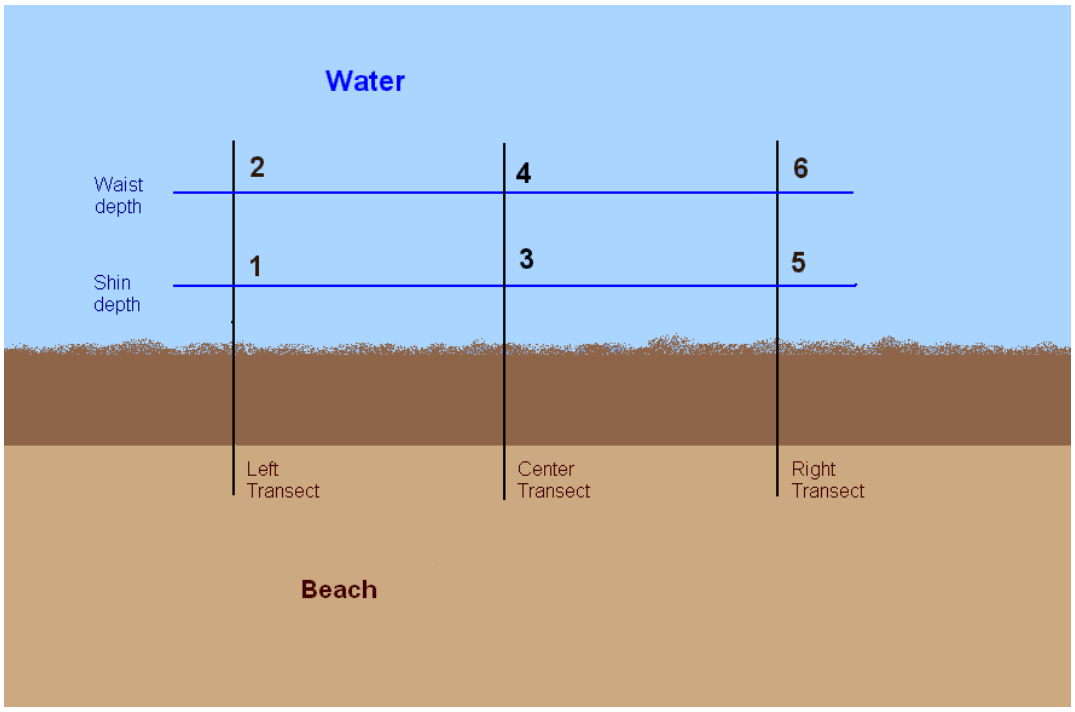
Sample Collection for Microbiological Analyses

Water Samples:

Three times a day, at 8:00 AM, 11:00 AM, and 3:00 PM, two water samples will be collected at the beach along each of the three transects perpendicular to the beach shoreline, one in waist-high water (1 ml deep) and one in shin-high water (0.3 m deep), for a total of 18 samples per day (*i.e.*, 6 grid locations x three times per day). See Figure 1a, which depicts the water sampling scheme. The location of the transects will be at least 20 meters apart or more, if the area used by the swimmers encompasses more than a total of 60 meters of shoreline. The samples will be collected on Saturdays and Sundays, and on 3 holiday weekdays, Monday May 25th, 2009 (1 day), Monday July 20th, 2009 (1 day) and Monday July 27th, 2009 (1 day) from May 15th, 2009 through August 2nd, 2009 (See Table 1), at Boquerón Beach, Puerto Rico. Westat will collect 18 beach water samples per day on Saturdays and Sundays, and on 2 holiday weekdays, (Friday, July 3, 2009 Monday September 7, 2009) from June 6, 2009 through September 7, 2009 (See Table 2), at Surfside Beach, South Carolina for microbiological analysis. It is intended that samples will be collected on the scheduled dates, but other dates may be substituted if rainfall or other problems prevent swimmers from going to the beach, prevent water sampling, or create hazardous conditions for the field personnel. Sample collectors will notify the WACOR or COR of adverse weather conditions or other problems and request guidance whether to begin or continue sampling on a given day or weekend. This is necessary because the samples must be collected when there are sufficient bathers at the beach to allow NHEERL to conduct their concurrent epidemiological/health study.

Global Positioning System (GPS) readings of the actual water collection locations and a photo of the sample collection sites will be taken.

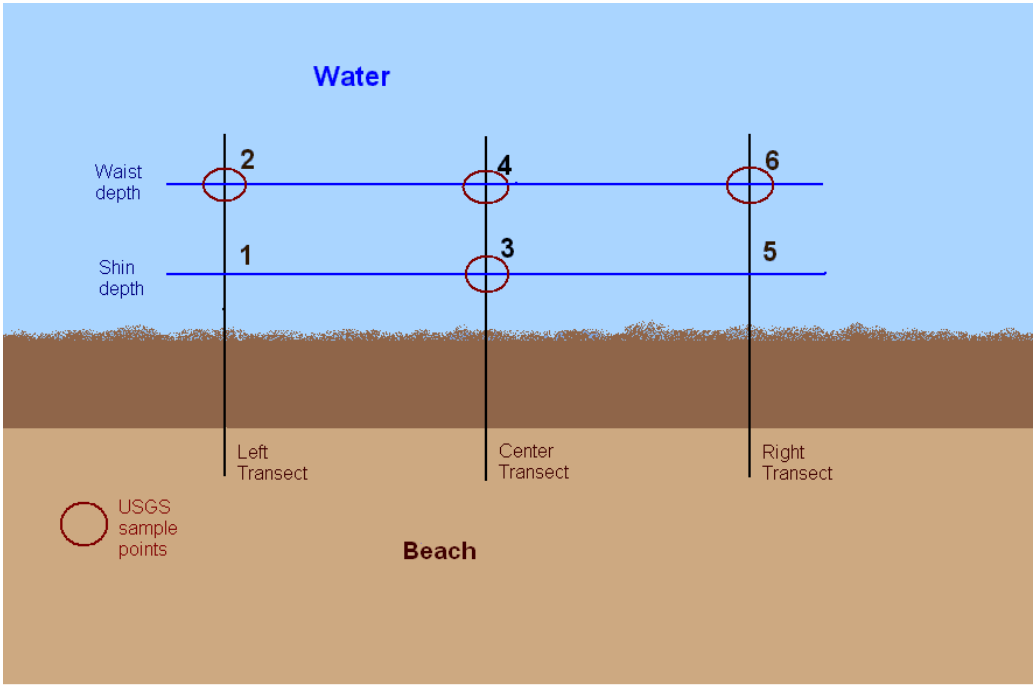
Figure 1a. Water Sampling Locations



Remote Chemical Analysis Samples

In addition, 3 1-liter plastic-coated glass bottles will be taken once a day, during the 11:00 AM collection at each of the three transect locations in waist-high water and at the center transect only in shin-high water (sampling points 2, 3, 4, and 6, as circled in Figure 1b) each Friday, Saturday, Sunday, and on 3 holiday weekdays (May 25th, July 20th, and July 27th) during the study for remote chemical analysis, described in a later section (for a total of 12 samples/sampling day). Figure 1b is a schematic that shows the collection of water samples at 11:00 am.

Figure 1b. USGS Water Sampling Locations at 11:00 am.



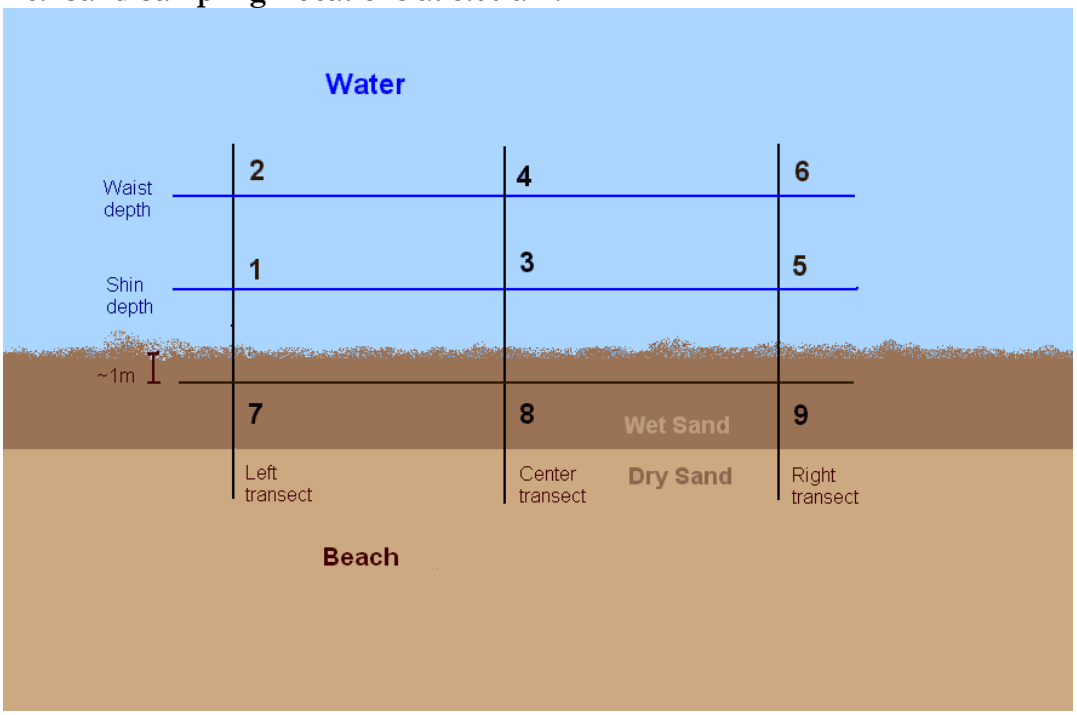
Sand Samples:

Westat will collect three sand samples per day at 8:00 AM along with the 8:00 AM water samples. The sand samples will be collected 1 meter from the lowest water level (when the waves have receded from the shoreline) at the same 3 transects where water samples are collected. See Figure

1c, which is a schematic that shows the collection of the sand samples at 8:00 am (sampling points 7, 8 and 9). The sand should be wet. If the sand is not wet at 1 meter from the water, the sand collection location will be moved the shortest possible distance toward the water to a location where the sand is wet. Westat will record the actual distance from the water. Global Positioning System (GPS) readings of the actual sand collection locations and a photo of the sample collection sites will be taken.

The sand samples will be collected on Saturdays and Sundays, and on 3 holiday weekdays, Monday May 25th, 2009 (1 day), Monday July 20th, 2009 (1 day) and Monday July 27th, 2009 (1 day) from May 15th, 2009 through August 2nd, 2009 at Boquerón Beach, Puerto Rico (**see Table 1**). Westat will collect the sand samples Saturdays and Sundays, and on 2 holiday weekdays, (Friday, July 3, 2009 Monday September 7, 2009) from June 6, 2009 through September 7, 2009 (See Table 2), at Surfside Beach, South Carolina for microbiological analysis. As with the water samples, it is intended that samples will be collected on the scheduled dates, but other dates may be substituted if rainfall or other problems prevent swimmers from going to the beach, prevent water sampling, or create hazardous conditions for the field personnel. Sample collectors will notify the WACOR or COR of adverse weather conditions or other problems and request guidance whether to begin or continue sampling on a given day or weekend. This is necessary because the samples must be collected when there are sufficient bathers at the beach to allow NHEERL to conduct their concurrent epidemiological/health study.

Figure 1c. Sand Sampling Locations at 8:00 am.



Composite Samples:

Westat will collect one additional bottle at each of the 6 grid locations (see Figure 1a) three times a day at 8AM, 11 AM and 3PM to be used for creation of composite samples. A total of 18 bottles will be collected per day. In Puerto Rico, these samples will be collected on Saturdays and Sundays, as well as 3 weekday holidays (Monday May 25th, 2009, Monday July 20, 2009, and Monday July 27, 2009) during the study period of May 15, 2009 to August 2, 2009. In South Carolina, these samples will be collected on Saturdays and Sundays, and on 2 holiday weekdays, (Friday, July 3, 2009 Monday September 7, 2009) during the study period from June 6, 2009 through September 7, 2009. At the lab these samples will be combined to create a composite sample, as described in the "Analytical Methods" section.

Sampling Methods

Water Samples:

See Standard Methods for the Examination of Water and Wastewater, 20th edition (1998), Section 9060, for recommendations on microbiological sampling (3). Briefly, samples will be collected in waist-high (1 m deep) and shin-high (0.3 m deep) water by serially immersing two (2) capped 1000-mL pre-sterilized, polypropylene bottles (or four 500-mL bottles) to the appropriate sample depth, removing the lids and allowing them to fill, re-capping lids (to prevent contamination from surface

water), raising them out of the water, removing the lids, and emptying them slightly to allow approximately 1 inch of head space before securing the lids. Sampling devices may be employed. Samples will be taken about 1 foot (0.3 m) under the surface of the water in waist-high water, and shin-high samples will be taken 6 inches (0.15 m) above the bottom of the water. The samples collected near the bottom must be taken with care so as not to introduce additional sand/solids/debris into the samples. Sample plans may have to be altered in extreme or unusual circumstances. If alterations of the sample method are considered, the WACOR or COR will be notified and guidance will be requested. Westat will utilize field protocols from Work Assignment 2-04 under contract EP-D-04-064. Such field protocols and sampling procedures will be submitted to the WACOR or COR for approval prior to the beginning of the study.

Three 1-liter water samples will be collected aseptically, as described above, at each location on the beach grid (Figure 1a) for microbial analysis:

1. 1 liter will be used for the membrane filter method and the ancillary measurements (which will be done last to prevent contamination).

2. 1 liter will be used for the rapid QPCR method.

3. 1 liter will be used for the Composite Samples

Two 1-liter plastic coated glass bottles will be used for the USGS remote chemical analysis, at the 11:00 AM sampling period, at sampling points 2, 3, 4 and 6 only.

Following collection, all samples will be placed in coolers and maintained on ice during transport and at
1 - 4° C during the time interval before they are analyzed or shipped (samples for chemical analyses only). No additional samples are collected for the determination of pH and turbidity. These measurements are made from the same samples used for membrane filtration after these analyses are completed to prevent contamination.

Any problems encountered while sampling or while taking ancillary measurements will be recorded on data collection sheets in comment fields or on additional sheets clearly identifying the date, time, sample location (on grid) and reported to the WACOR or COR if problems may affect the analytical results. In the event of problems, corrective actions taken (where possible) will be documented by the field team leader, along with the results of such actions.

Sand Samples

Westat will collect, transport, and process the sand samples according to the protocol provided by Kristen Brenner on April 2, 2007. The following text is taken from that protocol. Sand samples will be collected with sterile, 2 inch x 10 inch stainless steel liners (AMS, American Falls, Idaho, or the equivalent). The liner will be pushed into the sand at least 8 inches. The liners will be sterilized at the lab by rinsing them with water, wrapping them in aluminum foil and heating them in the drying oven at 170 °C overnight. The liners will remain wrapped in the aluminum foil until use. Liners containing the sand samples will be capped at both ends, placed in zip-lock plastic bags labeled using

a simplified version of the usual alpha-numeric system (See below.), and transported to the laboratory on ice. Samples will be stored in a refrigerator at 4 degrees C. until analyzed.

In the laboratory, sand samples will be aseptically transferred to sterile wide-mouth polypropylene bottles (500 ml or 1- liter, depending on the quantity of the sand), also labeled using the simplified version of the usual alpha-numeric labeling system. For each sand sample, 75 grams of sand will be aseptically weighed out in a sterile, **pre-tared**, wide-mouth 500-ml bottle (using sterile spatulas), and 300 ml of Standard Methods phosphate-buffered rinse/dilution water (3), measured with a sterile graduated cylinder, will be added to each bottle. Each bottle will be vigorously shaken 50 times (Please count.). Immediately after shaking, some of the contents of the bottle will be poured into two sterile 50-ml, disposable centrifuge tubes (Corning 430829 or the equivalent) and filled to the 50-ml mark. The tubes will be centrifuged for 5 minutes at ~3000 rpm (600 x g) to bring down the sand and sediment, and the supernatant will be removed using a sterile pipette and placed in a sterile 100-ml polypropylene bottle for subsequent analysis by Method 1600 (Attachment 1) and the Quantitative Polymerase Chain Reaction (QPCR) method (Attachment 6).

The accuracy of the 50-ml mark on the disposable tubes will be checked before the dry run by randomly choosing 5 tubes from the package, weighing each of the 5 tubes, and recording the weights. After 50 ml of distilled water is measured with a graduated cylinder and poured into each of the tubes, the tubes are again weighed. The weight of the distilled water (The difference between the two weights) in each tube should be close to 50 grams. Observe the position of the water meniscus with reference to the 50-ml mark on the tubes. In addition, 5 randomly chosen, **preweighed** tubes should be filled with distilled water so that the meniscus touches the top of the 50-ml line. Weigh the tubes again and determine the weight of the water by difference. If the mark is accurate, the weight of the distilled water should be close to 50 grams. Record all results, and send a copy of the results to the WACOR and Kristen Brenner, the technical point-of-contract for sand analyses.

During the dry run, aliquots of 10 ml and 1 ml of each undiluted sand extract and 1 ml of the 10^{-1} – 10^{-6} dilutions of each extract in phosphate-buffered dilution water (3) will be analyzed by EPA Method 1600 for *Enterococci* (Attachment 1). The number of filtrations for the actual study will be reduced after the normal range of concentrations in sand are determined during the dry run. Three 20-ml aliquots of each sample will be filtered, and the filters will be frozen, as described in the QPCR Method (Attachment 6), during the dry run. The sand extraction method described above and the volumes used for both tests may have to be adjusted, depending on the normal range of concentrations of *Enterococci* in the extracts during the dry run. Westat will obtain EPA's approval before changing the protocol or volumes analyzed.

In addition, the pH of each extract will be taken and recorded, and a 25-gram portion of each sand sample will be dried at 100 degrees C for several days to a week in a **preweighed** container. After the samples are dry, the containers should be weighed again to determine the dry weight of the sand samples by difference. Leftover sand samples, the bottles of the sand-buffer slurry, and extracts should be stored in the refrigerator until all the results have been obtained with all tests (about a week).

Sample Handling and Custody

Westat will utilize sample collection/custody forms modified in Work Assignment 2-04 under contract EP-D-04-064 as part of the general forms. A sample form is shown in Attachment 3. The distribution of each individual bottle taken at each location on the beach grid (Figure 1) must be documented on the custody forms. A copy of each airbill for the shipment of the samples for remote chemical analysis and a copy of the ASR custody form (Attachment 4) enclosed with each shipment of samples must be attached to the appropriate custody forms and submitted to USEPA. Each USGS sample must be cross-referenced with the NEEAR study locations.

Prior to sampling visits, tracking forms will be printed by a member of the site project team. The location, date, target collection time, field team leader, and information about all samples to be collected during that visit will be entered on the forms, by hand (or electronically, prior to printing). The forms will be printed on paper suitable for field work. Each cooler used to transport samples from the site to the lab will have a copy of the appropriately completed collection/custody form(s) in it or securely attached to it. Ideally, Westat will know in advance how many samples can fit in a given cooler and could, therefore, prepare specific tracking sheets for each cooler prior to going to the field. If more than one cooler is needed, the coolers will be numbered, and cooler numbers will be cross-referenced on the appropriate tracking sheet. Individual bottles for the rapid methods can be distributed after the samples are logged in at the laboratory, and the custody forms must be signed by each of the method analysts when portions/aliquots of the samples are removed.

Additional columns on the tracking forms include the actual collection time, the time samples arrive at the laboratory, and their storage location. Arrival time at the laboratory can be indicated by entering a time for the first sample on a custody sheet, and drawing a down arrow in the lab arrival time column for the rest of the samples. The field storage location may also be filled in this manner.

A different form (or forms) will be used to record the dates and times when analysis by QPCR and filtering begins (MF and QPCR), the dates and time plates are placed in the water bath (or filters are placed in the freezer for the QPCR method), the dates and time samples are removed from incubation (or freezer for the PCR method), and the analysis results. There will be spaces for associated initials for each of the sequential steps. The various "analysis" times will be treated on a batch basis; *i.e.*, a sample batch is all of the samples brought to the laboratory at the same time for analysis, such as all 6 morning samples.

Microbiological sample containers will be labeled with water resistant sample labels. The sample bottles will have IDs with consecutive numbers to facilitate handling in the laboratory and to prevent errors. However, Westat will be responsible for placing the requisite additional information onto sample bottles at the time of sampling to ensure that the samples can be clearly identified. It is recommended that the information (or at least alphanumeric information, such as suggested directly below) be added just prior to or just after sampling, as this should minimize the chance of getting samples in the wrong bottles. Information to be added would include the date, scheduled and actual time of collection, and some type of alphanumeric that identifies the sampling location, and the method(s) to be used.

Westat proposes to use the following sample labeling scheme for all water and sand samples. This scheme is the same as has been used in past years for water sampling and it was easily modified to accommodate the sand samples introduced this year. Microbiological sample containers will be labeled with water resistant sample labels using the following alphanumeric (9-character) scheme (to avoid confusion and duplicate sample numbers):

MMDDNSSXXB

Where:

MMDD is the date of the sample collection;
MM is the numeric month (1-12) and
DD is the day (01-31), e.g., 0614 for June 14,

N is the sample point at the beach;
1-6 for water samples (see Figure 1a.)and
7-9 for sand samples (see Figure 1c.)

SS is the method/bottle number, as follows:

01 = Membrane Filter Method 1600
02 = QPCR Methods
03 = Alternate Methods
4a = Chemical, bottle a
4b = Chemical, bottle b
4c = Chemical, bottle c
S1 = Sand container (If in the future, more than one sand sample will be taken from the same location at the same time, the second container would be S2.)
C1 = Composite Sample

XX is the planned time of day for the sample collection, as follows:

08 = 8:00 a.m.
11 = 11:00 a.m.
15 = 3:00 p.m.

B designates Boquerón Beach, which is the first initial of the beach.

Thus, for example, the water and sand samples collected on June 23 at 8:00 am would be labeled:

062310108B	062310208B
062320108B	062320208B
062330108B	062330208B
062340108B	062340208B
062350108B	062350208B
062360108B	062360208B
06237S108B	
06238S108B	
06239S108B	

Westat understands that sample containers may be reused after proper cleaning and resterilization or bottles, presterilized by the manufacturer, may be used. Westat will use only presterilized bottles.

Westat will obtain a copy of the manufacturer's sterilization certificate and/or record for each lot should be obtained. In addition, the sterility of a few randomly-chosen bottles from each lot should be tested before field use by adding sterile Trypticase Soy Broth to the bottles, incubating for 48-72 hours at 35° C, and observing the bottles for bacterial growth. Prior to leaving for the field, the sample team leader will check to see that there are an appropriate number of sample bottles and sample ID labels for the sampling visit (Bottles may have labels attached prior to sampling, if it is demonstrated that this has no deleterious effects on the labels.) for the sampling visit. There should also be extra, unlabeled sample containers, and a means to label them for back-up purposes. Copies of completed sample collection/custody sheets will be provided to the WACOR or COR daily along with their associated data sheets.

As stated above, following collection, samples are to be maintained on ice during transport and at 1 - 4° C until the time of analysis. This is the only preservation step. Microbiological analysis of water samples will commence within six hours of collection; it is critical that sample plates from the membrane filter methods be placed in the water bath within eight hours of sampling. In the event of any problems or irregular occurrences, it is imperative that the WACOR or COR be called immediately for guidance, and that the comments fields on the various data sheets be used to record problems/corrective actions, so that the effect on data quality can be considered. Examples of problems that might occur include sampling difficulties, failure to ice-down samples, missed holding/analysis times, longer than acceptable incubation times, problems with the instruments, etc.

With the rapid QPCR method, the critical step is the filtration of the water samples and storage of the filters in the freezer within the 8 hours after collection. Once the filters are frozen, analysis can be done as time allows. The times the QPCR method filters are frozen and stored, the location of the freezer(s), and the dates and times of the analyses will be recorded. If QPCR filters are analyzed in another lab, they must be shipped by overnight express on dry ice. The other laboratory will conform to the QC requirements of this document. Problems with the rapid methods, like those with the filter methods, must be reported and guidance requested from the WACOR or COR.

Samples may be disposed of following successful microbiological processing by each of the microbial methods, including the counting of all plates, successful pH and turbidity measurements, and completed analysis of samples by all methods except for the QPCR method. However, QPCR samples must be filtered and the filters frozen before the disposal of the samples. Contact the WACOR or COR about the disposition of the samples if unusual results are obtained.

Westat will maintain a dedicated sample record book that is used to record all sample IDs as samples are checked into the laboratory. The record book will also have columns for date checked in, storage locations, and disposal dates. Westat has the responsibility for ensuring that all sample IDs are recorded and will initial the record book for each batch of samples received to indicate that all expected samples were present. The USEPA may request that the record book or copies of pages from this record book be made available for examination. Westat is also responsible for verifying that the arrival time at the laboratory is entered in the appropriate column on the sample collection sheets, and will initial sample collection sheets in the appropriate space(s) to indicate such, and will note any leaking containers or other irregularities.

Analytical Methods

Microbiological Methods

1. Standard Membrane Filter Method *Enterococci* (Method 1600)

Attachment 1, "Method 1600: Membrane Filter Test Method for *Enterococci* in Water," EPA/821/R-97/004, May 1997 (4) describes the assay for *Enterrococci*. This method can also be found in **Attachment 2**. "Improved Enumeration Methods for Recreational Water Quality Indicators: *Enterococci* and *Escherichia coli*," EPA/821/R-97/004 (7). These attachments are detailed enough, including descriptions of required equipment, so that the membrane filter method can be performed. As such, these two attachments represent the standard operating procedures (SOPs) for the critical membrane filter data to be obtained from the field study. A 1-liter sample or two 500-ml water samples will be collected for use in performing the filtration method and the ancillary pH and turbidity measurements, which will be performed last to avoid contamination. All collected samples will be analyzed for *Enterococci* by the MF method using sample volumes of 100, 10 and 1 mL [except for special circumstances; for example, if plates at the standard sample volumes are all TNTC, or produce zero CFUs, then sample volumes may need to be adjusted.] The laboratory may have to adjust volumes using their own judgment if immediate communication with the WACOR or COR is not possible. In the event that the laboratory must adjust the volumes and adjust, the adjustment and documentation of reason must be indicated in the records submitted to the USEPA. Analysis of each sample will be initiated within 6 hours of its collection, and processing (filtration and plating) will be completed no later than 8 hours after collection.

Specific QC requirements to be incorporated into the assays (in place of the general guidance in the methods) can be found in the next section of this plan. Table 3 summarizes some of the key features of the method. Any modifications to the method, such as using auto-pipets or micro pipets instead of standard glass pipets, must be approved by the WACOR or COR prior to being implemented. Any other questions regarding the methods should also be addressed to the WACOR or COR prior to the start of field activity.

Table 3. Summary of the mEI Agar Method for *Enterococci*

Method	Medium	Incubation time and temperatures (° C)	Volumes analyzed (mL)	Detection limits (colonies per plate)	Ideal l# of colonies per membrane
Enterococci EPA 1600	mEI agar	24 hours ± 2 hours @ 41 +/- 0.5° C	100 10 1	1-200	20-60

On the sample collection/tracking sheets and final data sheets, laboratory analysts are responsible for entering times and their initials for the following sequential steps:

- Analysis start time.
- Time at which plates being incubation in the water bath.
- Time at which plates are removed from the water bath for counting.

These times will be entered by hand initially, and later entered into the database electronically. The times listed above, and initials, may be entered in a batch-wise manner. The laboratory is also responsible for entering dilution data, count data, QC data, etc. on data sheets. Responsibility for electronic data entry will be determined by Westat.

Samples are to be analyzed in batches. A batch will be considered to be all of the samples that were delivered to the laboratory at the same time. The plates for each batch of samples should start their incubation periods at the same time, and the microbiological control samples described below under "specific filtration control tests" will accompany each analysis batch.

For the membrane filter assay, the most critical quality control requirements are as follows:

- Prior to any sampling/filtering, an appropriate volume of TSA [Tryptic Soy Agar/Trypticase Soy Agar (Difco 0369-17-6, BD 4311043, Oxoid CM 0129B, or the equivalent)] will be prepared, and tested as described below. These plates will later by used for QC samples during sample runs. The recipe for TSA and the contamination screening for TSA plates is described below:

  Composition:

Tryptone	15 g
Soytone	5 g
NaCl	5 g
Agar	15 g

  Preparation: Add the dry ingredients listed above the 1000 mL of reagent-grade distilled water, and heat to boiling to dissolve the agar completely. Autoclave at 121° C (15 lbs pressure) for 15 min. Dispense the agar into 9 x 50 mm petri dishes (5 mL/plate).

  Test for contamination: Incubate all plates for 24 - 48 hr at 35° C to check for contamination. Discard any plates with growth. If ≥ 5% of the plates show contamination, discard all plates, and make new medium. Store plates in plastic bags at 4°C until needed. The final pH should be 7.3 ± 0.2. Records of preparation and testing will be maintained, and will be submitted to the WAM upon request.

- Each batch of mEI agar is to be pre-tested for performance (i.e., correct enzyme reaction) with known cultures of target (*e.g., Enterococcus faecium or Enterococcus fecalis*) and non-target (e.g., *Escherichia coli or Pseudomonas* species) organisms. Records of such tests are to be maintained by the laboratory and will be submitted to the COR and WACOR upon request.

- Specific filtration control tests, listed below, are to be performed each time a batch of samples are analyzed, and the results recorded. Results for all filter, agar or buffer controls, including counts (if any), will be reported with the sample results.

- Filter Control: Place one or more membrane filters on sterile TSA plates, and incubate the plates for 24 hours at 35° C. Absence of growth indicates sterility of the filter(s).

- Phosphate-Buffered Dilution Water Controls: Filter a 50-mL volume of sterile dilution water before beginning the sample filtrations and a 50-mL volume of dilution water after completing the sample filtrations. Place the filters on TSA plates, and incubate the plates for 24 hours at 35° C. Absence of growth indicates sterility of the dilution water.

- Agar Control: Place one or more plates of each medium, mEI and TSA, in the incubator. Incubate mEI at 41° C and TSA at 35° C for 24 hours to check for contamination. Absence of growth indicates sterility of the plates.

- Optional membrane test: Test new lots of membrane filters against an acceptable reference lot using the method of Brenner and Rankin (4). Although optional, this test is recommended. In lieu of performing this test, the laboratory should purchase filters from a reputable source. The USEPA has found (by the method referenced) that Sartorious filters have generally provided satisfactory performance; however, this does not mean other filters are unacceptable.

There are no specific sample IDs for the specific filtration control samples. On the hard copy format batch analysis sheets, their results will be reported with the following codes:

YYZ (MEDIA), where,

YY = AC, PB, or MF (for **A**gar **C**ontrol, **P**hosphate **B**uffer dilution water controls, or **M**embrane **F**ilter control).

Z = B or A, or nothing (used only for phosphate buffer dilution water controls; B for "**B**efore filtering" control, A for "**A**fter filtering" control).

(MEDIA) = mEI or TSA, the medium used for the control.

The methods contain other specific QC elements, such as requirements for laboratory water quality, specifying that thermometers be NIST-traceable, calling for daily confirmation of incubator and water bath temperatures. Such method specifications will be adhered to, and the adherence documented. All autoclave runs will contain maximum-registering thermometers to ensure appropriate temperatures are achieved. Additionally, at least weekly, autoclave runs will contain spore strips or vials, which will be incubated according to the manufacturer's instructions to check for proper sterilizer operation. Calibration records should be maintained for laboratory balances, pH meters, etc.

The method SOP (**Attachments 1 and 2**) contains procedures for verifying the correct identities of organisms. Verification tests are required for all samples (5 colonies/sample) from one day (either Saturday or Sunday) of the first weekend (6 sample locations x 3 times per day x 1 day = 18 samples

total) at the beach site. Results of the verification tests will be recorded and reported to the WACOR or COR with the other sample data in a mutually agreed upon manner.

It is expected that laboratories will follow generally accepted good microbiology laboratory practice, such as described in the USEPA Microbiology Methods Manual, Part IV, C (1); Section 9000 of the 20th edition of Standard Methods (3); or the QC section of the USEPA's "Manual for the Certification of Laboratories Analyzing Drinking Water" (5). Copies of any records associated with standard laboratory QC practices will be made available to the USEPA upon request.

2. Quantitative Polymerase Chain Reaction (QPCR) Method

Attachment 6 describes the procedures for the detection of *Enterococci* and *Bacteroides* in water samples based on the collection of these organisms on membrane filters, extraction of their total DNA, and polymerase change reaction (PCR) amplification (i.e., a process whereby the quantity of DNA is doubled in each cycle of amplification) of a genus-specific DNA sequence using the TaqMan™ PCR product detection system. The TaqMan™ system signals the formation of PCR products by a process involving the breakdown of a double-labeled fluorogenic probe that specifically attaches to the target sequence at a site between the two PCR primer recognition sequences. The reactions are performed in a specially-designed thermal cycling instrument that automates the detection and quantitative measurement of the fluorescent signals produced by probe degradation during each cycle of amplification. These signals are directly related numerically to the quantities of PCR products produced.

Westat understands that the attachment is detailed enough, including descriptions of required equipment, so that the method can be performed. As such, this attachment represents the standard operating procedure (SOP) for the critical data to be obtained from this portion of the field study. A 1-liter sample or two 500-ml water samples will be collected for use in this method. All collected samples will be analyzed for *Enterococci* and *Bacteroides* using sample volumes of 100 mL [except for special circumstances; for example, if this volume is found to be impractical to filter, then sample volumes may need to be adjusted]. Filtration of each sample will be initiated within 6 hours of its collections. Five (5) replicate filtrations will be performed, and the filters will be transferred to extraction tubes, as described in the protocol (Attachment 6), and stored at -20° C for an indefinite period. All filters will be properly labeled to identify the water sample they came from.

The local analytical lab will perform the 5 replicate filtrations and ship 3 filters to the PCR lab and 2 filters to Dr. Richard Haugland of USEPA (26 W. Martin Luther King Drive, Mail Location 314, Cincinnati, Ohio 45268-1314; Telephone: 513 / 569-7135), all by overnight express on dry ice on the Monday following the weekend the samples were collected.

The PCR lab will perform the extraction to obtain DNA to be used for QPCR analyses for all microorganisms (*Enterococci* and *Bacteroides*) as soon as possible using only one of the filters (**Attachment 6**), and two filters will be stored in the freezer as backups or for other/later analyses. At the end of this study, all remaining frozen filters will be sent on dry ice by overnight express to Dr. Richard Haugland of the USEPA.

Specific quality control (QC) requirements to be incorporated into these analyses are listed below, as well as those in the method protocol.

- QC requirements for sample collection and filtration are specified in the Microbiological Methods section.

- Cell suspensions of the calibrator strains, *Enterococcus faecalis*, American Type Cutlure Collection (ATCC) 29212, *Bacteroides fragilis* ATCC 25285, and reference strain, *Geotrichum candidum*, University of Alberta Microfungus Collection and Herbarium (UAMH) 7836, will be provided to the laboratory by the USEPA. The cell suspensions provided must be stored by the laboratory at -70° C, until used. Preliminary QPCR analyses must be performed using four tubes of these suspensions prior to the start of the study, and the results (C_T values and run files) must be reported to USEPA. Subsequent average results for these samples on each day of analysis should be within +2 C_T units of the average of the initial values (See paragraph below on monitoring the performance of the thermal cycling instrument and PCR reagents).

- Training for the laboratory on the highly specialized scientific PCR equipment will be provided by the government for validity of data. Westat will be responsible for ensuring that the PCR technician has documented experience in QPCR technology.

- Westat will be required to purchase PCR reagents, including primers and fluorescently-labeled probes. Primer and probe sequences will be provided by the USEPA.

- Westat will be required to monitor the performance of the thermal cycling instrument and PCR reagents based on ongoing calibrator sample analysis results. (See above.) In the event of failure to meet these performance criteria, Westat will be required to prepare and analyze a new set of calibrator extracts, identify the source of the problem (e.g., reagents or instruments), and take corrective action.

- Westat will be required to provide adequate facilities and carry out precautions necessary to minimize the likelihood of DNA contamination. Manipulation of samples and reagents will be performed in laminar flow hoods or workstations with UV light sources, and the areas will be disinfected before and after each use with 10% bleach. Disposable aerosol barrier pipette tips will be used for all liquid transfers. Tubes and other disposables that are not sterilized by the manufacturer must be autoclaved before use. All supplies and disposables will be DNA-free. Distilled water and other reagents must be verified to be free of target DNA in negative control analyses performed with each set of sample analyses.

- All pipettors used will be calibrated prior to commencing work and on a semiannual basis afterwards. It is recommended that the pipette calibration be verified weekly by weighing several different amounts of water (in the ranges use) pipetted into a properly tared container.

– This work assignment will also include four other combinations of reagents that will be tested for each organism by PCR method.

3. Composite Samples

Westat will create a composite sample by adding equal volumes from each of the three transects for the different depths. Westat will create a shin-depth composite sample by adding equal volumes from each of the shin-depth samples. Westat will create waist-depth composite sample by adding equal volumes from each of the waist-depth samples. Westat will create a total composite sample by adding equal volumes from all depth samples. There will be a total of 9 composite samples per day: 3 shin-depths (8AM, 11AM, and 3PM); 3 waist-depths (8AM, 11AM, and 3PM); and 3 all-depths (8AM, 11AM, and 3PM). These samples are created from parent samples (the additional 1-liter bottle collected at each grid location). These samples will be used to run QPCR (for *Enterococci* and *Bacteroides*) and Method 1600 Membrane Filtration analysis.

Ancillary Measurements

Ancillary measurements listed in Table 2 will be collected by a variety of means. Some are collected by simple observation; others involve the use of equipment, such as pH meters, wind gauges, and rain gauges. It is noted here that WACOR or COR approval of any deviation in methods is required.

For any ancillary data collection, especially that involving specific equipment, Westat will be responsible for documenting the exact methods used to collect the data, and to provide information about the calibration and QC procedures for any equipment. This documentation will be provided for approval to the USEPA WACOR or COR prior to the occurrence of any field sampling.

Appropriate field team members or lab team members are responsible for entering data on appropriate data collection sheets. Westat may propose additional QC activities related to sampling and analysis in their QAPP and work plan as necessary and appropriate. Any changes from the QC specified by individual method technical point-of-contacts must be confirmed with them before implementation.

Sample Collection and Custody for Remote Chemical Analysis

Three additional 1-liter water samples will be collected once a day, during the 11:00 AM collection period, in plastic-coated, ashed amber glass bottles, furnished by the U.S. Environmental Protection Agency, at each of the three transect locations in waist-high water and at the central location in shin-high water (sampling points 2, 3, 4 and 6 in **Figure 1b**) for a total of 12 1-liter samples (3 bottles at each of 4 locations). Samples will be collected at the marine water beach by the same methods used for collecting the water samples for microbial analysis. The bottles should be permanently labeled with the location of sample site, the date and time of collection, and designated "For Chemical Analysis (Unfiltered Water)." After cooling the samples on ice, the bottles will be packed in coolers with ice along with Analytical Services Requests (ASR) (**Attachment 4**) contained in double zip-lock bags, and shipped by overnight carrier to the U.S. Geological Survey (USGS) [Dr. Edward T.

Furlong, USFS, Denver Federal Center – Building 95, Denver, Colorado 80225-0046; Telephone (303) 236-3941; Fax: (303) 236-3499; E-Mail efurlong@usfs.gov] for chemical analyses and comparison of interlaboratory variation.

At the time of collection, an Analytical Services Request form (ASR), **Attachment 4**, will be completed in its entirety. The ASR should be placed in a waterproof covering and shipped with the sample to the appropriate laboratory. All sample bottles collected during a single weekend may be sent to the USGS in one shipment (by overnight express) on the Monday following the weekend, provided all samples are refrigerated during the entire time from collection until shipment. Coolers and packing material to ship samples will be provided by the USEPA (Dr. Susan Glassmeyer, 26 W. Martin Luther King Drive, Mail Location 564, Cincinnati, Ohio 45268-1564; telephone: 513 / 569-7757), and USEPA will provide airbills with the shipping costs billed to USEPA for the field personnel to use.

Quality Control (QC)

The most critical elements of quality control for the membrane filter method are those related to the microbiological assays. Sampling is straightforward; Westat must ensure that the proper samples are taken in the appropriately labeled containers. Holding time of samples will be considered critical. Samples that have not been placed in the water bath in the membrane filter method or completely filtered and placed in the freezer for use in the QPCR method within eight hours of collection will be considered to have produces invalid data. (However, all data will be collected, compiled, and reported to USEPA). The intent of this project is to collect all of the data for subsequent evaluation by the USEPA project team, who will ultimately determine its utility based on their collective expertise and experience. No data will be rejected outright by the persons performing the analysis. All data, including Too-Numerous-To-Count's (TNTC) and zero's in the membrane filter methods, will be reported to the USEPA. An estimation procedure for TNTC plates will be provided to the laboratory by USEPA (**Attachment 5**). The estimation data from the TNTC plates (i.e., the five counts from five squares on each filter) will all be submitted to USEPA along with the count data for the other samples. Westat will calibrate and maintain the instruments according to the methods and/or the manufacturer's recommendations. Westat will follow accepted good microbiology laboratory practice and maintain QC records. Westat will participate in any QA audits conducted, and will run one or more performance evaluation samples provided by the USEPA. Westat will contact the WACOR or COR when problems occur and document corrective actions taken in a report.

Corrective Actions

Failure to meet any QC requirements, including those associated with standard good laboratory practice, requires that appropriate corrective actions be taken. All QC failures, associated corrective actions, and their effectiveness, must be documented on a corrective action form, and submitted to the USEPA WACOR or COR as part of the weekly reports. Data associated with quality control problems will be clearly identified in such reports, along with an assessment as to the QC failure's potential effect(s) on data quality. The WACOR or COR will be notified of such problems/corrective actions as soon as possible to the time of the actual occurrence. All related sample and ancillary data will still be reported in the standard way, with the QC problems clearly noted on copies of the data deliverables.

Instrument/Equipment Testing, Inspection, and Maintenance

Any SOP for equipment/instrument which Westat may be required to develop or provide will describe standard maintenance procedures for equipment. Maintenance records will be described in the SOPs, and will be made available to USEPA upon request, including monitoring records of basic equipment such as incubators, refrigerators, etc.

For any equipment that might affect critical data (i.e., microbiological or ancillary data), Westat will prepare a short report for the WACOR or COR describing how the equipment was inspected and tested upon receipt. The report will be delivered within two weeks of equipment being placed in service.

Instrument/Equipment Calibration and Frequency

Any SOPs for instruments and equipment which Westat may be required to develop or provide will fully describe calibration and calibration verification procedures. This should include reference to any calibrations conducted using certified equipment and/or standards with known valid relationships to nationally recognized performance standards. Field instruments/gauges and laboratory measuring equipment, such as balances and volumetric measuring devices (e.g., micropipettes), will be professionally serviced/certified within the six months prior to the commencement of the field/laboratory activities for this project.

Tracking and Inspection/Acceptance of Supplies and Consumables

Westat will have a system for tracking supplies, reagents, etc. prior to the start of the field season.

The membrane filter methods (**Attachments 1 and 2**) describe the minimum requirements for the quality of chemicals and laboratory water. Quality control procedures for laboratory water outlined in the USEPA drinking water certification manual (5) are recommended. Westat will at least maintain basic records (i.e., resistively readings, filter changes, etc.) for their laboratory water systems.

The optional (but recommended) filter test (2); previously described, may be employed to test new membrane filter lots. All media prepared will be routinely tested for sterility. The goal is to have a clear association of all microbiological data with specific lots of all materials employed in performing analyses. All records associated with materials tracking and preparation will be made available to USEPA upon request.

Data Management

Some elements of data management for field data and laboratory data were previously outlined. Westat will initially hand-enter results on pre-printed forms that are approved by USEPA.

On an approximately daily basis, completed hand-entered data sheets will be sent to the WACOR or COR. Weekly submissions will also be submitted to the WACOR or COR.

Westat will maintain original copies of sampling and data worksheets until instructed by the WACOR or COR on the deposition of the worksheets. Westat will maintain two copies, on separate

storage media, of electronic versions of data until instructed by the WACOR or COR on the disposition of the data.

Readiness Review/Dry Runs

At least one week prior to actual sampling, sampling/analysis personnel will perform a readiness review/dry run at the beach site. One or more USEPA representatives may attend. A checklist modified in Work Assignment 2-04 under contract EP-D-04-064 will be utilized by Westat that details all equipment, supplies, worksheets, logbooks, etc. required to conduct sampling, ancillary data collection, microbiological analysis and data recording, and reporting at the beach site. A set of samples will be run. Data transmission will also occur as part of this effort. Westat will observe all activities in detail and record their observations.

Westat will be responsible for determining the results and any corrective action to be taken. After concurrence with the USEPA, a written report on the final approach to sampling/analysis/reporting will be provided to the WACOR or COR.

Site Visits/Technical Systems Audits

The USEPA may, at its discretion, perform a site visit at the beach site. The site visits may include technical systems audits (TSAs). Although any site visit or audit will likely be conducted by USEPA personnel, the possibility of using contractor support exists. A site visit or audit will be coordinated with Westat in advance.

Site visitors/auditors may recommend work stoppage if they observe what they deem to be critical failings on the part of Westat. Such a recommendation will be made to the WACOR or COR and the contracting officer. Work may only be stopped by the Contracting Officer until such time as effective corrective measures are implemented, verified effective, and approved.

Following the site visit/TSA, a report will be prepared by the personnel who conducted the visit. This report will be addressed to the WACOR or COR. Westat will be provided a copy of the report, and will be required to respond to any corrective action recommendations. Westat will be responsible for signing-off on the response. A close-out memo will be issued to Westat by the WACOR or COR following his/her approval of the response. However, USEPA does reserve the right to revisit any identified problem areas.

Routine Surveillance

Copies of any written reports generated by Westat on routine surveillance will be made available to the WACOR or COR.

Data Validation and Usability

According to USEPA Guidance for Quality Assurance Project Plans; USEPA QA/G-5 (6),

> "the process of data verification requires confirmation by examination or provision of objective evidence that the requirements of these specified QC acceptance criteria are met. In design and development, verification concerns the process of examining the

> result of a given activity to determine conformance to the stated requirements for that activity. For example, have the data been collected according to a specified method and have the collected data been faithfully recorded and transmitted? Do the data fulfill specified data format and metadata requirements?"

Regarding validation, G-5 states,

> "The process of data validation requires confirmation by examination and provision of objective evidence that the particular requirements for a specific intended use have been fulfilled: In design and development, validation concerns the process of examining a product or result to determine conformance to user needs."

Based on these definitions, verification is the responsibility of Westat; however, USEPA reserves the right to review the verification and will be responsible for validation.

Data Review, Verification, and Validation

All data will undergo several layers of review and verification, which is described in the **next** section. The previously described assessments are also a key component of verification. Validation is primarily considered part of reconciliation with project objectives. General principles guiding acceptance/selection, verification, and validation of data are discussed immediately below.

All microbiological data will be submitted to the USEPA (i.e., data from all sample volumes or dilutions, even if zero, uncountable or too numerous to count and all other forms of data, described above). The USEPA will decide whether or not data are acceptable, and will choose which data are included in the final data set for the project. The guiding principles for microbial data acceptance/selection will be:

- Legible data records.
- Dates and times correct.
- Sample IDs correct.
- Electronic and hard-copy data concur.
- Results are in an appropriate format.
- Results reasonable (i.e., not grossly wrong).
- CFU counts are in the ideal range, whenever possible.
- Dilutions with CFU counts outside ideal range are not grossly incompatible with those in the ideal range.
- Sample holding/analysis times were met.

- Associated QC sample results are acceptable.
- Specific acceptance criteria for the rapid methods adequate.

For ancillary data, the acceptance factors include:

- Legible data records.
- Dates and times correct.
- Electronic and hard-copy data concur.
- Results are in an appropriate format.
- Results reasonable (i.e., not grossly wrong).
- Associated QC sample results acceptable.
- Other factors support acceptance (or rejection).

For the remote chemical data the acceptance factors include:

- Legible ASR forms.
- Dates and times correct.
- Samples arrive in good condition at remote laboratory.

Verification and Validation Methods

Verification/Validation Roles and Responsibilities

Westat will inspect forms to see that all appropriate data fields have values entered, and that entries are legible and reasonable. Westat will also ensure that all planned samples have been collected. The verification of review will be indicated by entering their initials on the field data sheets in provided spaces. Westat is responsible for seeing that all forms are present and that they are delivered to the laboratory.

Westat will verify that all expected samples and field sheets are present upon arrival at the laboratory. Westat will periodically inspect the record book and make a record of any such inspections.

The laboratory is responsible for verifying that all microbiological (and other laboratory) data fields are legibly filled out with apparently reasonable data. They will enter their initials and the date in appropriate fields on the data sheets to indicate their review and acceptance. Spaces for date and initials will be provided on all data sheets. The laboratory's initials will indicate their inspection and acceptance of data sheets prior to their delivery to USEPA.

Transmission of deliverables is the *de facto* indicator that the data were completely reviewed and believed to be accurate. The laboratory personnel responsible for reviewing the data will be a person that is different than the person who originally keyed-in the data.

Making Corrections

On any hard copy data sheets, incorrect entries will be singly lined-out *(i.e.,* not obliterated) and correct results entered. If the party making the correction is not the person who made the original entry, then the date and initials of the person modifying the entry will be present next to the correction.

Attachments

Westat will utilize the attachments referenced in the work assignment:

Attachment 1. EPA Method 1600: Membrane Filter Test Method for *Enterococci* in Water (www.epa.gov/microbes)

Attachment 2. Improved Enumeration Methods for the Recreational Water Quality Indicators: *Enterococci* and *Escherichia coli*, EPA/821/R-97/004 (www.epa.gov/microbes)

Attachment 3. Sample Collection/Custody Form

Attachment 4. Analytical Services Request Form for Chemical Samples

Attachment 5. Estimation Procedure for Too-Numerous-To-Count Plates

Attachment 6. Rapid Polymerase Chain Reaction (PCR)-Based Methods for Measuring Total *Enterococci,* Total *Bacteroides*, and *Bacterioides* Thetoiotaomicron in Water Samples

Attachment 7. Method 1602

Saliva Samples:

The saliva study will be an additional effort designed to complement the basic NEEAR Water study which relies on water samples and questionnaires. The saliva study will be conducted at the Boqueron Beach, Puerto Rico site only. Saliva samples will be collected from active NEEAR water study participants using a simple sponge with a handle upon after they have completed the Part B questionnaire at the beach site. Active NEEAR water study participants includes persons who are actively and successfully participating in the current questionnaire process. Participants who are not able to complete the questionnaires successfully will become ineligible for the saliva collection. Participants will be mailed additional sponges to collect and return via mail to investigators 10-12 days and again six weeks later. The saliva sample goal for active NEEAR water study participants is between 2500-2600 participants, each contributing a maximum of three samples.

The NEEAR Water study targets and offers enrollment to all beachgoers between 11:00 AM and 5:00 PM. Beachgoers are approached by trained interviewers between these hours and asked if they would like to participate in a survey. Those who agree complete an initial baseline interview. After the Part B interview is completed, interviewers will ask participants in the NEEAR Water study first if they would be interested in providing saliva samples. They will briefly describe the study emphasizing the following points: 1) three samples will be collected, today, after 10 days, and after six weeks, and tested for germs that cause gastrointestinal and other illness; 2) sample collection is simple and takes about a minute (they will show the saliva sampling kit); and 3) they will be reimbursed $10 for each sample and won't incur any costs for shipping. Participants will be provided $10 for each saliva sample received, for a maximum of $30.

Westat will enroll active participants from the NEEAR water study survey in the saliva collection investigation. Adults 18 years of age and older shall be provided a consent form to review and a contractor interviewer shall be present to answer questions about the study. Unaccompanied minors (under 18 years of age) will not be eligible for enrollment. Parents will provide consent for children under 7, and will provide permission for all children under the age of 18. While the adults and adolescents are reading and signing their consent and assent forms, the contractor interviewer shall read the assent form to them and to any children age 7-14. Westat will store consent forms in a locked portable filing cabinet on the beach until they can be returned to the field office at the end of the study day. Bilingual interviewers (English/Spanish) shall be available at each site to facilitate enrollment of Spanish speaking subjects.

Westat will ship all saliva samples to the US EPA in Chapel Hill, NC, Attn: Elizabeth Sams, 104 Mason Farm Rd., Chapel Hill, NC 27514. All samples shall be shipped with appropriate containers and refrigeration. Westat will email the tracking numbers to sams.elizabeth@epa.gov, and hudgens.edward@epa.gov. Westat will include all shipments with appropriate sample logs and custodial forms. All shipment logs and custodial forms must be preapproved by the WACOR.

TASK 7
Antisera:

Westat will produce coliphage antibody that will be used for the quantification and typing of coliphage from bathing beach water. Westat will produce at least 150 ml of antisera for 7 different coliphage antigens. Westat will use the coliphage antigens and antisera preparation protocol by the US EPA for producing coliphage antiserum. Westat will aliquot the antisera in 5 ml volumes in containers suitable for freezing at very low temperatures. Westat will deliver 200 microliter-lml of antisera from each individual animal to the US EPA 2-3 weeks after the second injection indicated in the protocol.

DELIVERABLES

TASK 1

Deliverable 1 Electronic beach questionnaires (English and Spanish) shall be loaded to appropriate number of Beach Interviewing instruments by Friday, May 8, 2009. Electronic telephone interviews shall be loaded to the appropriate number of telephone data collection computers by Friday, May 15, 2009.

Deliverable 2 Beach Interviewer training sessions shall be completed by Friday, May 15, 2009 in Puerto Rico and by Friday June 5 in South Carolina. A report outlining the training schedule, verifying interviewer completion of the program, and Westat comments shall be due June 19, 2009. EPA/CDC representatives will have access to CAPI devices throughout training sessions.

Deliverable 3 Telephone Interviewer training sessions shall be completed by Monday, May 25, 2009. A report outlining the training schedule, verifying interviewer completion of the program, and contractor comments shall be due Friday, June 19, 2009. EPA/CDC representatives will have access to CATI devices throughout training sessions.

TASK 2

Deliverable 4 CATI telephone interviewer(s) shall implement trial interviews with at least three (3) members of the NEEAR Water Study project team designated by the WACOR or COR prior to Monday, May 25, 2009.

Deliverable 5 Westat will distribute $25 incentive checks and *Participation Status* letters to each household within 30 days of completing the phone interview.

TASK 3

Deliverable 6 Production Reports are due the close of business the Wednesday following the weekend of data collection. The exceptions to this are the weekends of May 22-25, 2009, July 17-20, 2009 and July 24-27, 2009 (Production Report is due the following Friday) (Production Report is due the following Tuesday). The final Performance Production Reports are due September 25, 2009.

Deliverable 7 The comprehensive database is due for each data collection weekend at close of business 10 calendar days after the final collection day. Westat will submit this database by individual data collection days. A final of the comprehensive database and final database report is due Friday, September 25, 2009.

Deliverable 8 A draft of the Final Report covering this work assignment evaluating the questionnaires, training and recommendations for improvement is due Friday, October 16, 2009. The Final Report, covering this work assignment, evaluating the questionnaires, training and recommendations for improvement is due Friday, October 30, 2009.

Deliverable 9 Transfer of project-generated products for Tasks 1-4 (including electronic products) are due Friday, November 20, 2009. These include data, computer-assisted interviews, databases, reports, and other materials created under this work assignment.

TASK 4

Deliverable 10 Westat will update QAPP modified in Work Assignment 2-04 as needed and provide intermediate updates prior to next data collection weekend (can be done electronically). A final QAPP is due at the end of the project, February 24, 2010.

TASK 5

Deliverable 11 Submit the final version quality assurance project plan modified in Work Assignment 2-04 for Task 5 and appropriate additional SOPs, as necessary, to USEPA by the end of the project, February 24, 2010.

Deliverable 12 Submit the final version of all data and result forms to USEPA by November 6, 2009.

Deliverable 13 Provide USEPA with the results of all analyses daily by facsimile, except the PCR, for the first two weekends and weekly (*i.e.*, after the completion of the analyses from each weekend) thereafter. If problems occur that result in the need to monitor the data more closely, results shall be provided daily. PCR results shall be sent to USEPA as soon as the analyses are completed.

Deliverable 14 Provide USEPA with a copy of the sampling records, sample custody forms, and ancillary data weekly.

Deliverable 15	Provide USEPA with a copy of the photographs at the end of the sampling at the beach.
Deliverable 16	Provide USEPA with a report of any problems encountered and the actions taken as soon as possible after the occurrence.
Deliverable 17	Provide USEPA with membrane filter verification data from all of the samples from one day (either the Saturday or Sunday) of the first weekend.
Deliverable 18	Provide USEPA with a final report on the readiness review/dry run, any problem encountered, and the actions taken.
Deliverable 19	Provide USEPA with a final Task 5 report within 30 days after the completion of all analyses from all methods. This report should include suggestions for improvement of future studies, maps of the beach sites with the transects indicated along with the permanent landmarks used to locate the transects, and beneficial procedures or other observations that might be helpful in future studies.
Deliverable 20	Transfer of project-generated products for Task 5 (including electronic products) are due Friday, January 15, 2010. These include data, computer-assisted interviews, databases, reports and materials created under this work assignment.

TASK 6

Deliverable 21	Westat will ship saliva samples to USEPA within the week of saliva sample collection.

TASK 7

Deliverable 22	Westat will ship coliphage antibody by February 25, 2010.

REPORTING REQUIREMENTS

Westat will furnish a copy of the Work Plan, as well as each section of the combined monthly technical and financial progress reports which relate to this Work Assignment directly to the Work Assignment Contracting Officer Representative at the same time progress reports are submitted to the Contracting Officer Representative and Contracting Officer.

Special reporting requirements include documentation of all sources and contacts so as to fully reference the sources of all information. Three copies of each final report shall be provided to the WACOR or COR. Deliverables shall be provided in hard copy and electronically CD ROM or via email. Reports shall be in Microsoft Word 2000 format or newer version, WordPerfect 9.0 or PDF format. A complete set of all deliverables must be submitted at the end of the work assignment. A complete checklist shall accompany this set of deliverables and the Format must be approved by the WACOR or COR. Westat will maintain liaison with the WACOR or COR either by phone or email.

Acceptance criteria: The quality of the data shall be judged by its internal consistency and agreement with well established scientific knowledge, conformity with approved protocols, and completeness both in terms of satisfying the requirements of the work assignment and in terms of adequately characterizing the collected data in order to ensure correct interpretation of the data users.

PERIOD OF PERFORMANCE

The period of performance of this work assignment is from the date of issue through February 24, 2010.

WORK ASSIGNMENT CONTRACTING OFFICER REPRESENTATIVE DESIGNATION

The WACOR will be Elizabeth A. Sams, Environmental Health Scientist, US EPA/NHEERL (MD-58C), Research Triangle Park, NC 27711. 919-843-3161 (phone) and 919-966-0655 (fax). Sams.elizabeth@epa.gov

NOTICE REGARDING GUIDANCE PROVIDED UNDER THIS WORK ASSIGNMENT

Guidance is strictly limited to technical and analytical support. Westat will not engage in activities of an inherently governmental nature such as the following:

1. Formulation of Agency policy;
2. Selection of Agency priorities;
3. Development of Agency regulations.

Should WEstast receive any instruction from an EPA staff person that Westat ascertains to fall into any of these categories or goes beyond the scope of the contract or work assignment, Westat will immediately contact the COR or the Contract Officer.

Westat asserts that the work under this work assignment does not contain any real or apparent personal or organizational conflict of interest. Westat will certify that none exist at the time the work plan is submitted to the EPA.

VIII. Personnel

Westat proposes **Ms. Karen Della Torre** (PL-4) as the Work Assignment Lead (WAL) and Project Leader. Ms. Della Torre shall oversee all aspects of this work assignment. She has over 15 years of experience in the environmental field,serving as program/contract director, a work assignment manager, and senior analyst in support of EPA, including experience in managing high-visibility heath and environmental studies. Also, Ms. Della Torre served as the Project Director for the NEEAR Water Study from 2002-2008, has performed site supervision at West Beach (2003), Silver Beach (2004), Washington Park (2004), Edgewater Beach (2005), Fairhope Beach (2007) and Goddard Beach (2007), and designed the CAPI data collection system for the study.
Ms. Della Torre holds degrees in computer systems, medical engineering, an M.B.A. and is certified as a Project Management Professional.

We propose **Mr. Kurt Patrizi** (PL-3) to serve as field director for this work assignment. Mr. Patrizi holds degrees in Environmental Resource Management and Environmental Sciences and Engineering and has 19 years of experience in environmental regulation, management, policy,

analysis, training, and planning. For over 13 years Mr. Patrizi has served as a work assignment manager, program/contract director, and senior analyst in support of EPA. Mr. Patrizi served as the beach field director for the NEEAR Pilot Study during 2002 and has performed site supervision at West Beach (2003), Silver Beach (2004), Washington Park (2004), Edgewater Beach (2005), Fairhope Beach (2007) and Goddard Beach (2007).

We propose **Dr. Robert Clickner** (PL-4) to provide technical guidance for this project as necessary. Dr. Clickner is an Associate Director at Westat and a senior statistician with 35 years of experience in the development, implementation, and management of statistical and environmental research projects, including four years experience directing the Beaches water quality studies for EPA**.** Dr. Clickner has also designed, conducted and analyzed biostatistical experiments involving pesticides and other environmental contaminants. His project management activities have included the development and maintenance of project completion plans, quality assurance plans, schedules, and budgets; management and coordination of multiple subcontractors, including numerous laboratories; staff assignments; review of deliverables; and client coordination and communication**.** He has developed and conducted international workshops on methodologies for human exposure assessment field studies.

We propose **Ms. Amy Kominski** (PL-2) as Research Assistant to perform beach assessment tasks. Ms. Kominski is a biologist, research assistant, with experience in collecting epidemiologic research data. Prior to working at Westat, she worked at The National Institutes of Health in Bethesda, Maryland. While at The NIH she worked in a clinical microbiology lab and has experience designing and managing public health studies, including; protocol development and implementation of large volume, multi-site research projects. Her specific experience includes infection control studies involving antibiotic resistant bacteria, specifically *Enterococci* and *Staphylococcus aureus*. Ms Kominski is proficient in general laboratory biochemical testing methods, quality assurance and control, antibiotic susceptibility testing and state-of-the-art molecular assays. Since joining Westat, Ms. Kominski has been involved with the work done at Fairhope Beach (2007) and Goddard Beach (2007) and has performed water sampling pilot studies and beach assessment studies.

Westat proposes **Mr. David Barmettler** (PL-2) to serve as site manager for the Boqueron Beach site. In this role, he will manage all data collection activities performed at the site. Mr. Barmettler has extensive experience in supporting international public health studies and providing technical assistance to a number of epidemiologic studies. Mr. Barmettler served EPA in the Community Study of Common Infections in Lawrence, MA, providing site supervision for the collection of epidemiologic data and saliva samples. Mr. Barmettler has performed beach site assessments at Goddard Beach, Huntington Beach, and Washington Park Beach. He has coordinated travel plans, logistic arrangements, and equipment shipments to USAID missions in various countries. Mr. Barmettler holds an MPH and is fluent in Spanish.

Westat **proposes Dr. Sharon Jasim-Hanif** (PL-2) as saliva study protocol developer and manager. Having completed a Ph.D. in Environmental Engineering and a M.Sc. in Environmental Technology, she has extensive experience in scientific, laboratory, and field studies research. Dr. Jasim-Hanif has provided support to many U.S. Environmental Protection Agency studies and has served as Data Collection Supervisor for a wide range of Epidemiological field studies. For the National Epidemiological and Environmental Assessment of Recreational Water Study, Dr. Jasim-Hanif served as on-site Field Supervisor for the in-person interviews with beachgoers at the Edgewater Beach, Biloxi study site. She served as Field Supervisor for the water sample collection at

Edgewater Beach, Biloxi (MS), Washington Park, Michigan City (IN) and Silver Beach, St. Joseph, (MI) study sites. She is skilled in study and protocol design, fieldwork operations, data analysis and management, literature review, and public outreach and health communication. She also has experience interviewing, training, and supervising staff.

Westat proposes **Ms. Sara Hader** (PL-2) as quality assurance specialist for the environmental sample collection. Ms. Hader has 4 years of experience in study design, field data collection, data analysis, and program evaluation for environmental and occupational health studies. She has managed data and sample collection, and the packaging and shipment of environmental samples. Ms. Hader holds a B.S. in microbiology and has served as quality assurance specialist for water and sand sample collection at three previous NEEAR Water Study sites.

Westat proposes **Ms. Naa Adjei** (PL-2) as quality assurance specialist for the environmental sample collection. Ms. Adjei has 3 years of experience in scientific study design, data collection, and data analysis. She has performed beach sites assessments. Ms. Adjei holds a B.S. in neurobiology.

We propose Mr. **Ron Hirschhorn** (PL-3) as the systems and data manager for the survey. Mr. Hirschhorn is an Associate Director with Westat who has overseen the development of many large-scale field data collection and transmission systems. Mr. Hirschhorn holds degrees in mathematics and has over 25 years of experience in systems analysis and design. Mr. Hirschhorn served as the systems data manager for the NEEAR 2004 and 2005 and 2007 study beaches.

Ms. Helen Jewells (PL-2) will be the Telephone Research Center (TRC) Manager for the telephone interviews and has more than fifteen years of experience supervising telephone data collection staff and conducting telephone interviews. She is responsible for training; scheduling and monitoring interviewers; and editing, coding, and other data cleaning. She also has extensive experience with recruiting, survey-design editing, sample reconciliation, and policies and procedures for refusal conversion. Ms Jewells served as the TRC interviewing manager for the 2004 and 2005 and 2007 study beaches.

We propose **Ms.Stephane Ridore, Mr. Marcus Walker, Mr. Michael Seppy, and Mr. Eddy Mattingly** (PL-1) to server as the user support specialists and to serve as interviewer trainers. Both have over 3 years of experience in computer applications, user support, and software testing.

Appendix C

Choosing the Best Membrane Filter Count

MERB SOP 041

CHOOSING THE BEST MEMBRANE FILTER COUNT FOR THE CALCULATION OF THE FINAL CONCENTRATION OF MICROORGANISMS PER 100 ML

A count of 20-60 is the ideal counting range for **any original Escherichi coli or Enterococcus membrane filter colony count, regardless of the volume of sample tested**. Whether a count falls within the ideal counting range will ultimately affect the final concentration, but the ideal counting range does not directly apply to it. To obtain the final concentration, we look at the original counts to determine which count to use in the calculation; then we adjust by the dilution factor to obtain the final concentration (colonies/100 milliliters). The detailed procedure is as follows:

1. Look at the **actual colony count** for the **largest volume** of sample tested, 100 milliliters in this case.
2. If the count falls **within the ideal counting range of 20-60**, calculate the final concentration (colonies/100 milliliters) by multiplying the count by the dilution factor. However, when the largest volume tested is 100 milliliters, the count does not have to be adjusted (that is, the count **is** the final concentration). If the largest volume is 10 milliliters or 1 milliliter, the count would have to be multiplied by 10 or 100, respectively.
3. If the count in the **largest volume** of sample is **outside the ideal counting range of 20-60 on the "low side" (that is, < 20)**, calculate the final concentration (colonies/100 milliliters) by multiplying that count by the dilution factor, if any. If the largest volume is 100 milliliters, the count **is** the final concentration. If the largest volume is 10 milliliters or 1 milliliter, that count would have to be multiplied by 10 or 100, respectively.
4. If the count in the **largest volume** of sample is **outside the ideal counting range of 20-60 on the "high side" (that is, > 60)**, look at the actual colony count for the **next (second) largest volume** of sample. In this case, it is 10 milliliters.
5. If the count in the **second largest volume** of sample falls **within the ideal counting range of 20-60**, calculate the final concentration (colonies/100 milliliters) by multiplying that count by the dilution factor. In this case, the dilution factor is 10.
6. If the count in the **second largest volume** of sample is **outside the ideal counting range of 20-60 on the "low side" (that is, < 20) and the count for the largest volume of sample is > 60 but < 100**, **use the count from the largest volume rather than the count from the second largest volume** to calculate the final concentration (colonies/100 milliliters) by multiplying the chosen count by the dilution factor, if any. If the largest volume tested is 100 milliliters, the count **is** the final concentration.
7. If the count in the **second largest volume of sample** is **outside the ideal counting range of 20-60 on the "high side" (that is, > 60)**, look at the count from the **next (third) largest volume** and **repeat the same steps as in 5-7** . Since the third largest volume is 1 milliliter, the dilution factor is 100.
8. If the count in the **third largest volume** of sample falls **within the ideal counting range of 20-60**, calculate the final concentration (colonies/100 milliliters) by multiplying that count by the dilution factor. In this case, the dilution factor is 100.
9. If the count in the **third largest volume** of sample is **outside the ideal counting range of 20-60 on the "low side" (that is, < 20) and the count for the second largest volume of sample is > 60 but < 100**, **use the count from the second largest volume rather than the count from the third largest volume** to calculate the final concentration (colonies/100 milliliters) by multiplying the chosen count by the dilution factor. In this case, the dilution factor would be 10.
10. If the count in the **third largest volume of sample** is **outside the ideal counting range of 20-60 on the "high side" (that is, > 60)**, look at the count from the **next (fourth) largest volume**, if any, and **repeat the same steps as before** .
11. If there is **no additional (fourth largest) volume** and the count for the third largest sample was **too-numerous-to-count**, record as **TNTC, greater than 100 multiplied by the dilution factor for the third largest sample (100 in this case) or > 10,000**.

Appendix D

Quality Assurance Project Plan: Water Sampling and Testing

EPA Contract No. EPD-09-040

Work Assignment 0-01, Task 5

Quality Assurance Project Plan (QAPP)
Revised Draft

For

The National Epidemiological and Environmental Assessment of Recreational Water Study for Beaches Program

Boquerón Beach, Puerto Rico

And

Surfside Beach, South Carolina

U.S. Environmental Protection Agency (EPA)
EPA Contracting Officer Representative: Elizabeth Sams

Submitted by:
Westat Work Assignment and Project Director: Karen Della Torre
Westat Water Quality Project Leader: Robert Clickner

December 7, 2009

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1. INTRODUCTION AND OVERVIEW

1.1 Introduction

Westat is submitting this Quality Assurance Project Plan (QAPP) in fulfillment of the requirements of Work Assignment 0-01, Task 5. Under Work Assignment 0-01, Task 5,Westat conducted and completed beach water sampling from May 15, 2009 through August 2, 2009 at Boquerón Beach, Boquerón, Puerto Rico and from June 6, 2009 through September 7, 2009 at Surfside Beach, Surfside, South Carolina, followed by laboratory analyses and database development. In summary, this QAPP seamlessly covers the work performed during this task.

1.2 Background Information

In order to meet some of the requirements of the Clean Water Action Plan, the Beach Action Plan and the Beach Act of 2000, this beach study was initiated in 2003 to assist the Office of Water in formulating new health and risk guidelines for recreational water.

This study is being conducted jointly by the National Exposure Research Laboratory, Microbiological and Chemical Exposure Assessment Research Division (NERL/MCEARD), the National Health and Environmental Effects Research Laboratory (NHEERL) and the Centers for Disease Control and Prevention (CDC).

This information is being collected as part of a research program consistent with the Sec. 3(a)(v)(1) of the Beaches Environmental Assessment and Coastal Health Act of 2000 and the strategic plan for EPA's Office of Research and Development (ORD) and the Office of Water entitled "Action Plan for Beaches and Recreational Water." The Beaches Act and ORD's strategic plan has identified research on effects of microbial pathogens in recreational waters as a high-priority research area with particular emphasis on developing new water quality indicator guidelines for recreational waters. This data collection is for a series of epidemiological studies to evaluate exposure to and effects of microbial pathogens in marine and fresh recreational waters as part of the EPA's research program on exposure and health effects of microbial pathogens in recreational waters. The information collected by this study program will be used to estimate the relationship between water quality indicators and health effects. The questionnaire health data will be compared with routinely collected water quality measurements. The

analysis will focus on determining whether any water quality parameters are associated with increased prevalence of swimming-related health effects.

1.3 Study Beach Site

The study period from took place from May 15, 2009 through August 2, 2009 at Boquerón Beach, Boquerón, Puerto Rico and from June 6, 2009 through September 7, 2009 at Surfside Beach, Surfside, South Carolina. This work assignment implements field procedures for the water quality data collection at a beach study site and the follow-up telephone interviews. Westat traveled to these sites.

Boquerón, Puerto Rico Beach Contact: Eddie Nieves Santiago, Assistant Park Superintendent Boquerón Beach, PR at 787-851-1900.

Surfside, South Carolina Beach Contact: Ed Booth, Town Manager of Surfside Beach, SC at 843-913-6111.

The beach sites were selected based upon specific criteria listed below:

- The beach was an officially designated recreational area near a large population center.
- The beach generally meets the state or local water quality standards.
- The beach was contaminated by a human source of pollution.
- The beach has a large attendance (e.g., 300-400 swimmers/day)
- The age range of the swimmers was broad (i.e., includes children, teenagers, and adults).
- The swimming season was at least 90 days long.

1.4 Project/Task Organization

This study, which was necessary to meet the requirements of the Congressionally-mandated Beach Act of 2000, was administered out of the United States Environmental Protection Agency's

(USEPA) National Exposure Research Laboratory, Microbiological and Chemical Exposure Assessment Research Division (NERL/MCEARD), Cincinnati, Ohio. The project was funded by the office of Research and Development. The water quality monitoring study was conducted by Westat, and the concurrent health study was conducted and funded by the National Health & Environmental Effects Research Laboratory (NHEERL), with the assistance of Westat.

Westat assembled a team consisting of experienced Westat environmental researchers, qualified locally hired staff to collect the water samples and four laboratories to analyze the water samples. The Westat staff are described in the personnel section of this QAPP. It was necessary to use four laboratories because some of the analytical methods must be performed within eight hours of the sample collection, which required a laboratory close to the beaches where sampling took place. In addition, certain analytical procedures, for example qPCR, require special expertise and licensing, which was possessed by only relatively few laboratories. Thus, the laboratories were:

- **The University of Puerto Rico Microbiology Lab** onsite at The Department of Marine Sciences, UPR, Isla Magueyes, La Parguera, Puerto Rico, performed the Method 1600: *Enterococci* in Water by Membrane Filtration using membrane-*Enterococcus* Indoxyl-β-D-Glucoside Agar (Method 1600 membrane filtration) (EPA 821/R-02/022), froze, preparatory filtration steps of the Rapid Quantitative Polymerase Chain Reaction (QPCR), pH, turbidity, conductivity and salinity analyses on the samples collected at Boquerón, Beach. Additionally, the UPR Microbiology Lab collected shipped samples for the Cyanobateria analyses performed by Green Water Laboratory, Palatka, Florida.

- **Environmental Systems Testing Services,** Conway, South Carolina, performed the Method 1600: *Enterococci* in Water by Membrane Filtration using membrane-*Enterococcus* Indoxyl-β-D-Glucoside Agar (Method 1600 membrane filtration) (EPA 821/R-02/022), froze, preparatory filtration steps of the Rapid Quantitative Polymerase Chain Reaction (QPCR), pH, turbidity, conductivity and salinity analyses on the samples collected at Surfside, Beach.

- **Green Water Laboratory**, Palatka, Florida performed analysis of water samples collected at Boquerón Beach, Puerto Rico for the presence of Cyanobateria.

- **EMSL Analytical, Inc**., Westmont, New Jersey, completed the qPCR analyses on all samples collected at both Boquerón and Surfside Beaches.

1.5 Proficiency/Certification

There was no specific training anticipated by USEPA for the current approved membrane filter *Enterococci* method (mEI Agar; see References 7 and 8). Westat ensured that analysts were proficient in the above method and each of the rapid methods [Rapid Quantitative Polymerase Chain Reaction (QPCR) and Human Specific QPCR (See Reference 9).]. The laboratory analysts who performed the assays were proficient in each method and consulted with USEPA personnel, the technical advisors, and the manufacturers of the instruments to ensure proper knowledge and use of the analytical methods. Westat required laboratory staff to process one or two performance evaluation samples (unknowns) for all methods during the study. Westat ensured that the sub-contract laboratories followed general field and lab safety protocols throughout the study period.

1.6 Safety Protocol

Westat utilized a safety plan specific to EPD-09-040, Work Assignment 0-01.

2. DOCUMENTS AND RECORDS

2.1 Data Deliverables

Westat used sample collection/custody forms, approved by USEPA. Westat maintained sample collection/custody sheets in a binder in the laboratory where the analysis was performed. Westat's laboratories initially recorded membrane filter count data and lot number on field or laboratory worksheets, then entered and saved data electronically in an Excel spreadsheet. Microsoft Word was used for the text in reports. The worksheets, database format, data sheets, or spreadsheets for this method and all other methods in this study were approved by the USEPA. A separate spreadsheet file was employed for each day of sample collection. The file name incorporated the dates or range of dates of collection. Separate database forms were used to record the following information:

- Field measurement data (See below),
- Sample collection time/analysis start time/incubation start and end time (See Microbiological Method Section), and
- Microbiological data for each sample/volume and associated quality control (QC) samples.

All data from each of the rapid methods (counts, C_T values, setup values, plots, growth curves, graphs, computer files, notes, statistical parameters and analyses, etc.) were recorded, saved, and submitted to USEPA in a hard copy (where applicable) and in electronic form. A copy of all computer files was sent to USEPA electronically by E-Mail with a cover letter outlining the contents of the files. Additions or changes to the worksheet were approved by USEPA.

A hard copy format of hand-entered sample collection and custody sheets, data sheets, and all other forms of data (graphs, bit maps, C_T values, setup values, plots, growth curves, notes, QC data, statistical parameters and analyses, etc.) for each sampling event was provided to the COR and WACOR no later than the Monday following each 3-day weekend period (Friday, Saturday and Sunday) of sampling each week at the beach site. Westat also entered the data electronically into the database forms/spreadsheets, and, after the analyses were completed at the beach site, the electronic file(s) were delivered to the COR and WACOR along with all other forms of data (graphs, bit maps, plots, curves, setup values, notes, QC data, statistical parameters and analysis, etc). The delivery was accompanied by a brief cover memo outlining the delivery contents by method. The memo also indicated if there were any

unusual circumstances or known problems surrounding the deliverable, such as QC problems in any of the methods.

Critical data that were reported included the bacterial counts from the membrane filter tests; the complete run files for the QPCR method, including C_T and setup values, positive and negative controls, etc; the complete run files. All membrane filter plates were examined and counted [If possible, plates with up to 200 colony forming units (CFUs) were to be considered countable, although the ideal number of CFUs is 20-80.], and the results for all plates were reported, including zeros and those "too-numerous-to-count" (TNTC). An estimation procedure for TNTC plates were provided to the laboratory by USEPA. The estimation data from the TNTC plates (*i.e.*, the five counts from five squares on each filter) was submitted to USEPA along with the count data for the other samples. QC data was reported with the sample data for all methods.

In addition to the water samples that were collected, ancillary data was collected for each sampling visit. These are shown in Table 1. Certain ancillary data items were entered using specific measuring instruments. Table 2 describes the measuring instruments, including model, range accuracy and calibration procedures. All field data was entered with permanent non-running ink. In addition to the items detailed in Table 1 and 2, any items/activities specific to the beaches were added.

Any QC results associated with the collection of ancillary data were also reported (QC samples were specified in Methods/SOPs describing ancillary data collection methods).

Table 1. Measurements to be recorded at/for each sampling visit

Measurement	Description	Units/Format	MQOs
Date and Time	Date and Time of day	Mm/dd/yy; hh:mm	±5 minutes
Air temperature	Measured by thermometer at a fixed location every visit	°C	±1°
Water temperature	Measured by thermometer at a fixed sampling location at appropriate depth for thermometer on every visit	°C	±1°
Cloud Cover	Sunny, Mostly Sunny (20-50% cloud cover), Cloudy (50-70% cover) Mostly Cloudy (70-99% cover), Overcast	S, MS, C, MC, O	Field Person or Team Consensus
Rainfall	Measured by rain gauge near sampling area; collected each day at time of sampling and any time rain is known to have occurred at the beach since the last measurement was taken. Current conditions such as rain, lightning, hail, etc. noted	Rain in inches; other observations noted in comments field	±0.25 Inches
Wind speed	Sustained speed measured by wind gauge; gusts indicated in comments fields	Miles per hour	± 5 mph
Wind direction	Compass direction to nearest semi-quadrant leeward measured on wind gauge	N, NE,E, SE, S, SW, W, or NW	Recorders judgement
Current Direction	Described in relation to shoreline facing out	Descriptive (onshore, right, etc.)	Field Person or Team Consensus
Wave height, if applicable	Meter stick measurement at central sampling point. This is the distance from the low point (trough) to the high point (peak) of the wave	Meters	±0.2 M
Bather density	Number of bathers in the water, in the sampling area, and number of "bathers" on beach, within outer transects to edge of beach on land side	Categorical; <20, 20-100, 100-200, >200	Field Person or Team Consensus
Boats	Number/approximate number of boats in the water, within approximately 500 M of sampling area	Categorical; None, 1-5, 5-10, 10-20, 20-30, etc., etc.	Field Person or Team Consensus
Animals/Birds	Animals and birds potentially affecting the water (within approximately 20M of the sampling area in the water or laterally within 20M of the outer transects on the beach); also includes number of fowl or other birds in the air near the sampling area	Types of Animals, Numbers of Animal Types on beach and in water	Field Person or Team Consensus
Debris	Description of any debris floating in the water or washed on shore within the bathing area	Categorical; "None," "Very Little," "Little," "Lots," describe types	Field Person or Team Consensus

Table 1. Measurements to be recorded at/for each sampling visit (continued)

Measurement	Description	Units/Format	MQOs
pH	Each sample measured after microbiological analysis processing, per "Standard Methods" (3) or equivalent. *Equipment utilized for this measurement must be preapproved by WACOR or COR	pH units	± 0.2 units
Turbidity	Each sample measured by nephlometer after microbiological analysis processing, per Standard Methods (3) or equivalent *Equipment utilized for this measurement must be preapproved by WACOR or COR	Nephlometric Turbidity Units (NTUs)	Range dependent; see Standard Methods 2130B
Salinity	Each sample measured after microbiological analysis processing, per "Standard Methods" (3) or equivalent and Measured on site concurrently with temperature and air current	Parts per thousand	Field Person
Conductivity	Each sample measured after microbiological analysis processing, per "Standard Method" (3) or equivalent	microSiemens or milliSiemens as appropriate	Field Person
UV Reading	Measured by UV device	Units/Format	MQOs
Geographical Position	GPS Unit Coordinates were taken in 3 places for each of the 3 transects. Total of 9 positions for each sample run (8:00 Am, 11:00 AM, 3:00 PM)	Lat/Long	Field Person or Team Consensus
Swim Advisory Flags	Flags put on beach by lifeguards or other official to indicate if swimming is advised, cautioned against, or unallowed for bacteria levels, weather, or roughness of water. Usually Green for Safe, Yellow for Advisory, and Red for Unsafe/Not Allowed	Indicate if advisory is due to bacteria, weather, or roughness of water.	Field Person

Table 2. Measurements recorded at/for each sampling visit and equipment used

Measurement	MQOs	Instrument	Calibration
Air temperature	±1°	Kestrel 4000 Pocket Weather Tracker Range: -29 to 70°C Accuracy: +/-1°C	Factory calibrated: Temperature response of unit was verified in comparison with a Eutechnics 4600 Precision Thermometer or a standard Kestrel 4000 Pocket Weather Tracker calibrated weekly with the Eutechnics 4600.
Water temperature	±1°	YSI Model 30/30M Range: -5 to 95°C Accuracy: +/- 0.1°C	Factory calibrated. Calibration checked and adjusted weekly in field according to manufacturer's protocol using 50 mS/cm calibration solution.
Rainfall	± 0.25 Inches	Cole-Parmer 03319-00 Range: 0.00 to11.00 in Accuracy: +/- 0.01 in	Rain measured in graduated cylinder calibrated during manufacturing. No further calibration required.
Wind speed	± 5 mph	Kestrel 4000 Pocket Weather Tracker Range: 0.8 to 89.0 Accuracy: 3% of reading	Factory calibrated: The impeller installed in the unit was individually tested in a subsonic wind tunnel operating at approximately 6.1 m/s monitored by a Gill Instruments Model 1350 ultrasonic time-of-flight anemometer. Low-speed function of impeller further verified following wind tunnel testing.
Salinity	Not specified	YSI Model 30/30M Range: 0 to 80 ppt Accuracy: +/-2%, or +/-0.1ppt	Factory calibrated. Calibration checked and adjusted weekly in field according to manufacturer's protocol using 50 mS/cm calibration solution.
Conductivity	Not specified	YSI Model 30/30M Range: 0 to 49.99 mS Accuracy: +/-0.5%	Factory calibrated. Calibration checked and adjusted weekly in field according to manufacturer's protocol using 50 mS/cm calibration solution.
UV Reading	Not specified	UVP, Inc. UVX Radiometer Range: 0 to 1999µW/cm^2 Accuracy: +/-5%	Calibrated according to manufacturer's recommendations. Both the sensor and the radiometer are calibrated every year, prior to the start of sampling. Equipment is shipped to manufacturer for calibration.
Geographical Position	Not specified	Garmin GPS 76 Accuracy: < 15 m	Factory calibrated. Time zone set for specific beach.

Photographic Data

To aid researchers in determining conditions at the beach that may not be readily apparent from the ancillary data recorded, photographs of the sample locations at the beach area were taken for the record at least once a day during the sampling period at the beach site. While the work assignment required taking photographs only once a day, Westat took them at every sample collection, as in past years, because the conditions on the beach changed substantially over the course of the day from 8:00 am to 3:00 pm. Photographs were of sufficient quantity and quality to estimate the number of bathers on the beach and in the water.

Photos were taken with a digital camera. They were labeled with the beach name, date, target sample collection time, and actual time of photograph. The camera was configured to have the date (and time, if possible) displayed on the image. The beach name, target sample time, and actual time of photograph (when unable to configure camera to display on image) were part of the image name. Submission of photographs to the WACOR or COR occurred at the end of the sampling period for the beach via appropriate means, expected to be delivery of a CD-ROM with the digital photographs.

Weather Station Data

EPA and Westat setup HOBO Weather Stations at suitable locations near the beach sites to automatically collect data on sequential weather parameters. At Boquerón Beach, the weather station was initially setup and tested by EPA Athens on a handicap access ramp on the beach (Figure 1a). However, EPA moved the weather station prior to the start of the study because the location on the ramp was easily accessible to potential vandals. EPA Athens relocated the weather station to the top of a lifeguard stand located on the beach (Figure 1b). Shortly after the study began, Westat reported that some of the parameters measured by the Boquerón weather station were not being recorded or were out of normal range. After a series of attempts to fix the weather station, Westat set-up an additional weather station on the opposite side of the lifeguard stand (Figure 1c).

Prior to setting up another weather station, Westat made a series of adjustments and replacements in attempt to alleviate the problematic weather station parts that were not measuring and recording the data properly. All attempts made to correct the problems were reported to EPA and methods were developed in consortium with EPA.

On Thursday, June 4, 2009 Westat recognized that rain and barometric pressure data were not being measured by the device. As a first attempt at troubleshooting the problem, Westat instructed field staff as well as local Westat tech support staff to check all connections to and from the instrument to verify that this was not the problem. Westat field staff reported that the cable connections were intact, and the device was still not measuring rain and barometric pressure. EPA informed Westat that the weather station manufacturer said that the faulty readings were likely due to bad sensors. EPA suggested turning switching out the rain and barometric pressure sensors with replacement parts belonging to EPA and located in the storage facility on site.

On June 11, 2009 Westat instructed field staff to locate the extra rain gauge and pressure sensor and install them on the weather station. Westat field staff located the replacement parts.

On June 25, 2009 EPA again suggested that the port that connected the sensors might have been faulty and to switch the ports in which the cables were connected before installing the replacement sensor parts. Westat instructed field staff to attempt to fix the weather station by testing the rain gauge and barometric pressure sensors in different ports and to follow up with the replacement of the rain gauge and pressure sensor if switching ports did not work.

On the June 26, 2009 the cables were tested in all ports by Westat field staff. While all other parameters were successfully recorded, the rain and barometric pressure data was not recorded. Westat staff also connected the replacement EPA rain gauge and barometric pressure sensor and found that still neither parameter was recorded. After testing all of the possibilities, Westat sent another replacement rain gauge and pressure sensors as well as a replacement logger (that were not in use) from the South Carolina field office to Boquerón, PR. The Westat replacement rain gauge and barometric pressure sensors were connected.

On June 28, 2009 Westat field staff forwarded weather station data to Westat home office which indicated that data for rain and barometric pressure was still not being measured.

On July 9, 2009 Westat field staff examined the weather station again and observed loose connections in the data logger box. A photograph was taken and sent it to Westat home office. EPA replied with a photo of where the cables should be connected on the instrument. The next day, field staff attempted to fix the rain gauge and barometric pressure sensors by connecting to an additional logger. Westat staff noted that the logger was not measuring rain due to the fact that the cable came out once the

door was closed. The cable was replaced and the Westat logger was measuring rain and barometric pressure data.

On July 11, 2009 Westat field staff checked the weather station again and found that all parameters, with the exception of UV and battery status, were not being recorded by the EPA logger. Westat's logger was reading rain data and barometric pressure.

July 13, 2009 EPA suggested a few different methods to troubleshoot the malfunctioning weather station. Westat tried each suggestion to get the device logging properly, but was unsuccessful.

July 15, 2009 Westat sent all remaining weather station parts from the South Carolina field house to Boquerón, PR. These replacement sensors were set up on the Westat logger.

July 20, 2009 Westat reported to EPA that the additional weather station was installed and all parameters were being collected.

In Surfside Beach, the weather station was setup at the end of a fishing pier just south of the sampling area (Figure 1d).

Westat collected weather parameters/units from May 15, 2009 through August 2, 2009 at Boquerón Beach, Boquerón, Puerto Rico and from June 6, 2009 through September 7, 2009 at Surfside Beach, Surfside, South Carolina.. The parameters collected include:

- Dew Point (F)
- Barometric Pressure (in Hg)
- Rain (in)
- Relative Humidity (%)
- Solar Radiation (W/m^2)
- UV Voltage (in Volts)
- Temperature (F)
- Wind Speed (mph)
- Gust Speed (mph)

- Wind Direction (compass point degrees)

Westat's weather station was set to observe conditions at 1 minute intervals and to record data at 10 minute intervals.

Figure 1a. View of the initial weather station setup at Boquerón Beach, PR

Figure 1b. Relocation of the weather station, Boquerón Beach

Figure 1c. Westat's setup of an additional weather station

Figure 1d. Surfside Beach weather station on fishing pier

General Laboratory Quality Control Records

Laboratories were expected to maintain records of general laboratory quality control activities, such as are described in this QAPP and the references. Such records may become deliverables upon an amendment to the work assignment.

Data Formats

The exact format of all data fields were approved by USEPA prior to data collection. Formats were based on those specified for this project in the work assignment, in the references, or in the forms recommended by the manufacturers of the instruments. Where possible, database/spreadsheet templates had fields preformatted.

Quality Assurance Plan and Revisions

All project personnel received copies of the most current version of the Quality Assurance Project Plan (QAPP) prior to dry run.

Other Records

Various other documents and records (*e.g.*, SOPs, reports, method validation records, laboratory QC, and maintenance records) are discussed in this document in appropriate sections. The USEPA reserves the right to request copies of any documents and records from Westat that could affect this project. Any records that are received, and any records generated by Westat became part of the overall project file.

3. DATA GENERATION AND ACQUISTION

3.1 Overview of Sample Collection for Microbiological Analyses

Before beginning sample collection at the beach sites, the sampling locations were identified. These locations were in waist-high water (1 meter deep), shin-high water (0.3 meters deep), and in wet-sand along pre-selected transects. The transects were lines perpendicular to the shoreline, selected to represent the water areas frequented by the beachgoers. At both Boquerón and Surfside Beach there were three transects, with shin-high, waist-high, and wet-sand samples along each transect, for a total of nine sampling locations (Figure 2a).

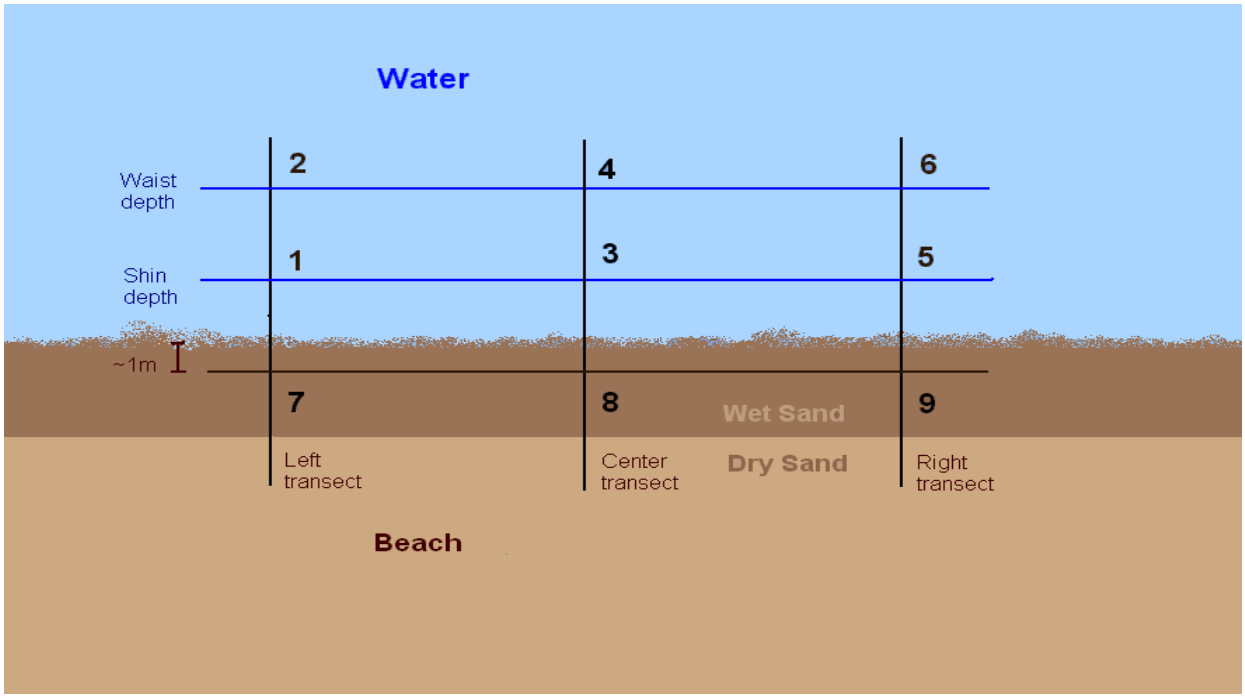


Figure 2a. Sample transects schematic

At Boquerón Beach, Westat collected beach water samples on specified weekend (Saturday and Sunday) sampling days during the study period of May 16th, 2009 through August 2nd, 2009, including two weekday holidays (Mondays), May 25th, 2009 and July 27th, 2009 for microbiological analysis by three methods [the current approved membrane filter (MF) *Enterococci* method (mEI Agar), Rapid Quantitative Polymerase Chain Reaction (QPCR), and for Cyanobacteria analysis].

At Surfside Beach, Westat collected beach water samples on specified weekend (Saturday and Sunday) sampling days during the study period of June 6, 2009 through Sept. 7, 2009, including two weekday holidays, Friday July 3, 2009 and Monday, Sept. 7, 2009 for microbiological analysis by two methods [the current approved membrane filter (MF) *Enterococci* method (mEI Agar), Rapid Quantitative Polymerase Chain Reaction (QPCR)].

Individual Water Samples

Three times a day, at 8:00 AM, 11:00 AM, and 3:00 PM, water samples were collected at both beach sites along each of the three transects perpendicular to the beach shoreline, one in waist-high water (1 ml deep) and one in shin-high water (0.3 m deep), for a total of 18 samples collected per day (*i.e.*, 6 grid locations x three times per day). See Figure 2b, which depicts the water sampling scheme. Water samples were collected at the points numbered 1 through 6. The location of the transects were at least 20 meters apart or more, if the area used by the swimmers encompasses more than a total of 60 meters of shoreline. It was intended that samples were collected on the scheduled dates, but other dates may have been substituted if rainfall or other problems prevented swimmers from going to the beach, prevented water sampling, or created hazardous conditions for the field personnel. Sample collectors notified the WACOR or COR of adverse weather conditions or other problems and requested guidance whether to begin or continue sampling on a given day or weekend. This was necessary because the samples needed to be collected when there were sufficient bathers at the beach to allow NHEERL to conduct their concurrent epidemiological/health study.

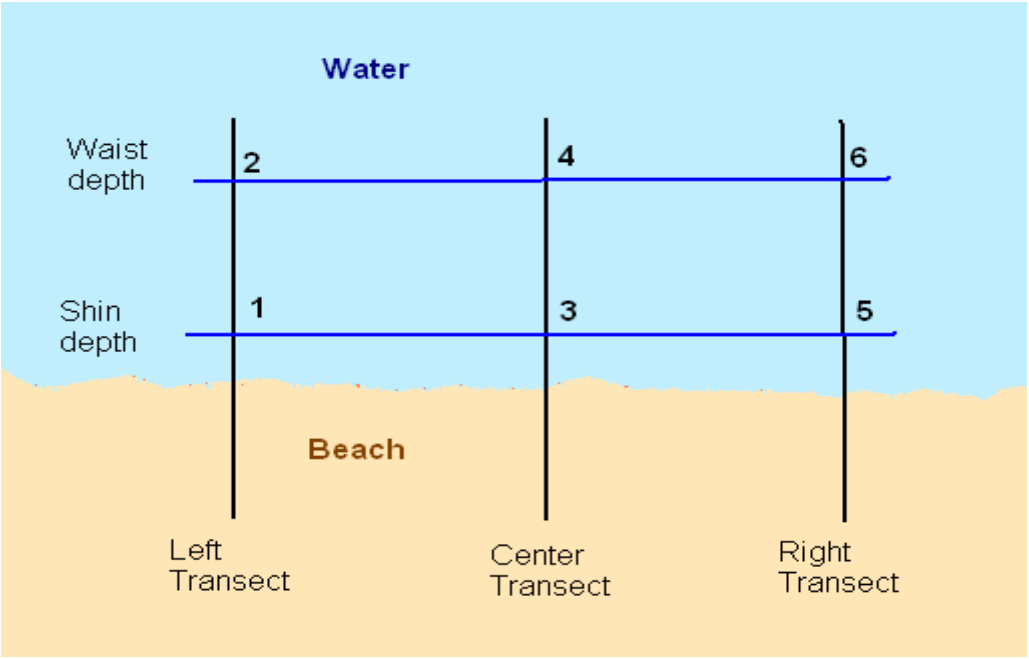


Figure 2b. Water sampling locations

Composite Samples

At both beach sites, additional samples were collected at the same six points and at the same three times of day as the beach water individual samples. These additional samples were used to develop composite samples. One plastic 1-liter bottle was collected at each of the six locations on the grid in Figure 2b for microbial analysis. At the lab these samples were combined to create a composite sample, as described in the "Compositing Samples Protocol" and briefly here:

- Two 300 mL portions from the 3 bottles collected at points 1, 3, and 5 were combined to form two shin composite samples, one for MF and one for PCR.

- Two 300 mL portions from the 3 bottles collected at points 2, 4, and 6 were combined to form two waist composite samples, again, one for MF and one for PCR.

- 150mL from each bottle collected at points 1-6 was combined to form two total composite samples, one for MF and one for PCR.

It was intended that samples were collected on the scheduled dates, but other dates may have been substituted if rainfall or other problems prevented swimmers from going to the beach, prevented water sampling, or created hazardous conditions for the field personnel. Sample collectors notified the WACOR or COR of adverse weather conditions or other problems and requested guidance whether to begin or continue sampling on a given day or weekend. This was necessary because the samples needed to be collected when there were sufficient bathers at the beach to allow NHEERL to conduct their concurrent epidemiological/health study.

Cyanobacteria Samples

At Boquerón Beach only, Westat collected water samples in pre-prepared polypropylene bottles for Cyanobacteria analysis. Specifically, samples were collected from the waist depth location at each of three transects (sampling points 2, 4, and 6, see Figure 2c) at the 11AM sampling time on weekend and holiday water sampling days. Nine bottles total were filled. At each of the three sites the following bottles were filled: two 237 ml bottles, and one 40 ml bottle. At the local lab, 1% Lugols iodine was added to the 40mL bottle as a preservative. The water collectors completed documentation of sample collection in an EPA-provided sample log. Samples were refrigerated or kept on ice within 30 minutes of collection and until they were shipped to Green Water Laboratories in Palatka, Florida.

As with the other water samples, it was intended that the cyanobacteria samples were collected on the scheduled dates, but other dates may have been substituted if rainfall or other problems prevented swimmers from going to the beach, prevented water sampling, or created hazardous conditions for the field personnel. Sample collectors notified the WACOR or COR of adverse weather conditions or other problems and requested guidance whether to begin or continue sampling on a given day or weekend. This was necessary because the samples were collected when there were sufficient bathers at the beach to allow NHEERL to conduct their concurrent epidemiological/health study.

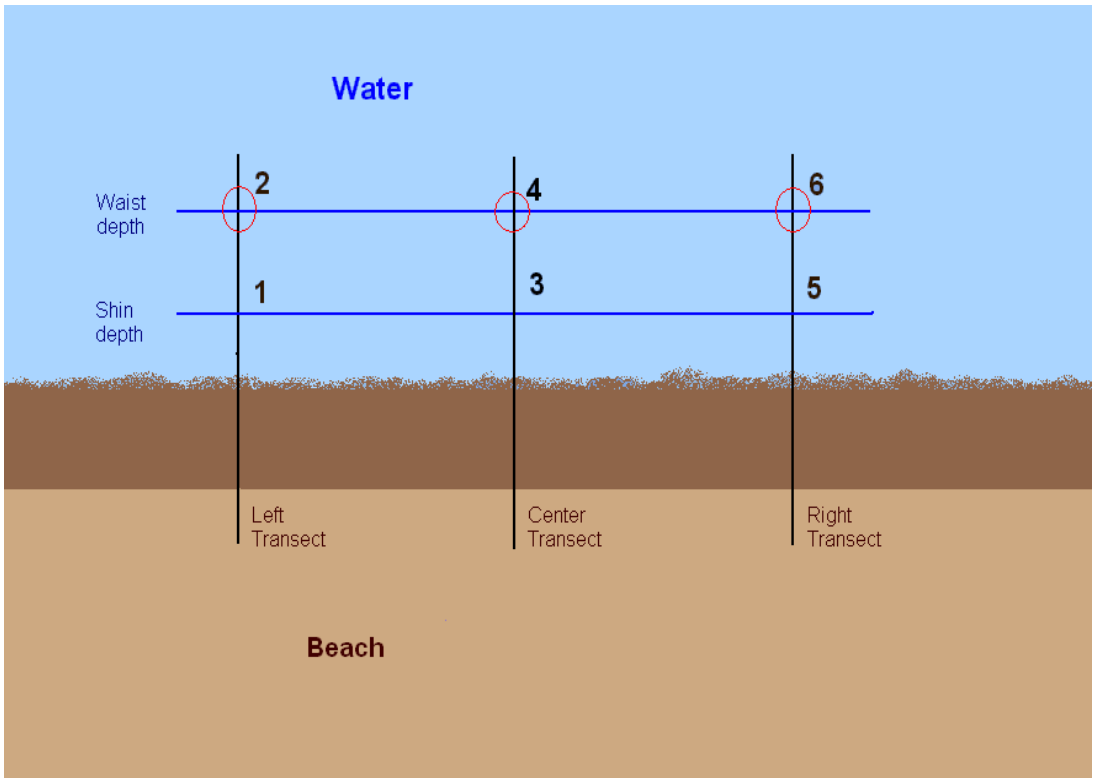


Figure 2c. Water sampling locations for Cyanobacteria at 11:00 am.

Sand Samples:

Westat collected three beach sand samples per day on Saturdays, Sundays, and on weekday holidays [Monday May 25, 2009 and Monday July 27, 2009 for Boquerón Beach and Friday July 3, 2009 and Monday September 7, 2009 for Surfside Beach] for both beach sites, for microbiological analysis by two methods [the current approved membrane filter (MF) *Enterococci* method (mEI Agar) and Rapid Quantitative Polymerase Chain Reaction (QPCR)]. These samples were taken in the designated area of the beach associated with the water quality samples. Westat employed a "scoop" method to collect the samples.

Westat collected three sand samples per day at 8:00 AM along with the 8:00 AM water samples. Samples of wet sand were collected 1 meter from the lowest water level (when the waves receded from the shoreline) at the same 3 transects where water samples were collected. See Figure 2d, which is a schematic that shows the collection of the water and sand samples at 8:00 am. Sand samples were collected at points 7, 8 and 9. When the sand was not wet at 1 meter from the water, the sand collection location was moved the shortest possible distance toward the water to a location where the sand

was wet. Westat recorded the actual distance from the water. Global Positioning System (GPS) readings of the actual sand collection locations and a photo of the sample collection sites were taken.

As with the water samples, it was intended that sand samples were collected on the scheduled dates, but other dates may have been substituted if rainfall or other problems prevented swimmers from going to the beach, prevented water sampling, or created hazardous conditions for the field personnel. Sample collectors notified the WACOR or COR of adverse weather conditions or other problems and requested guidance whether to begin or continue sampling on a given day or weekend. This was necessary because the samples were collected when there were sufficient bathers at the beach to allow NHEERL to conduct their concurrent epidemiological/health study.

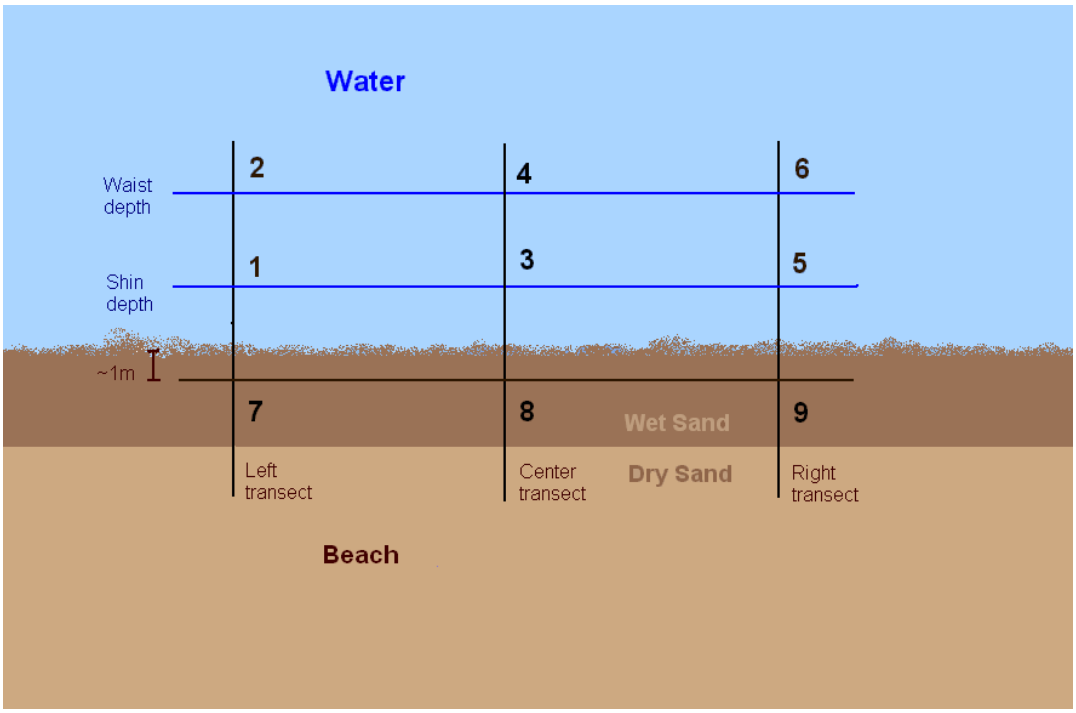


Figure 2d. Sand sampling locations (points 7, 8 and 9) at 8:00 am

Table 3 details the number of samples collected and Table 4 details the number of samples analyzed at Boquerón Beach. Table 5 details the number of samples collected and Table 6 details the number of samples analyzed at Surfside Beach. Any variance in the number of samples collected is due primarily to weather-related complications that prevented the collection of samples during the planned collection times. These situations are documented in the following tables.

Table 3: Boquerón Beach sampling schedule: Number of samples collected by type of analysis

				Water Samples						
Weekend	**Day**	**Date**	**Day**	**MF**	**PCR**	**Composite**	**Cyano**	**Sand samples**	**Total**	**Notes**
1	1	5/16/2009	Sat	18	18	18	9	3	66	
	2	5/17/2009	Sun	18	18	18	9	3	66	
2	3	5/23/2009	Sat	18	18	18	9	3	66	
	4	5/24/2009	Sun	18	18	18	9	3	66	
	5	* 5/25/2009	Mon	18	18	18	9	3	66	
3	6	5/30/2009	Sat	18	18	18	9	3	66	
	7	5/31/2009	Sun	18	18	18	9	3	66	
4	8	6/6/2009	Sat	18	18	18	9	3	66	
	9	6/7/2009	Sun	18	18	18	9	3	66	
5	10	6/13/2009	Sat	18	18	18	9	3	66	
	11	6/14/2009	Sun	18	18	18	9	3	66	
6	12	6/20/2009	Sat	18	18	18	9	3	66	
	13	6/21/2009	Sun	18	18	18	9	3	66	
7	14	6/27/2009	Sat	18	18	18	9	3	66	
	15	6/28/2009	Sun	18	18	18	9	3	66	
8	16	7/4/2009	Sat	18	18	18	9	3	66	
	17	7/5/2009	Sun	18	18	18	9	3	66	
9	18	7/11/2009	Sat	18	18	18	9	3	66	
	19	7/12/2009	Sun	18	18	18	9	3	66	
10	20	7/18/2009	Sat	18	18	18	9	3	66	
	21	7/19/2009	Sun	18	18	18	9	3	66	
	22	*7/20/2009	Mon	6	6	6	-	3	21	8AM Sample Collected Only
11	23	7/25/2009	Sat	18	18	18	9	3	66	
	24	7/26/2009	Sun	18	18	18	9	3	66	
	25	* 7/27/2009	Mon	18	18	18	9	3	66	
12	26	8/1/2009	Sat	18	18	18	9	3	66	
	27	8/2/2009	Sun	18	18	18	9	3	66	
Totals				**468**	**468**	**468**	**234**	**78**	**1,737**	

MF = Membrane Filtration Enterococci Method
PCR = Polymerase Chain Reaction Method
Composite = Composite Samples (generated from beach water samples)
Cyano= Cyanobacteria
Sand Sample = Sand Samples
* Indicates a weekday sample

Table 4: Number of samples analyzed at Boquerón Beach

		Beach Water (separate samples were collected)		Beach Composite (collected sample was split)		Sand Sample (collected sample was split)	
Weekend	**Date**	**MF**	**PCR**	**MF**	**PCR**	**MF**	**PCR**
1	5/16/2009	18	18	9	9	3	3
	5/17/2009	18	18	9	9	3	3
2	5/23/2009	18	18	9	9	3	3
	5/24/2009	18	18	9	9	3	3
	*5/25/2009	18	18	9	9	3	3
3	5/30/2009	18	18	9	9	3	3
	5/31/2009	18	18	9	9	3	3
4	6/6/2009	18	18	9	9	3	3
	6/7/2009	18	18	9	9	3	3
5	6/13/2009	18	18	9	9	3	3
	6/14/2009	18	18	9	9	3	3
6	6/20/2009	18	18	9	9	3	3
	6/21/2009	18	18	9	9	3	3
7	6/27/2009	18	18	9	9	3	3
	6/28/2009	18	18	9	9	3	3
8	7/4/2009	18	18	9	9	3	3
	7/5/2009	18	18	9	9	3	3
9	7/11/2009	18	18	9	9	3	3
	7/12/2009	18	18	9	9	3	3
10	7/18/2009	18	18	9	9	3	3
	7/19/2009	18	18	9	9	3	3
	7/20/2009	6	6	3	3	3	3
11	7/25/2009	18	18	9	9	3	3
	7/26/2009	18	18	9	9	3	3
	*7/27/2009	18	18	9	9	3	3
12	8/1/2009	18	18	9	9	3	3
	8/2/2009	18	18	9	9	3	3
	Total	**474**	**474**	**237**	**237**	**81**	**81**

Beach Water = Water Samples
Beach Composite = Composite Samples (generated from beach water samples)
Sand Sample = Sand Samples
Cyanobacteria = Remote Analysis of Cyanobacteria
MF = Membrane Filtration Enterococci Method
PCR = Polymerase Chain Reaction Method

Table 5: Surfside Beach sampling schedule: Number of samples collected by type of analysis

				Water Samples						
Weekend	Day	Date	Day	MF	PCR	Composite	Cyano	Sand samples	Total	Notes
1	1	6/6/2009	Sat	-	-	-	NA	-	0	All Collections Cancelled- Weather
	2	6/7/2009	Sun	18	18	18	NA	3	57	
2	3	6/13/2009	Sat	18	18	18	NA	3	57	
	4	6/14/2009	Sun	18	18	18	NA	3	57	
3	5	6/20/2009	Sat	18	18	18	NA	3	57	
	6	6/21/2009	Sun	18	18	18	NA	3	57	
4	7	6/27/2009	Sat	18	18	18	NA	3	57	
	8	6/28/2009	Sun	18	18	18	NA	3	57	
5	9	*7/3/2009	Fri	18	18	18	NA	3	57	
	10	7/4/2009	Sat	18	18	18	NA	3	57	
	11	7/5/2009	Sun	18	18	18	NA	3	57	
6	12	7/11/2009	Sat	18	18	18	NA	3	57	
	13	7/12/2009	Sun	18	18	18	NA	3	57	
7	14	7/18/2009	Sat	18	18	18	NA	3	57	
	15	7/19/2009	Sun	18	18	18	NA	3	57	
8	16	7/25/2009	Sat	18	18	18	NA	3	57	
	17	7/26/2009	Sun	18	18	18	NA	3	57	
9	18	8/1/2009	Sat	18	18	18	NA	3	57	
	19	8/2/2009	Sun	12	12	12	NA	3	39	3PM Collection Cancelled- Weather
10	20	8/8/2009	Sat	18	18	18	NA	3	57	
	21	8/9/2009	Sun	18	18	18	NA	3	57	
11	22	8/15/2009	Sat	18	18	18	NA	3	57	
	23	8/16/2009	Sun	18	18	18	NA	3	57	
12	24	8/22/2009	Sat	12	12	12	NA	3	39	3PM Collection Cancelled- Weather
	25	8/23/2009	Sun	18	18	18	NA	3	57	
13	26	8/29/2009	Sat	18	18	18	NA	3	57	
	27	8/30/2009	Sun	18	18	18	NA	3	57	
14	28	9/5/2009	Sat	18	18	18	NA	3	57	
	29	9/6/2009	Sun	18	18	18	NA	3	57	
	30	* 9/7/2009	Mon	18	18	18	NA	3	57	
Totals				**510**	**510**	**510**	**NA**	**87**	**1,617**	

MF = Membrane Filtration Enterococci Method
PCR = Polymerase Chain Reaction Method
Composite = Composite Samples
Cyano= Cyanobacteria
Sand Sample = Sand Samples
* Indicates a weekday sample

Table 6: Number of samples analyzed at Surfside Beach

		Beach Water (separate samples were collected)		**Beach Composite** (collected sample was split)		**Sand Sample** (collected sample was split)	
Weekend	**Date**	MF	PCR	MF	PCR	MF	PCR
NA	6/1/2009 Dry Run	18	18	9	9	3	3
1	6/6/2009	-	-	-	-	-	-
	6/7/2009	18	18	9	9	3	3
2	6/13/2009	18	18	9	9	3	3
	6/14/2009	18	18	9	9	3	3
3	6/20/2009	18	18	9	9	3	3
	6/21/2009	18	18	9	9	3	3
4	6/27/2009	18	18	9	9	3	3
	6/28/2009	18	18	9	9	3	3
5	7/3/2009	18	18	9	9	3	3
	7/4/2009	18	18	9	9	3	3
	7/5/2009	18	18	9	9	3	3
6	7/11/2009	18	18	9	9	3	3
	7/12/2009	18	18	9	9	3	3
7	7/18/2009	18	18	9	9	3	3
	7/19/2009	18	18	9	9	3	3
8	7/25/2009	18	18	9	9	3	3
	7/26/2009	18	18	9	9	3	3
9	8/1/2009	18	18	9	9	3	3
	8/2/2009	12	12	6	6	3	3
10	8/8/2009	18	18	9	9	3	3
	8/9/2009	18	18	9	9	3	3
11	8/15/2009	18	18	9	9	3	3
	8/16/2009	18	18	9	9	3	3
12	8/22/2009	12	12	6	6	3	3
	8/23/2009	18	18	9	9	3	3
13	8/29/2009	18	18	9	9	3	3
	8/30/2009	18	18	9	9	3	3
14	9/5/2009	18	18	9	9	3	3
	9/6/2009	18	18	9	9	3	3
	* 9/7/2009	18	18	9	9	3	3
	Total	**528**	**528**	**264**	**264**	**90**	**90**

Beach Water = Water Samples
Beach Composite = Composite Samples (generated from beach water samples)
Sand Sample = Sand Samples
MF = Membrane Filtration Enterococci Method
PCR = Polymerase Chain Reaction Method

3.2 Determination of Transects

Boquerón Beach, Puerto Rico

Figure 3 is a schematic diagram of Boquerón Beach that identifies the transect locations and corresponding sampling points (numbered as 1-9). The sampling area is defined as points located in the area within the wheelchair water access structure (far left, when standing on the shore and facing the ocean) and the long pier (far right, when standing on the shore facing the ocean). Each transect was identified by the alignment of two permanent structures. Samples 1, 3 and 5 were the shin-depth water sampling locations. Samples 2, 4, and 6 were the waist-depth sampling locations. Samples 7, 8, and 9 were the sand sampling locations. The left transect was the left-most sampling point when standing on the shore and facing the water. Samples 1, 2, and 7 were taken at the left transect. The right transect was the right-most sampling point when standing on the shore and facing the water. Samples 5, 6, and 9 were taken at the right transect. The center transect was in between the left and right transects. Samples 3, 4, and 8 were taken at the center transect.

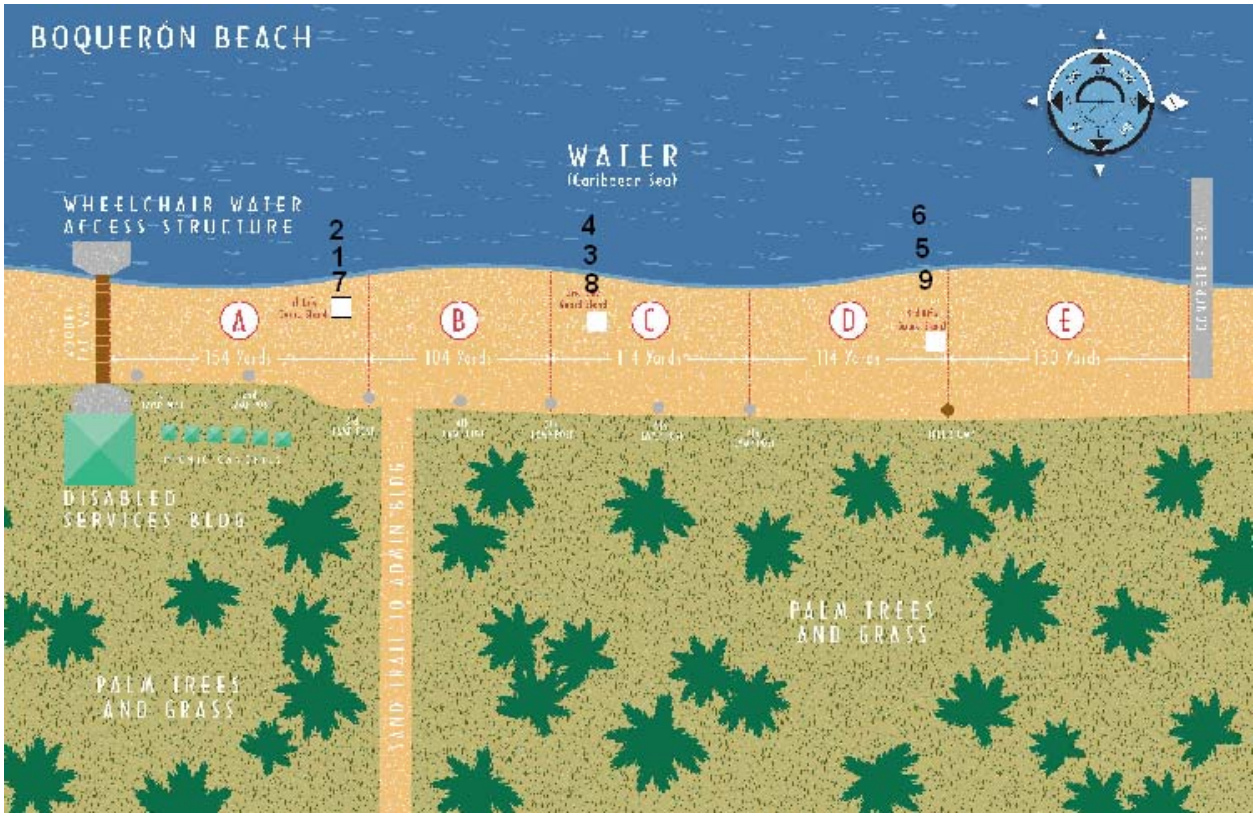


Figure 3: Schematic of Boquerón Beach

Left Transect – Samples 1, 2 and 7

The left transect was located approximately N 18.01938 and W -67.17223. The structures used to identify the transect were the furthest most left lifeguard stand (red circle) and a palm tree (yellow circle) that was located behind it, as shown in Figure 4a, below. The sample point was defined by the alignment of the center of the ramp on the lifeguard stand with the palm tree so that the palm tree was in the center of the lifeguard stand. A close-up view of the structures that defined the left transect can be viewed in Figures 4b and 4c. Figure 4d depicts the transects lined up. Sample 1 was collected in shin-deep water where these two structures appear aligned, as in Figure 4d. Sample 2 was collected in waist-deep water along the same transect. The sand sample 7 was collected one meter from the water's edge in wet sand along the same transect.

Figure 4a: Left transect (view from the water)

Figure 4b: Left transect (Palm Tree)

Figure 4c: Left transect (Lifeguard Chair Ramp)

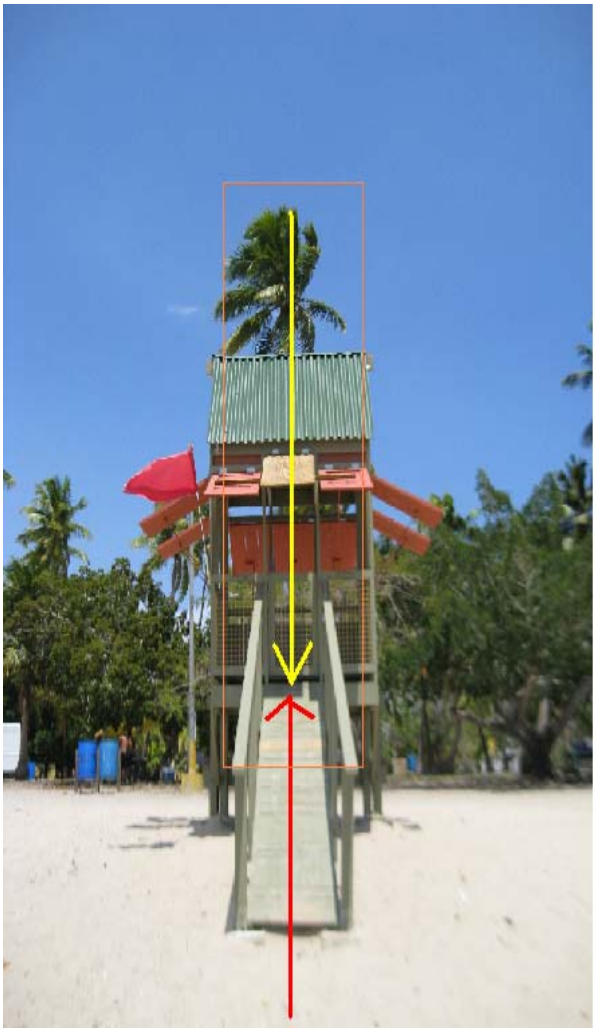


Figure 4d: Left transect (Lined Up)

Center Transect – Samples 3, 4, and 8

The center transect was located approximately N 18.02076 and W -67.17220. The structures used to identify the transect were the second or center lifeguard stand (red circle) and a palm tree (yellow circle) that was located behind it, as shown in Figure 5a, below. It was defined by the alignment of the ramp handrail of the lifeguard platform with the palm tree. The handrail that was lined up with the tree was the left-side rail (when sampler faced the lifeguard stand, and had their back to the water). A close-up view of the structures that defined the center transect can be viewed in Figures 5b and 5c. Figure 5d depicts the transects lined up. Sample 3 was collected in shin-deep water where these two structures appear aligned, as in Figure 5d. Sample 4 was collected in waist-deep water along the same transect. Sand sample 8 was collected one meter from the water's edge in wet sand along the same transect.

Figure 5a: Center transect, long view (view from water)

Figure 5b: Center transect (Palm Tree)

Figure 5c: Center transect (Handrail)

Figure 5d: Center transect (Lined-Up)

Right Transect – Samples 5, 6, and 9

The right transect was located approximately 30 feet south of N 18.02201 and W -67.17231. The transect was identified by two palm trees approximately 30 feet south of the lifeguard stand (Figure 6a) that are in a line perpendicular to the shoreline. Originally, the transect was planned to be in front a lifeguard stand, similar to the other transects. However, during training it was discovered that there were slippery rocks in the water that made collection of the water samples hazardous. As a result the transect was moved about 30 feet to avoid the rocks. The front tree is the second large tree to the right of the lifeguard stand, when viewed from the shore (Figure 6b). The rear tree is several yards behind it (Figure 6c). Figure 6d shows the two trees lined up. Sample 5 was collected in shin-deep water where these two structures appear aligned, as in Figure 6d. Sample 6 was collected in waist-deep water along the same transect. Sand sample 9 was collected one meter from the water's edge in wet sand along the same transect.

Figure 6a: Right transect (view from the water)

Figure 6b: Right transect (Front Tree)

Figure 6c: Right transect (Rear Tree)

Figure 6d: Right transect (Trees Aligned)

Surfside Beach, South Carolina

Figure 7 is a schematic diagram of Surfside Beach that identifies the transect locations and corresponding sampling points (numbered as 1-9). The sampling area was defined as points located in the area within 7th Ave N and 2nd Ave N. Each transect was identified by the alignment of two permanent structures. Samples 1, 3 and 5 were the shin-depth water sampling locations. Samples 2, 4, and 6 were the waist-depth sampling locations. Samples 7, 8, and 9 were the sand sampling locations. The left transect was the left-most sampling point when standing on the shore and facing the water. Samples 1, 2, and 7 were taken at the left transect. The right transect was the right-most sampling point when standing on the shore and facing the water. Samples 5, 6, and 9 were taken at the right transect. The center

transect was in between the left and right transects. Samples 3, 4, and 8 were taken at the center transect.

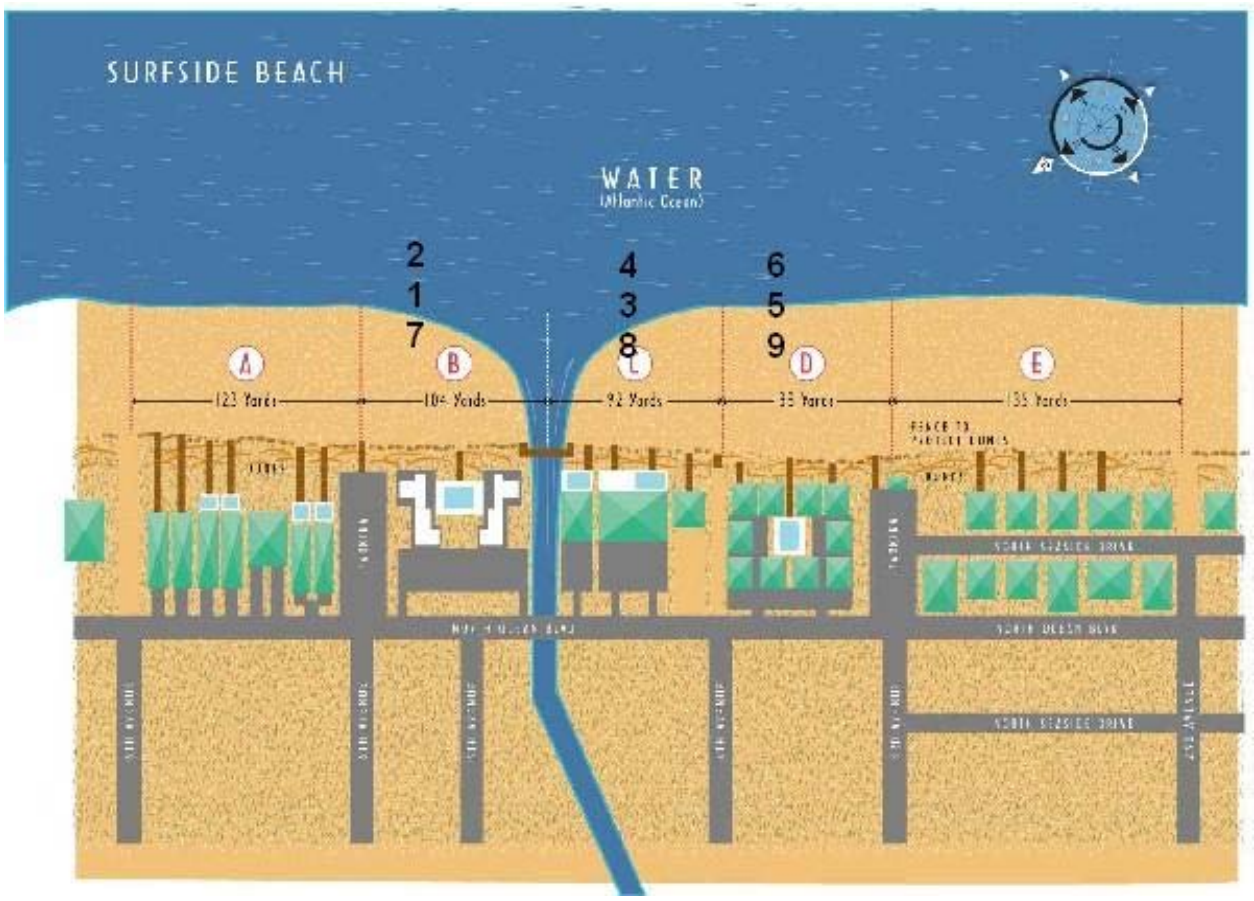


Figure 7: Schematic of Surfside Beach

Left Transect – Samples 1, 2 and 7

The left transect was located approximately **N 33.60884** and **W - 78.96704**. The structures used to identify the transect were the edge of a grey condo located closest to the 6th Ave N. entrance to of the beach (red circle) and the end of a fence located in front of the condo that blocked the sand dunes (yellow circle), as shown in Figure 8a, below. It was defined by the alignment of the far left edge of the condo (when the sampler faced the building/back to the water) ramp with the fence edge so that the fence was directly in line with the building. A close-up view of the structures defining the left transect can be viewed in Figures 8b and 8c. Figure 8d shows the transects lined up. Sample 1 was collected in shin-deep water where these two structures appear aligned, as in Figure 8d. Sample 2 was collected in waist-deep

water along the same transect. Sand sample 7 was collected one meter from the water's edge in wet sand along the same transect.

Figure 8a: Left transect (view from the water)

Figure 8b: Left transect (Edge of Building)

Figure 8c: Left transect (Fence Post)

Figure 8d: Left transect (Lined Up)

Center Transect – Samples 3, 4, and 8

The center transect was located approximately **N 33.60811** and **W-78.96767**. The structures used to identify the transect were the center of a grey condo building (red circle) and the railing of a wooden walkway (yellow circle) that was located in front of it, as shown in Figure 9a, below. The transect was defined by the alignment of the center of the condo building with the right railing of the walkway (when sampler faced the building). A close-up view of the structures that defined the center transect can be viewed in Figures 9b and 9c. Figure 9d shows the transects lined up. Sample 3 was collected in shin-deep water where these two structures appear aligned, as in Figure 9d. Sample 4 was collected in waist-deep water along the same transect. Sand sample 8 was collected one meter from the water's edge in wet sand along the same transect.

Figure 9a: Center transect, (view from water)

Figure 9b: Center transect (Center of Condo)

Figure 9c: Center transect (Handrail of Walkway)

Figure 9d: Center transect (Lined-Up)

Right Transect – Samples 5, 6, and 9

The right transect was located approximately N **33.60763 and W -78.96815**. The structures used to locate the sampling location were the corner of a yellow house (red line) and the second floor windows (yellow circle) in the grey house behind the yellow house. Both structures can be viewed in Figure 10a. The transect was defined by the alignment of the side of the yellow house so that it blocks the 2 of the 3 windows of the grey house behind it. A close-up view of the structures defining the center transect can be viewed in Figures 10b and 10c. Figure 10d shows the transects lined up. Sample 5was collected in shin-deep water where these two structures appear aligned, as in Figure 10d. Sample 6 was collected in waist-deep water along the same transect. Sand sample 9 was collected one meter from the water's edge in wet sand along the same transect.

Figure 10a: Right transect (View from the water)

Figure 10b: Right transect (Yellow House)

Figure 10c: Right transect (Rear House Windows)

Figure 10d: Right transect (Structures Aligned)

3.3 Sampling Methods

Water Samples:

See Standard Methods for the Examination of Water and Wastewater, 20th edition (1998), Section 9060, for recommendations on microbiological sampling (3). Briefly, samples were collected in waist-high (1 m deep) and shin-high (0.3 m deep) water by serially immersing (2) capped 1000-mL pre-sterilized, polypropylene bottles to the appropriate sample depth, removing the lids and allowing them to fill, raising them out of the water, and emptying them slightly to allow approximately 1 inch of head space before replacing the lids. Samples were taken about 1 foot (0.3 m) under the surface of the water in waist-high water, and shin-high samples were taken 6 inches (0.15 m) above the bottom of the water. The samples collected near the bottom were taken with care so as not to introduce additional sand/solids/debris into the samples. Sample plans were only altered in extreme or unusual circumstances. When alterations of the sample method were considered, the WACOR or COR were notified and guidance was requested. Westat utilized field protocols from Work Assignment 2-04 of the previous

contract. Such field protocols and sampling procedures were submitted to the WACOR or COR for approval prior to the beginning of the study.

Water samples were collected aseptically, as described above, at each location on the beach grids (Figure 3 for Boquerón Beach and Figure 7 for Surfside Beach) for microbial analysis:

- 1 liter was used for the membrane filter method and the ancillary measurements (which were done last to prevent contamination).
- 1 liter was used for the rapid QPCR method.
- 1 liter was used for the composite samples.
- Two 237 ml bottles and one 40 ml bottle were used for the Cyanobacteria samples at the 11:00 AM sampling period, at sampling points 2, 4, and 6 only. These samples were only collected at the Boquerón Beach site.

Following collection, all samples were placed in coolers and maintained on ice during transport and at 1 - 4° C during the time interval before they were analyzed or shipped. No additional samples were collected for the determination of pH and turbidity. These measurements were made from the same samples used for membrane filtration after these analyses were completed to prevent contamination.

Any problems encountered while sampling or while taking ancillary measurements were recorded on data collection sheets in comment fields or on additional sheets clearly having identified the date, time, sample location (on grid) and reported to the WACOR or COR if problems may have potentially affected the analytical results. In the event of problems, corrective actions taken (where possible) were documented by the field team leader, along with the results of such actions.

Sand Samples

Westat collected, transported, and processed the sand samples according to the protocol provided by EPA on April 2, 2007. Sand samples were collected with sterile, 2 inch x 10 inch stainless steel liners (AMS, American Falls, Idaho, or the equivalent). The liner was pushed into the sand at least 8 inches. The liners were sterilized at the lab by rinsing them with water, wrapping them in aluminum foil or suitable bag and heating them in the drying oven at 170°C overnight. The liners remained wrapped in the aluminum foil until use. Liners containing the sand samples were capped at both ends, placed in zip-

lock plastic bags labeled using an alpha-numeric system (See below.), and transported to the laboratory on ice. Samples were stored in a refrigerator at 4 degrees C. until analyzed.

In the laboratory, sand samples were aseptically transferred to sterile wide-mouth polypropylene bottles (500 ml or 1- liter, depending on the quantity of the sand), also labeled using the simplified version of the usual alpha-numeric labeling system. For each sand sample, 75 grams of sand was aseptically weighed out in a sterile, pre-tared, wide-mouth 500-ml bottle (using sterile spatulas), and 300 ml of Standard Methods phosphate-buffered rinse/dilution water (3), measured with a sterile graduated cylinder, was added to each bottle. Each bottle was vigorously shaken 50 times. Immediately after shaking, some of the contents of the bottle were poured into two sterile 50-ml, disposable centrifuge tubes (Corning 430829 or the equivalent) and filled to the 50-ml mark. The tubes were centrifuged for 5 minutes at ~3000 rpm (600 x g) to bring down the sand and sediment, and the supernatant was removed using a sterile pipette and placed in a sterile 100-ml polypropylene bottle for subsequent analysis by Method 1600 (Reference 8) and the Quantitative Polymerase Chain Reaction (QPCR) method (Reference 9).

The accuracy of the 50-ml mark on the disposable tubes was checked before the dry run by randomly choosing 5 tubes from the package, weighing each of the 5 tubes, and recording the weights. After 50 ml of distilled water was measured with a graduated cylinder and poured into each of the tubes, the tubes were again weighed. The weight of the distilled water (The difference between the two weights) in each tube was required to be close to 50 grams. The position of the water meniscus was observed with reference to the 50-ml mark on the tubes. In addition, 5 randomly chosen, pre-weighed tubes were filled with distilled water so that the meniscus touched the top of the 50-ml line. The tubes were weighed again and the weight of the water was determined by difference. For the mark to be accurate, the weight of the distilled water was required to be close to 50 grams. All results were recorded, and a copy of the results was sent to the WACOR and Kristen Brenner, the technical point-of-contract for sand analyses.

During the dry run, aliquots of 10 ml and 1 ml of each undiluted sand extract and 1 ml of the 10^{-1} – 10^{-6} dilutions of each extract in phosphate-buffered dilution water (3) was analyzed by EPA Method 1600 for *Enterococci*. The number of filtrations for the actual study was reduced after the normal range of concentrations in sand were determined during the dry run. Three 20-ml aliquots of each sample was filtered, and the filters were frozen, as described in the QPCR Method, during the dry run. The sand extraction method described above and the volumes used for both tests may have been adjusted,

depending on the normal range of concentrations of *Enterococci* in the extracts during the dry run. Westat obtained EPA's approval before changing the protocol or analyzing volumes.

3.3.1 Preparation

On the day prior to collection, all the EPA single-use polyethylene water collection bottles, stainless steel sand liners, the related forms, Cyanobacteria sample collection bottles (Boquerón Beach only), and other materials were pre-assembled and pre-labeled for both days of the collection weekend. These items included:

- **Bottles and sand collection containers for the Saturday 8:00 AM collection:** 18 pre-labeled polyethylene bottles and 3 pre-labeled sterilized stainless steel liners per beach.
- **Bottles for the Saturday 11:00 AM collection:** 18 pre-labeled polyethylene bottles and 9 Cyanobacteria sample collection bottles (for Boquerón Beach only).
- **Bottles for the Saturday 3:00 PM collection**: 18 pre-labeled polyethylene bottles per beach.
- **Bottles and sand collection containers for the Sunday 8:00 AM collection:** 18 pre-labeled polyethylene bottles and 3 pre-labeled sterilized stainless steel liners per beach.
- **Bottles for the Sunday 11:00 AM collection:** 18 pre-labeled polyethylene bottles and 9 Cyanobacteria sample collection bottles (for Boquerón Beach only).
- **Bottles for the Sunday 3:00 PM collection**: 18 pre-labeled polyethylene bottles per beach.
- **6 coolers per beach site** (one each for the 8:00, 11:00, & 3:00 EPA collections, one for the sand collections, one for the Cyanobacteria collections (Boquerón Beach only), and one for the icepacks)
- Filling the freezer with **icepacks**
- Labeling the 4-page **lab transmittal sheets** and **ancillary data forms**
- Checking the operation of the **Digital camera**
- Checking the **GPS device**
- Checking the **UV meter**

- Checking the **YSI 30-M Temperature/Salinity/Conductivity meter, in water-resistant bag**
- Checking the **Handheld Weather Monitor**
- Checking the **Compass**
- Checking the **Meter Rule**
- Checking the **Rubber Mallet**
- Checking the **Water Thermometer** in the waterproof pouch with a probe
- Checking the **Sterlized end caps for sand collection**
- Checking the **Collection clipboard with wax pencil**
- Checking the **Procedures Manual**
- Checking the **EPA collection log book with paper forms**
- Checking the **4 Storage containers** (one with supplies to take to the beach, and one each to hold the bottles for the Sunday 8:00, 11:00, & 3:00 collections until they can be put in the coolers after the Saturday collection)

Once all sample containers for a collection time period were labeled (see Labeling Procedures), the set of collection containers were put into their respective coolers reserved and labeled for each time period (8:00, 11:00, and 3:00). The collection bottles and sand containers for the Sunday collections were first put into storage containers, until after the Saturday collections were completed and the Sunday collection bottles and sand containers could be transferred to their correct coolers in preparation for the next day. To assist in preventing the incorrect collection of a sample in the wrong container, the containers for each time period were separated by sample points and placed in different mesh bags.

All of the collection materials and supplies such as paper towels, batteries, life vests, life saver ring, mesh bags to hold collection bottles, scuba gloves, plastic bags to put the water collection bottles into, paper ties to close the plastic bags, extra polyethylene (EPA), stainless steel liners (sand) and glass (USGS) water collection bottles were put into a large plastic storage container used to assist in transporting to the beach collection site.

3.3.2 Sample Handling and Custody

Westat utilized sample collection/custody forms modified from forms used at previous beach sites as part of the general forms. The distribution of each individual bottle taken at each location on the beach grid was documented on the custody forms.

Prior to sampling visits, tracking forms were printed by a member of the site project team. The location, date, target collection time, field staff, and information about all samples to be collected during that visit was entered on the forms, by hand (or electronically, prior to printing). The forms were printed on laminated paper suitable for field work. Each cooler used to transport samples from the site to the lab had a copy of the appropriately completed collection/custody form(s) in it or securely attached to it. Westat knew in advance how many samples could fit in a given cooler and could, therefore, prepare specific tracking sheets for each cooler prior to going to the field. When more than one cooler were needed, the coolers were labeled, and cooler labels were cross-referenced on the appropriate tracking sheet. Individual bottles for the rapid methods were distributed after the samples were logged in at the laboratory, and the custody forms were signed by each of the method analysts when portions/aliquots of the samples were removed.

Additional columns on the tracking forms include the actual collection time, the time samples arrived at the laboratory, and their storage location. Arrival time at the laboratory was indicated by entering a time for the first sample on a custody sheet, and drawing a down arrow in the lab arrival time column for the rest of the samples. The field storage location was filled in this manner.

A different form (or forms) was used to record the dates and times when analysis by QPCR and filtering began (MF and QPCR), the dates and time plates were placed in the incubator (or filters were placed in the freezer for the QPCR method), the dates and time samples were removed from incubation (or freezer for the PCR method), and the analysis results. There were spaces for associated initials for each of the sequential steps. The various "analysis" times were treated on a batch basis; *i.e.*, a sample batch was all of the samples brought to the laboratory at the same time for analysis, such as all 6 morning samples.

3.3.3 Labeling Procedures

Microbiological sample containers were labeled with water resistant sample labels. The sample bottles had IDs with consecutive numbers to facilitate handling in the laboratory and to prevent errors. However, Westat was responsible for placing the requisite additional information onto sample bottles at the time of sampling to ensure that the samples could be clearly identified. It was recommended that the information (or at least alphanumeric information, such as suggested directly below) be added just prior to or just after sampling, as this would minimize the chance of getting samples in the wrong bottles. Information added included the date, scheduled and actual time of collection, and some type of alphanumeric that identified the sampling location, and the method(s) used.

Westat used the following sample labeling scheme for all water and sand samples. This scheme is the similar to one that has been used in past years for sampling. Microbiological sample containers will be labeled with water resistant sample labels using the following alphanumeric (10-character) scheme (to avoid confusion and duplicate sample numbers):

FMMDDXXNSS

Where:

F designates the beach area. B for Boquerón Beach and S for Surfside Beach

MMDD is the date of the sample collection;
MM is the numeric month (1-12) and
DD is the day (01-31), e.g., 0614 for June 14,

XX is the planned time of day for the sample collection, as follows:

08 = 8:00 am
11 = 11:00 am
15 = 3:00 pm

N is the sample point at the beach;
1-6 for water samples and
7-9 for sand samples
(see Figure 2a)

SS is the method of analysis planned for the sample/bottle number, as follows:

01 = Membrane Filter Method 1600
02 = QPCR Methods
S1 = Sand container

C1 = Beach Sample to be Composited
5a = Cyanobacteria, bottle a (473 mL) *
5b = Cyanobacteria, bottle b (237 mL) *
5c = Cyanobacteria, bottle c (40 mL) *

* At Boquerón Beach Site Only

The following provides examples of the sample IDs that would be used for samples collected on Saturday June 20. Examples of all necessary labels are given.

Boquerón Beach, Puerto Rico

8:00 am:

Water:
MF: B062008101, B062008201, B062008301, B062008401, B062008501, B062008601

PCR: B062008102, B062008202, B062008302, B062008402, B062008502, B062008602

Sand:
B0620087S1, B0620088S1, B0620089S1

At the lab the sand samples were processed and each split into two new "children" samples that were analyzed by MF and PCR.

Beach Composite:
B0620081C1, B0620082C1, B0620083C1, B0620084C1, B0620085C1, B0620086C1

At the lab the composite samples were combined in this fashion:
Shin Composite = B0620081C1 + B0620083C1 + B0620085C1
Waist Composite = B0620082C1 + B0620084C1 + B0620086C1
TotalComposite = B0620081C1+B0620082C1+B0620083C1+B0620084C1+B0620085C1+B0620086C1

Each new child sample (8 AM shin composite, 8 AM waist composite, and 8 AM total composite) were split into 2 more children samples (total of 6) and analyzed for MF and PCR.

11:00 am:

Water:
MF: B062011101, B062011201, B062011301, B062011401, B062011501, B062011601

PCR: B062011102, B062011202, B062011302, B062011402, B062011502, B062011602

Composite:
B0620111C1, B0620112C1, B0620113C1, B0620114C1, B0620115C1, B0620116C1

At the lab the composite samples were combined in this fashion:
Shin Composite = B0620111C1 + B0620113C1 + B0620115C1

Waist Composite = B0620112C1 + B0620114C1 + B0620116C1
TotalComposite = B0620111C1+B0620112C1+B0620113C1+B0620114C1+B0620115C1+B0620116C1

Each new child sample (11 AM shin composite, 11 AM waist composite, and 11 AM total composite) were split into 2 more children samples (total of 6) and analyzed for MF and PCR.

Cyanobacteria Water Sample:
B06201125a, B06201125b, B06201125c, B06201145a, B06201145b, B06201145c, B06201165a, B06201165b, B06201165c

3:00 pm:

Water:
MF: B062015101, B062015201, B062015301, B062015401, B062015501, B062015601

PCR: B062015102, B062015202, B062015302, B062015402, B062015502, B062015602

Composite:
B0620151C1, B0620152C1, B0620153C1, B0620154C1, B0620155C1, B0620156C1

At the lab the composite samples were combined in this fashion:
Shin Composite = B0620151C1 + B0620153C1 + B0620155C1
Waist Composite = B0620152C1 + B0620154C1 + B0620156C1
TotalComposite = B0620151C1+B0620152C1+B0620153C1+B0620154C1+B0620155C1+B0620156C1

Each new child sample (3PM shin composite, 3PM waist composite, and 3PM total composite) were split into 2 more children samples (total of 6) and analyzed for MF and PCR.

Surfside Beach, South Carolina

8:00 am:

Water:
MF: S062008101, S062008201, S062008301, S062008401, S062008501, S062008601

PCR: S062008102, S062008202, S062008302, S062008402, S062008502, S062008602

Sand:
S0620087S1, S0620088S1, S0620089S1

At the lab these samples were processed and each split into two new "children" samples that were analyzed by MF and PCR.

Beach Composite:
S0620081C1, S0620082C1, S0620083C1, S0620084C1, S0620085C1, S0620086C1

At the lab the composite samples were combined in this fashion:
Shin Composite = S0620081C1 + S0620083C1 + S0620085C1
Waist Composite = S0620082C1 + S0620084C1 + S0620086C1
Total Composite = S0620081C1+S0620082C1+S0620083C1+S0620084C1+S0620085C1+S0620086C1

Each new child sample (8:00 am shin composite, 8:00 am waist composite, and 8:00 am total composite) were split into 2 more children samples (total of 6) and analyzed for MF and PCR.

11:00 am:

Water:
MF: S062011101, S062011201, S062011301, S062011401, S062011501, S062011601

PCR: S062011102, S062011202, S062011302, S062011402, S062011502, S062011602

Composite:
S0620111C1, S0620112C1, S0620113C1, S0620114C1, S0620115C1, S0620116C1

At the lab the composite samples were combined in this fashion:
Shin Composite = S0620111C1 + S0620113C1 + S0620115C1
Waist Composite = S0620112C1 + S0620114C1 + S0620116C1
Total Composite = S0620111C1+S0620112C1+S0620113C1+S0620114C1+S0620115C1+S0620116C1

Each new child sample (11:00 am shin composite, 11:00 am waist composite, and 11:00 am total composite) were split into 2 more children samples (total of 6) and analyzed for MF and PCR.

3:00 pm:

Water:
MF: S062015101, S062015201, S062015301, S062015401, S062015501, S062015601

PCR: S062015102, S062015202, S062015302, S062015402, S062015502, S062015602

Composite:
S0620151C1, S0620152C1, S0620153C1, S0620154C1, S0620155C1, S0620156C1

At the lab the composite samples were combined in this fashion:
Shin Composite = S0620151C1 + S0620153C1 + S0620155C1
Waist Composite = S0620152C1+ S0620154C1 + S0620156C1
Total Composite = S0620151C1+S0620152C1+S0620153C1+S0620154C1+S0620155C1+S0620156C1

Each new child sample (3:00 pm shin composite, 3:00 pm waist composite, and 3:00 pm total composite) were split into 2 more children samples (total of 6) and analyzed for MF and PCR.

Westat understood that sample containers could be reused after proper cleaning and resterilization or bottles, presterilized by the manufacturer, could be used. Westat only used presterilized bottles. Westat obtained a copy of the manufacturer's sterilization certificate and/or recorded for each lot was obtained. In addition, the sterility of a few randomly-chosen bottles from each lot was tested before field use by adding sterile Trypticase Soy Broth to the bottles, incubating for 48-72 hours at 35° C, and observing the bottles for bacterial growth. Prior to leaving for the field, the sample team leader checked to see that there were an appropriate number of sample bottles and sample ID labels for the sampling visit (Bottles had labels attached prior to sampling, as it was demonstrated that this had no deleterious effects on the labels.) for the sampling visit. There were extra, unlabeled sample containers, and a means to label them for back-up purposes. Copies of completed sample collection/custody sheets were provided to the WACOR or COR daily along with their associated data sheets.

3.3.4 Water Collection at the Beach

All the necessary equipment and coolers (see preparation) were brought to the beach. Then for each time collection period the appropriate cooler with the collection bottles was taken down on to the beach and used to store the empty and filled water sample bottles. The sample collection started at the 1st transect on the left-side of the beach. For each transect, the appropriate mesh bag was taken out with the correct pre-labeled bottles. To enter the water, the water collectors lined themselves up with the line-of-site markers for the transect point, and then walked in a straight line out to the appropriate water depth to collect the samples. The Supervisor marked the GPS coordinates and time for each of the six collection points while the water collectors collected the water samples. Table 3 summarizes the different samples collected at the three sampling time periods (8AM, 11AM and 3PM) for both beach sites.

Table 7: Summary of samples collected at each beach site

Type of Sample	Boquerón Beach	Surfside Beach
Sand – microbiology	3 locations 1 time (8 am) 1 cylinder, stainless steel MF, QPCR	3 locations 1 time (8 am) 1 cylinder, stainless steel MF, QPCR
Beach water – microbiology, individual samples	6 locations 3 times (8 am, 11 am, 3 pm) 2 bottles, 1 L plastic each MF, QPCR	6 locations 3 times (8 am, 11 am, 3 pm) 2 bottles, 1 L plastic each MF, QPCR
Beach water – microbiology, composite samples	6 locations 3 times (8 am, 11 am, 3 pm) 1 bottle, 1 L plastic Composite (MF, QPCR)	6 locations 3 times (8 am, 11 am, 3 pm) 1 bottle, 1 L plastic Composite (MF, QPCR)
Cyanobacteria Samples	3 locations (waist deep) 1 time (11 am) 3 bottles, prepared and supplied by GW lab, 237 mL, 237 mL, 40 mL Refrigerate and ship to GW lab	NA

Sample Collection Steps

In the water, the water collectors retrieved from the mesh bag and verified each of the IDs on the bottles before collecting a sample at each of the six sample points. Staying in-line with the line-of-site poles and transect points, the water collectors first pointed themselves into the direction of the current and took the water sample pointing into the current holding the bottle in front of and away from their body. (See Figures below for more detail).

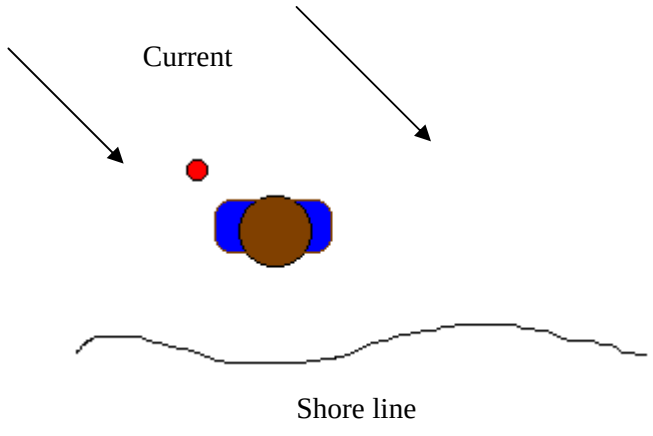


Figure 11. An example of sample collection point in water

1. The water collectors oriented and prepared themselves to collect the water samples pointing into the direction of the current, away from their body.

Figure 12. Start of collection

2. The collection bottle ID was checked for each bottle used at each sample point and the cap was slightly loosened. The cap, however, was NOT removed.

Figure 13. Submersion of bottle at shin depth

3. The bottle was submersed to a depth of approximately 6 inches for the shin level sample, and the cap removed to fill the bottle while holding the bottle perpendicular to the ocean bottom. It was important to make sure the bottle was not very close to the ocean bottom. The start time of the sample collection was recorded by the field supervisor on the Water Sample Collection Log.

Figure 14. Removal of excess water

4. Once the bottle was filled, it was recapped, and brought to the surface, to prevent surface water contamination. Then a small amount of water was removed, so that the bottle was filled to the appropriate 1-L mark at shoulder of the collection bottle.

Figure 15. Tightly recapping bottle

5. The bottle was tightly re-sealed, taking extra precautions to eliminate extended air exposure, and the filled collection bottles were placed into mesh bag, until they could be put back in the cooler.

Figure 16. Collection of waist level samples

6. After the shin samples were collected, the water collectors traveled out (staying in-line with the transect points and the line-of-site poles) to the 1 meter collection point for the waist level samples. The collection steps 1 – 5 were repeated. Again, the bottle ID was verified for the sample point before collection, and the sample was collected into the current.

Figure 17. Continuation of waist level sample collection

7. When repeating steps 1 – 5, field staff also made sure the bottle was only uncapped after being submerged to the 12 inch collection depth for waist level samples, was recapped before re-surfacing the sample (to prevent the sample from being contaminated by surface water), a portion of the sample was poured out so the sample level was close to the 1-L mark on the shoulder of the bottle, and the bottle was tightly resealed, to prevent loss of any sample collected.

8. Then steps 1 – 7 were repeated for the other shin and waist level samples for the other transect points. NOTE: When collecting the middle transect samples, the water temperature, salinity and conductivity were measured by submersing the entire probe to the collection point depth and recording the temperature reading of the water during the sample collection. The average wave height was also estimated and recorded between the middle sample points.

3.3.5 Sand Collection at the Beach

All the necessary equipment (see preparation) was brought to the beach. For each transect point, the appropriate cooler with the collection containers was taken down on to the beach and used to store the empty and filled water sand collection containers. The sample collection started at the 1st transect on the left-side of the beach. Three sand samples were collected only during the 8:00 AM water collection time according to the following protocol. The sand samples were transported to the lab in a cooler and stored in a refrigerator until analyzed.

Saturday and Sunday 8:00 AM Sand Collection:

1. The sand samples were collected 1 meter from the lowest water level (when the waves have receded from the shoreline) at the same 3 transects where water samples were to be collected. The meter stick was laid down on the sand and the sand collection sleeve was pushed in the ground just at the top of the meter stick, so that the hole created by the sleeve was not within the meter distance but the edge of the hole was a meter away. The sand should have been wet. If the sand was not wet at 1 meter from the water, the sand collection location was moved the shortest possible distance toward the water to a location where the sand was wet.

2. The meter stick was used to record the actual distance from the water on the sand ancillary data sheet once the sampling location had been identified. Also anything unusual or particular about the sand directly surrounding the sand sampling location was recorded on the ancillary data form.

3. A Global Positioning System (GPS) reading of the actual sand collection locations was taken and the associated GPS recorded name on the ancillary data form was recorded.

4. The first plastic bag labeled "7S1" from the cooler was obtained. Then, the covered sterile stainless steel sleeve was taken out.

5. The steel sleeve was wrapped in aluminum foil or a paper bag. If it was wrapped in foil, a knife was used to remove the first two inches of aluminum foil from one end of the sleeve. The top of the aluminum foil was removed by tracing/cutting 2 inches from the top and then pulling off the end (similar to opening the top of a wine bottle). The inside or the lip of the sterile steel sleeve was not touched If it was in a paper bag, the bag was opened and the sleeve removed, taking care not to touch either end.

6. The brown paper bag with the sterilized tops was opened. One sterile cap was removed while trying not to touch any of the other caps as much as possible or touching the inside or lip of the cap itself.

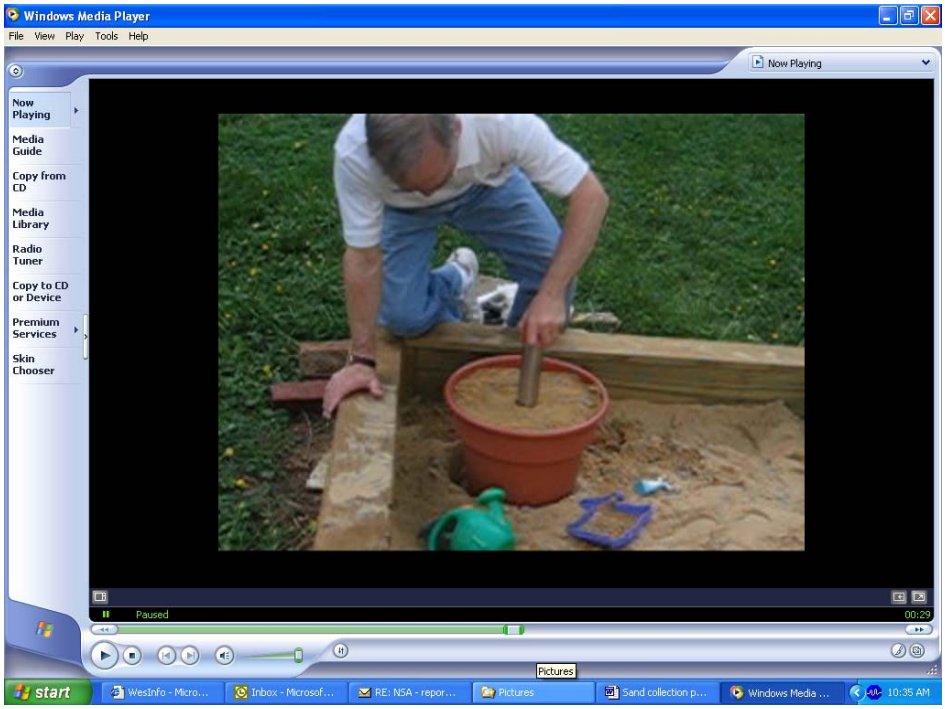
7. The sterile cap was attached to the exposed end of the still covered sterile steel sleeve.

8. The rest of the aluminum foil was removed from the stainless steel sleeve and the uncapped and now exposed end of the sleeve was not touched.

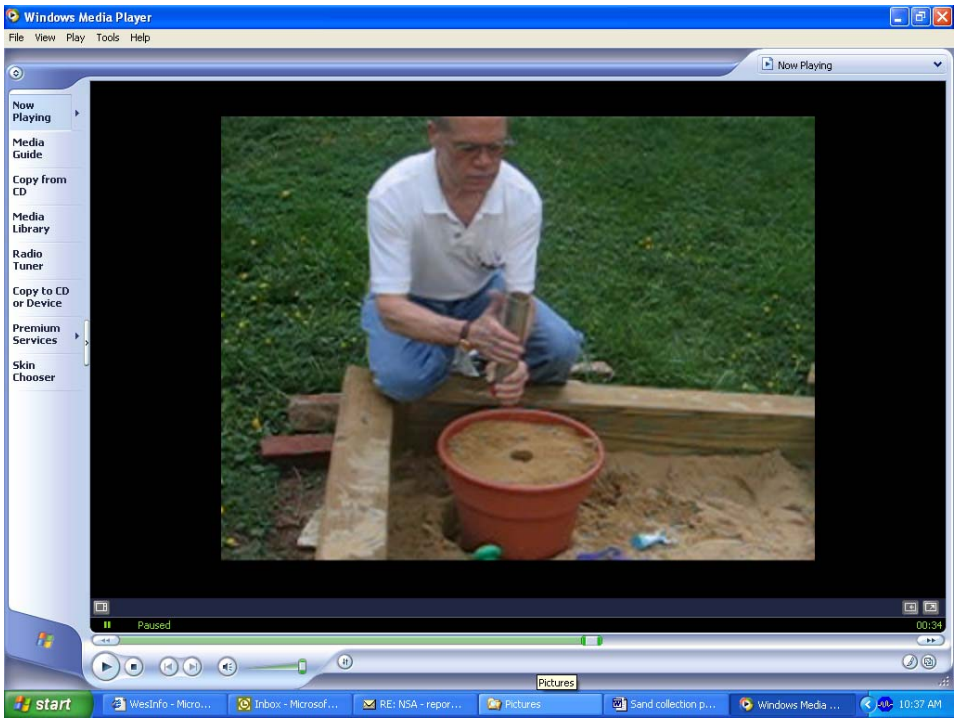
9. By hand, the sleeve was pushed straight down into the pre-determined sand sampling location, at least 8 inches down into the sand. See figure below. If necessary the rubber-headed mallet was used to tap the sleeve into the sand.

10. The sleeve was pushed into sand until only the cap showed.

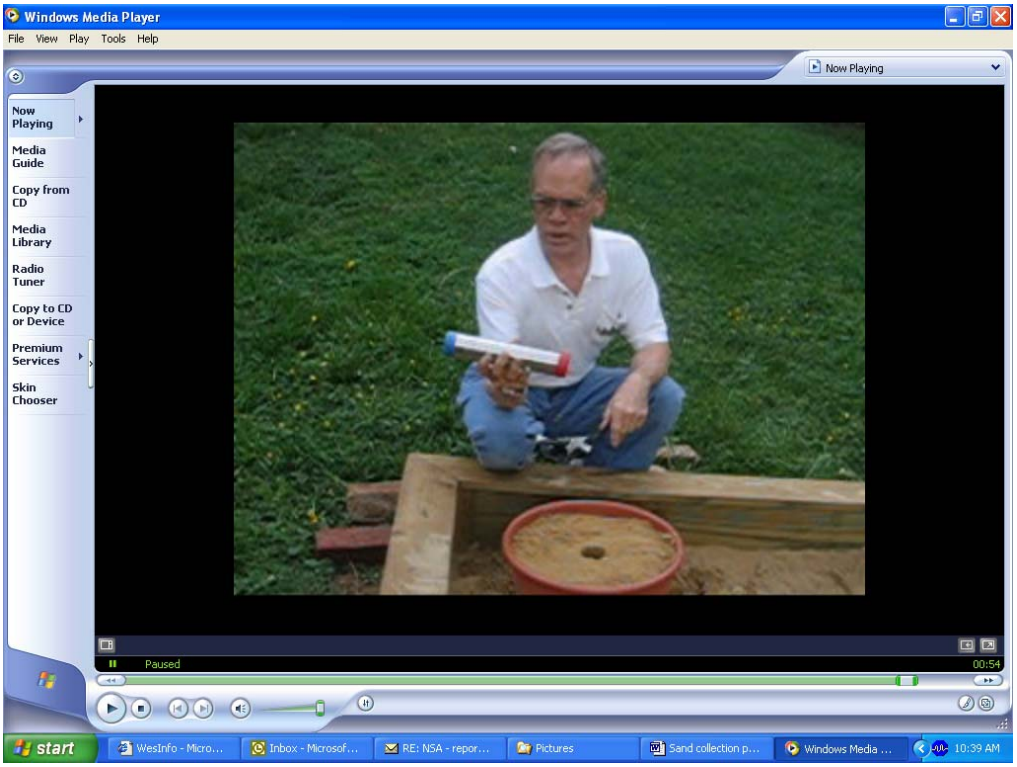
11. The sleeve was pulled out of sand.



12. The side of the sleeve was tapped to slightly pack sand and the liner was checked that it had enough sand (it should have had about 8 inches of sand).



13. The other end cap on was put on the sleeve.



14. The sleeve was capped and full of sand back and was placed in the same pre-labeled zip-lock bag and then placed back in the cooler.

15. After the sand sample was collected, a picture of the sand sampling location was taken that included the hole where the sand sample was removed and the water's edge. See figure below.

16. The above steps 4-15 were repeated for each of the sand sampling locations, the bag labeled "8S1" was removed for the second transect, and the bag labeled "9S1" was removed for the third transect.

3.3.6 Ancillary Data

Ancillary measurements listed in Table 1 were collected by a variety of means. Some were collected by simple observation; others involved the use of equipment, such as pH meters, wind gauges, and rain gauges. It is noted here that WACOR or COR approval of any deviation in methods was required.

For any ancillary data collection, especially that involving specific equipment, Westat was responsible for documenting the exact methods used to collect the data, and to provide information about the calibration and QC procedures for any equipment. This documentation was provided for approval to the USEPA WACOR or COR prior to the occurrence of any field sampling.

Appropriate field team members or lab team members were responsible for entering data on appropriate data collection sheets. Westat may have proposed additional QC activities related to sampling and analysis in their QAPP and work plan as necessary and appropriate. Any changes from the QC specified by individual method technical point-of-contacts were confirmed with them before

implementation. The ancillary data was typically taken by the field team supervisor as the water samples were collected. Due to the nature of the environment where the water collection occurred, the data collected was put down on a clipboard using plastic-covered forms and a grease pencil. This was to due to the fact that the actual paper forms might get ruined from the splashing water from waves or from wind and rain. The data was then transferred from the clipboard plastic sheet covered forms to the real forms immediately after the sample collection.

The data collected included air temperature and wind speed measured by the handheld weather monitor, and wind direction using the compass. The UV measurement was made prior to the start of each sampling time. Then estimates of cloud cover was made, along with an estimated count of animals on the beach or in the water, the number of boats in the water, number of bathers at the beach or in the water, amount of debris on the beach or in the water, rainfall measured by the rain gauge, the water temperature, salinity and conductivity at the middle sample points, current direction and average wave height, and the GPS point measurements at each sample point during the collection of the first bottle. Additionally, approximately 5-7 digital pictures were taken of the surrounding area at each transect to document and serve as backup for the collected ancillary data. If any problem or anything of interest was located or occurred at the collection site, it was recorded in comments on the collection form, and digital images were taken, if necessary.

The photographs were downloaded from the cameras to the field office computer and saved as jpg images with the following file naming convention. The images were backed up onto CD.

L2007MMDD_#

Where:
L = location or Beach (B for Boquerón Beach and S for Surfside Beach)
M = month = 05, 06, 07, 08, or 09.
DD = day = 01 to 31
# = the automatic picture counter supplied by the camera software.

3.3.7 After the Collection

After the collection, the water sample bottles sand collection containers were returned to their respective coolers. Before transport of the samples to the water quality lab, the containers were all

put through a quality control and quality assurance check to ensure all the samples were collected and the caps on the containers were on tight to prevent a loss of any water sample. The bottles and sand containers were then placed into plastic bags and tied off with twist ties to prevent any separation of ID labels and the water bottle samples during transport to the analytical lab. The bottles and sand containers were put back into the cooler for transport with icepacks all around to cool the samples down. All the ancillary data was transferred to the proper forms, double-checking to ensure that the data variable values were correctly transcribed. The Sample Collection Forms were filled out and signed by the sample collection team members and Lab Transmittal forms were filled out, noting any comments about any sample collected and initialed by the person who actually collected the sample. The water collector who was transporting the samples to the lab then signed the Transmittal form denoting that they took custody of the samples. The field team supervisor performed a QA/QC of these documents and then signed them after reviewing them. The Lab Transmittal forms were then taken with the water and sand samples to the analytical lab, where the lab would sign the transmittal form taking custody of the samples from the water collector for filing with the study documentation and faxing to EPA.

3.3.8 GPS points and GPS ID

The GPS points were marked as way points and stored in the GPS measuring device used during the water and sand sample collection. After the water and sand sample collection, the points were retrieved and the data values for each point were recorded on the Ancillary Data forms. To be able to identify and store each of the marked GPS points, a unique ID scheme was developed and implemented. At the end of each collection period, or at the end of the day, the GPS IDs originally assigned by the GPS unit were changed to correspond and uniquely identify the captured GPS points for each collection period on each of the collection days. Listed below is the labeling scheme used to identify the collection period and locations of stored GPS points:

MDDPTTL

MDDPTTL

Where:

MDD was the date of the sample collection;

M was the numeric month and DD was the day, *e.g.*, 614 for June 14,

P was the sample collection point at the beach (1 – 9), and

TT was the planned time of day for the sample collection, using the following:

08 = 8:00 AM
11 = 11:00 AM
15 = 3:00 PM

L is the beach site location (B = Boquerón Beach and S= Surfside Beach)

3.3.9 End of Day /Weekend Procedures

At the end of each collection weekend, several things happened. First, all the collection forms and logs were photocopied to be sent to EPA. The originals were sent to Westat's main offices to be entered into the database maintained at the main campus. Also, the GPS IDs in the GPS unit were changed to the study IDs, and noted on the Ancillary Data Forms. If water sample collection was to continue on the next day, such as on Saturdays with collections scheduled to continue on Sundays, the coolers and mesh bags were rinsed, cleaned, dried and prepared for the following day's sample collection (putting the appropriate pre-labeled bottles into the right cooler and mesh bags). The icepacks would also be wiped down and returned to the freezer to be reused. The digital pictures taken were downloaded from the camera to the laptop at the end of the day (or sometimes only at the end of the entire collection weekend). Lastly, if not done already, the all of the Cyanobacteria samples (Boquerón Beach only) were parafilmed across the cap and neck of the bottle, to prevent any loss of a water sample during storage or transport.

3.3.10 End of the Collection Weekend Shipping Procedures

Westat's subcontract laboratories were provided with a document that detailed the required shipping procedures. The document highlighted the standard shipping procedures for all data, samples, or filters sent from the local laboratory to the various analytical laboratories during the course of the Water Quality Study in the summer of 2009.

Types of Samples

During this time period, the subcontract laboratory was responsible for shipping the following items to the respective locations:

1. **Sand Sample Extracts** (300 g of sand was removed from each sand sample prior to processing)
   Shipped to: Emylee Prevette
   University of North Carolina Env. Sci and Engineering
   Room 1108
   135 Dauer Drive Route 1A
   Chapel Hill, NC 27599
   Electronic COCs were sent to Elizabeth Sams.

2. **Water Sample PCR Filters** (total of 7 filters per sample)
   Shipped to: EMSL Analytical in Westmont, NJ (3 filters) c/o Charlie Li
   USEPA in Cincinnati, OH (2 filters) c/o Rich Haugland
   USEPA in Athens, GA (2 filters) c/o Marirosa Molina

3. **Sand Sample PCR Filters**
   Shipped to: EMSL Analytical in Westmont, NJ (3 filters) c/o Charlie Li

4. **Transmittal Sheets, Pictures & MF /Turbidity/Ancillary Data Result Sheets**
   Shipped to: Westat in Rockville, MD c/o Robert Clickner

5. *** Cyanobacteria Samples ***
   Shipped to: Greenwater Labs in Palatka, FL c/o Amanda Foss

   * Only for the Puerto Rico Beach Site *

Samples, Filters and Data were shipped out on Mondays following a sample collection weekend via FedEx overnight priority delivery to their respective locations. On weekends where there was a Monday holiday, shipments went out on Tuesdays. Integrity of the shipment was maintained by following the protocol specifications regarding the method for shipping and maintaining the appropriate temperatures.

Westat's subcontract laboratories were provided with a flowchart that depicted the process for each sample from collection through processing or analysis and onto shipping/transport. See Appendix A, Flowchart of Samples Collected.

Labeling

All child samples were labeled with the waterproof polyester labels supplied by Westat. These labels were made of the same material as the labels supplied for the collection of the samples. The child samples included the composited samples and the QPCR filters.

Chain of Custody

All shipments were accompanied with a detailed chain of custody (COC) that indicated every item that was included in the shipment, number of duplicates, sample id, sampling location, collection time, freeze time, etc.

A Chain of Custody template (See Appendix B) was provided by Westat for the study dates. There were separate Chain of Custody for each recipient; the intended recipient was indicated in the Chain of Custody heading. The information that was pre-filled consisted of the sample ID, sampling date, nominal collection time, sample location, sample type.

At the laboratory, lab technicians were responsible for completing the following fields on the Chain of Custody:

- Actual number of bottles/filters with that ID;
- Actual date processed/analyzed;
- Shipping date; and,
- Any relevant comments.

As a form of quality control, someone other than they person who packed the shipping container would double check the shipment and corresponding Chain of Custody to verify that it was correct.

In addition to the packed Chain of Custody, the subcontract laboratories sent out electronic versions as well.

Packing and Shipping Bottles and Filters

When packing the shipments, the subcontract laboratory was provided with the following checklist to ensure a safe shipment and to help maintain the integrity of the sample.

- Ensure all bottles/filters are properly labeled.
- Seal the bottle caps and necks with parafilm or electric tape.

- Double-bag each bottle in sealed zip-lock bags. This is not needed for the QPCR filters, since they were packed in compartmentalized shippers.
- Individually wrap each bottle in bubble wrap or other protective container/packing material. This was not needed for the QPCR filters.
- Place plenty of ice or ice packs or dry ice (as appropriate) on the bottom and sides of the cooler or shipping container.
- Add the samples and additional ice/packs/dry ice and bubble wrap.
- Lay more ice/packs/dry ice and bubble wrap on top of the samples before shipping.
- Add the printed COC.
-

Sample-Specific Procedures and Shipping Requirements

The following paragraphs describe the specific shipping requirements that were followed for the different type of items that were shipped throughout this study.

Data Sheets and Lab Transmittal Forms: The local lab shipped copies of all laboratory transmittal forms and data sheets (MF results, ancillary data, pH and turbidity reading) to Westat on the Monday or Tuesday after all analyses were complete.

Sand Samples: Sand samples collected during the 8AM sample collection time on Saturdays, Sundays and Holidays during the study period were taken to the local lab for analysis via MF and processing for PCR. Prior to processing the sand, 300g of each sample was ascetically weighed out and transferred to a separate plastic container. These sand samples (created from the parent sand samples) were stored until in the lab refrigerator, until shipped on Monday. During the Monday shipment the samples were sent on ice packs to the USEPA Lab in Chapel Hill, NC.

PCR Filters: PCR filters were frozen and stored at temperatures of at least -20º C until shipped on Monday. Filters were sent frozen and on dry ice to EMSL Laboratories in Westmont, NJ, USEPA Lab in Athens, GA, and the USEPA Lab in Cincinnati, OH. Filter blanks were sent along with the samples, one blank filter for every 6 samples was shipped, in accordance with the June 5, 2009 email from Kris Brenner. Filter blanks were included on the chain of custody forms.

4. ANALYTICAL METHODS

Following collection, samples were maintained on ice during transport and at 1 - 4° C until the time of analysis. This was the only preservation step. Microbiological analysis of water and sand samples commenced within six hours of collection. Further, it was critical that sample plates from the membrane filter methods be placed in the incubator within eight hours of sampling. This was accomplished for all samples. In the event of any problems or irregular occurrences, it was imperative that the WACOR or COR be called immediately for guidance, and that the comments fields on the various data sheets was used to record problems/corrective actions, so that the effect on data quality could be considered. Examples of problems that could occur included sampling difficulties, failure to ice-down samples, missed holding/analysis times, longer than acceptable incubation times, problems with the instruments, etc.

With the rapid QPCR method, the critical step was the filtration of the water samples and storage of the filters in the freezer within the 8 hours after collection. Once the filters were frozen, analysis could be done as the time allowed. Again, this was accomplished for all samples. The times the QPCR method filters were frozen and stored, the location of the freezer(s), and the dates and times of the analyses were recorded. If QPCR filters were analyzed in another lab, they were shipped by overnight express on dry ice. The other laboratory conformed to the QC requirements of this document. Problems with the rapid methods, like those with the filter methods, were reported and guidance requested from the WACOR or COR.

Samples were disposed of following successful microbiological processing by each of the microbial methods, including the counting of all plates, successful pH, conductivity, salinity and turbidity measurements, and completed analysis of samples by all methods except for the QPCR method. However, QPCR samples were filtered and the filters frozen before the disposal of the samples. The WACOR or COR was contacted about the disposition of the samples if unusual results were obtained.

Westat maintained a dedicated sample record book that was used to record all sample IDs as samples were checked into the laboratory. The record book also had columns for date checked in, storage locations, and disposal dates. Westat was responsible for ensuring that all sample IDs were recorded and initialed the record book for each batch of samples received to indicate that all expected samples were present. The USEPA could request that the record book or copies of pages from this record book be made

available for examination. Westat was also responsible for verifying that the arrival time at the laboratory was entered in the appropriate column on the sample collection sheets, and initialed sample collection sheets in the appropriate space(s) to indicate such, and noted any leaking containers or other irregularities.

4.1 Microbiological Methods

4.1.1 Standard Membrane Filter Method *Enterococci* (Method 1600)

Reference 8, EPA/821/R-97/004, describes the membrane filtration assay for *Enterrococci*. This method can also be found in Reference 7: "Improved Enumeration Methods for Recreational Water Quality Indicators: *Enterococci* and *Escherichia coli*," EPA/821/R-97/004. These references are detailed enough, including descriptions of required equipment, so that the membrane filter method can be performed. As such, these two references represent the standard operating procedures (SOPs) for the critical membrane filter data to be obtained from the field study. A 1-liter sample was collected for use in performing the filtration method and the ancillary pH, conductivity, salinity and turbidity measurements, which was performed last to avoid contamination. All collected samples were analyzed for *Enterococci* by the MF method using sample volumes of 100, 10 and 1 mL [except for special circumstances; for example, if plates at the standard sample volumes were all TNTC, or produced zero CFUs, then sample volumes needed to be adjusted.] In the event that the laboratory needed to adjust the volumes, the adjustment and documentation of reason was indicated in the records submitted to the USEPA. Analysis of each sample was initiated within 6 hours of its collection, and processing (filtration and plating) was completed no later than 8 hours after collection.

Specific QC requirements to be incorporated into the assays (in place of the general guidance in the methods) can be found in the next section of this plan. Table 8 summarizes some of the key features of the method. Any modifications to the method, such as using auto-pipets or micro pipets instead of standard glass pipets, was approved by the WACOR or COR prior to being implemented. Any other questions regarding the methods were also addressed to the WACOR or COR prior to the start of field activity.

Table 8. Summary of the mEI Agar Method for *Enterococci*

Method	Medium	Incubation time and temperatures (° C)	Volumes analyzed (mL)	Detection limits (colonies per plate)	Ideal l# of colonies per membrane
Enterococci EPA 1600	mEI agar	24 hours ± 2 hours @ 41 +/- 0.5° C	100 10 1	1-200	20-60

On the sample collection/tracking sheets and final data sheets, laboratory analysts were responsible for entering times and their initials for the following sequential steps:

- Analysis start time.
- Time at which plates being incubation in the water bath.
- Time at which plates are removed from the water bath for counting.

These times were entered by hand initially, and later entered into the database electronically. The times listed above, and initials, were entered in a batch-wise manner. The laboratory was also responsible for entering dilution data, count data, QC data, etc. on data sheets. Responsibility for electronic data entry was determined by Westat.

Samples were analyzed in batches. A batch was considered to be all of the samples that were delivered to the laboratory at the same time. The plates for each batch of samples started their incubation periods at the same time, and the microbiological control samples described below under "specific filtration control tests" accompanied each analysis batch.

For the membrane filter assay, the most critical quality control requirements are as follows:

- Prior to any sampling/filtering, an appropriate volume of TSA [Tryptic Soy Agar/Trypticase Soy Agar (Difco 0369-17-6, BD 4311043, Oxoid CM 0129B, or the equivalent)] was prepared, and tested as described below. These plates were later used for QC samples during sample runs. The recipe for TSA and the contamination screening for TSA plates is described below:

  Composition:

Tryptone	15 g
Soytone	5 g
NaCl	5 g
Agar	15 g

  Preparation: Add the dry ingredients listed above to the 1000 mL of reagent-grade distilled water, and heat to boiling to dissolve the agar completely. Autoclave at 121° C (15 lbs pressure) for 15 min. Dispense the agar into 9 x 50 mm petri dishes (5 mL/plate).

  Test for contamination: Incubate all plates for 24 - 48 hr at 35° C to check for contamination. Discard any plates with growth. If ≥ 5% of the plates show contamination, discard all plates, and make new medium. Store plates in plastic bags at 4°C until needed. The final pH was 7.3 ± 0.2. Records of preparation and testing were maintained, and were submitted to the WAM upon request.

- Each batch of mEI agar was pre-tested for performance (i.e., correct enzyme reaction) with known cultures of target (*e.g., Enterococcus faecium or Enterococococcus fecalis*) and non-target (e.g., *Escherichia coli or Pseudomonas* species) organisms. Records of such tests were maintained by the laboratory and was submitted to the COR and WACOR upon request.

- Specific filtration control tests, listed below, were performed each time a batch of samples was analyzed, and the results recorded. Results for all filter, agar or buffer controls, including counts (if any), were reported with the sample results.

- Filter Control: Place one or more membrane filters on sterile TSA plates, and incubate the plates for 24 hours at 35° C. Absence of growth indicates sterility of the filter(s).

- Phosphate-Buffered Dilution Water Controls: Filter a 50-mL volume of sterile dilution water before beginning the sample filtrations and a 50-mL volume of dilution water was filtered after completing the sample filtrations. Place the filters on TSA plates, and incubate the plates for 24 hours at 35° C. Absence of growth indicates sterility of the dilution water.

- Agar Control: Place one or more plates of each medium, mEI and TSA, in the incubator. Incubate mEI at 41° C and the TSA at 35° C for 24 hours to check for contamination. Absence of growth indicated sterility of the plates.

- Optional membrane test: Test new lots of membrane filters against an acceptable reference lot using the method of Brenner and Rankin (4). Although optional, this test was recommended. In lieu of performing this test, the laboratory purchased filters from a reputable source. The USEPA has found (by the method referenced) that Sartorious filters have generally provided satisfactory performance; however, this does not mean other filters were unacceptable.

There were no specific sample IDs for the specific filtration control samples. On the hard copy format batch analysis sheets, their results were reported with the following codes:

YYZ (MEDIA), where,

YY = AC, PB, or MF (for Agar Control, Phosphate Buffer dilution water controls, or Membrane Filter control).

Z = B or A, or nothing (used only for phosphate buffer dilution water controls; B for "Before filtering" control, A for "After filtering" control).

(MEDIA) = mEI or TSA, the medium used for the control.

The methods contain other specific QC elements, such as requirements for laboratory water quality, specifying that thermometers be NIST-traceable, calling for daily confirmation of incubator and water bath temperatures. Such method specifications were adhered to, and the adherence documented. All autoclave runs contained maximum-registering thermometers to ensure appropriate temperatures were achieved. Additionally, at least weekly, autoclave runs contained spore strips or vials, which were incubated according to the manufacturer's instructions to check for proper sterilizer operation. Calibration records were maintained for laboratory balances, pH meters, etc.

The method SOP contained procedures for verifying the correct identities of organisms. Verification tests were performed for all samples (5 colonies/sample) from one day (either Saturday or Sunday) of the first weekend (6 sample locations x 3 times per day x 1 day = 18 samples total) at the beach site. Results of the verification tests were recorded and reported to the WACOR or COR with the other sample data in a mutually agreed upon manner.

It was expected that laboratories would follow generally accepted good microbiology laboratory practice, such as described in the USEPA Microbiology Methods Manual, Part IV, C (1);

Section 9000 of the 20th edition of Standard Methods (3); or the QC section of the USEPA's "Manual for the Certification of Laboratories Analyzing Drinking Water" (5). Copies of any records associated with standard laboratory QC practices were made available to the USEPA upon request.

4.1.2 Quantitative Polymerase Chain Reaction (QPCR) Method

Reference 9 describes the procedures for the detection of total *Enterococci* and total *Bacteroides* in water samples based on the collection of these organisms on membrane filters, extraction of their total DNA, and polymerase change reaction (PCR) amplification (i.e., a process whereby the quantity of DNA is doubled in each cycle of amplification) of a genus-specific DNA sequence using the TaqMan™ PCR product detection system. The TaqMan™ system signals the formation of PCR products by a process involving the breakdown of a double-labeled fluorogenic probe that specifically attaches to the target sequence at a site between the two PCR primer recognition sequences. The reactions were performed in a specially-designed thermal cycling instrument that automated the detection and quantitative measurement of the fluorescent signals produced by probe degradation during each cycle of amplification. These signals were directly related numerically to the quantities of PCR products produced.

The protocol was detailed enough, including descriptions of required equipment, so that the method could be performed. As such, this reference represents the standard operating procedure (SOP) for the critical data obtained from this portion of the field study. A 1-liter sample was collected for use in this method. All collected samples were analyzed for total *Enterococci and* total *Bacteroides* using sample volumes of 100 mL [except for special circumstances; for example, if this volume was found to be impractical to filter, then sample volumes may have been adjusted]. Filtration of each sample was initiated within 6 hours of its collections. Seven (7) replicate filtrations were performed, and the filters were transferred to extraction tubes, as described in the protocol and stored at -20° C for an indefinite period. All filters were properly labeled to identify the water sample they came from.

The local lab performed the 7 replicate filtrations and shipped 3 filters to the PCR lab, 2 filters to Dr. Richard Haugland of USEPA, and 2 filters to Dr. Mariosa Molina of USEPA. All silters were sent by overnight express on dry ice on the Monday following the weekend the samples were collected.

The PCR lab performed the extraction to obtain DNA to be used for QPCR analyses for all microorganisms (total *Enterococci and* total *Bacteroides*) as soon as possible using only one of the

filters, and two filters were stored in the freezer as backups or for other/later analyses. At the end of this study, all remaining frozen filters were sent on dry ice by overnight express to Dr. Richard Haugland of the USEPA.

Specific quality control (QC) requirements that were incorporated into these analyses are listed below, as well as those in the method protocol.

- QC requirements for sample collection and filtration are specified in the Microbiological Methods section.

- Cell suspensions of the calibrator strains, *Enterococcus faecalis*, American Type Cutlure Collection (ATCC) 29212, *Bacteroides fragilis* ATCC 25285, and reference strain, *Geotrichum candidum*, University of Alberta Microfungus Collection and Herbarium (UAMH) 7836, were provided to the laboratory by the USEPA. The cell suspensions provided were stored by the laboratory at -20° C, or preferably at -70° C, until used. Preliminary QPCR analyses were performed using four tubes of these suspensions prior to the start of the study, and the results (C_T values and run files) were reported to USEPA. Subsequent average results for these samples on each day of analysis were within +2 C_T units of the average of the initial values (See paragraph below on monitoring the performance of the thermal cycling instrument and PCR reagents).

- Training for the laboratory on the highly specialized scientific PCR equipment was provided by the government for validity of data. Westat was responsible for ensuring that the PCR technician had documented experience in QPCR technology.

- Westat purchased PCR reagents, including primers and fluorescently-labeled probes. Primer and probe sequences were provided by the USEPA.

- Thermal cycling instrumentation (SMART Cycler TD System, Cepheid, Sunnyvale, California) was provided by the USEPA. Westat monitored the performance of the thermal cycling instrument and PCR reagents based on ongoing calibrator sample analysis results. (See above.) In the event of failure to meet these performance criteria, Westat prepared and analyzed a new set of calibrator extracts, identify the source of the problem (e.g., reagents or instruments), and take corrective action.

- Westat provided adequate facilities and carry out precautions necessary to minimize the likelihood of DNA contamination. Manipulation of samples and reagents was performed in laminar flow hoods or workstations with UV light sources, and the areas were disinfected before and after each use with 10% bleach. Disposable aerosol barrier pipette tips were used for all liquid transfers. Tubes and other disposables that were not sterilized by the manufacturer were autoclaved before use. All supplies and disposables were DNA-free. Distilled water and other reagents were verified to be free of target DNA in negative control analyses performed with each set of sample analyses.

- All pipettors used were calibrated prior to commencing work and on a semiannual basis afterwards. It was recommended that the pipette calibration be verified weekly by weighing several different amounts of water (in the ranges use) pipetted into a properly tared container.

- This work assignment also included four other combinations of reagents that were tested for each organism by PCR method.

5. QUALITY CONTROL (QC)

The most critical elements of quality control for the membrane filter method were those related to the microbiological assays. Sampling was straightforward; Westat was required to ensure that the proper samples were taken in the appropriately labeled containers. *Holding time of samples was considered critical*. Samples that had not been placed in the water bath in the membrane filter method or completely filtered and placed in the freezer for use in the QPCR method within eight hours of collection was considered to have produced invalid data. (However, all data was collected, compiled, and reported to USEPA). The intent of this project was to collect all of the data for subsequent evaluation by the USEPA project team, who ultimately determine its utility based on their collective expertise and experience. No data was rejected outright by the persons performing the analysis. All data, including Too-Numerous-To-Count's (TNTC) and zero's in the membrane filter methods, was reported to the USEPA. An estimation procedure for TNTC plates was provided to the laboratory by USEPA. The estimation data from the TNTC plates (i.e., the five counts from five squares on each filter) were all submitted to USEPA along with the count data for the other samples. Westat calibrated and maintained the instruments according to the methods and/or the manufacturer's recommendations. Westat followed accepted good microbiology laboratory practice and maintained QC records. Westat participated in all QA audits conducted, and ran one or more performance evaluation samples provided by the USEPA. Westat contacted the WACOR or COR when problems occurred and documented corrective actions taken in a report.

5.1 Corrective Actions

Failure to meet any QC requirements, including those associated with standard good laboratory practice, requires that appropriate corrective actions be taken. All QC failures, associated corrective actions, and their effectiveness, were documented on a corrective action form, and submitted to the USEPA WACOR or COR as part of the weekly reports. Data associated with quality control problems was clearly identified in such reports, along with an assessment as to the QC failure's potential effect(s) on data quality. The WACOR or COR was notified of such problems/corrective actions as soon as possible to the time of the actual occurrence. All related sample and ancillary data was still reported in the standard way, with the QC problems clearly noted on copies of the data deliverables.

5.2 Instrument/Equipment Testing, Inspection, and Maintenance

Any SOP for equipment/instrument which Westat was required to develop or provide described standard maintenance procedures for equipment. Maintenance records were described in the SOPs, and were made available to USEPA upon request, including monitoring records of basic equipment such as incubators, refrigerators, etc.

For any equipment that might have affected critical data (i.e., microbiological or ancillary data), Westat prepared a short report for the WACOR or COR describing how the equipment was inspected and tested upon receipt. The report was delivered within two weeks of equipment being placed in service.

5.3 Instrument/Equipment Calibration and Frequency

Any SOPs for instruments and equipment which Westat may be required to develop or provide would fully describe calibration and calibration verification procedures. This included reference to any calibrations conducted using certified equipment and/or standards with known valid relationships to nationally recognized performance standards. Field instruments/gauges and laboratory measuring equipment, such as balances and volumetric measuring devices (e.g., micropipettes), were professionally serviced/certified within the six months prior to the commencement of the field/laboratory activities for this project.

5.4 Tracking and Inspection/Acceptance of Supplies and Consumables

Westat had a system for tracking supplies, reagents, etc., and submitted its procedures to the WACOR or COR for approval prior to the start of the field season.

The membrane filter methods describe the minimum requirements for the quality of chemicals and laboratory water. Quality control procedures for laboratory water outlined in the USEPA drinking water certification manual (5) were recommended. Westat maintained basic records (i.e., resistively readings, filter changes, etc.) for their laboratory water systems.

The optional (but recommended) filter test (2); previously described, may be employed to test new membrane filter lots. All media prepared was routinely tested for sterility.

The goal was to have a clear association of all microbiological data with specific lots of all materials employed in performing analyses. All records associated with materials tracking and preparation were made available to USEPA upon request.

5.5 Data Management

Some elements of data management for field data and laboratory data were previously outlined. Westat initially hand-entered results on pre-printed forms that were approved by USEPA.

On an approximately daily basis, completed hand-entered data sheets were sent to the WACOR or COR. Weekly submissions were also submitted to the WACOR or COR.

Westat maintained original copies of sampling and data worksheets until instructed by the WACOR or COR on the deposition of the worksheets. Westat maintained two copies, on separate storage media, of electronic versions of data until instructed by the WACOR or COR on the disposition of the data.

5.6 Readiness Review/Dry Runs

At least one week prior to actual sampling, sampling/analysis personnel performed a readiness review/dry run at the beach site. USEPA representatives attended. A checklist modified in Work Assignment 2-04, under the previous contract, was utilized by Westat that detailed all equipment, supplies, worksheets, logbooks, etc. required to conduct sampling, ancillary data collection, microbiological analysis and data recording, and reporting at the beach site. A set of samples was run. Data transmission also occurred as part of this effort. Westat observed all activities in detail and recorded their observations.

Westat was responsible for determining the results and any corrective action that needed to be taken. After concurrence with the USEPA, a written report on the final approach to sampling/analysis/reporting was provided to the WACOR or COR.

5.7 Site Visits/Technical Systems Audits

The USEPA performed a site visit at the beach site. The site visit included a technical systems audit (TSAs). The site visit or audit was coordinated with Westat in advance.

Site visitors/auditors may recommend work stoppage if they observe what they deem to be critical failings on the part of Westat. Work may be stopped until such time as effective corrective measures were implemented, verified effective, and approved.

Following the site visit/TSA, a report was prepared by the personnel who conducted the visit. This report was addressed to the WACOR or COR. Westat was provided a copy of the report, and was required to respond to any corrective action recommendations. Westat was responsible for signing-off on the response. A close-out memo was issued to Westat by the WACOR or COR following his/her approval of the response. However, USEPA reserved the right to revisit any identified problem areas.

5.8 Routine Surveillance

Copies of any written reports generated by Westat on routine surveillance was made available to the WACOR or COR.

6. DATA VALIDATION AND USABILITY

According to USEPA Guidance for Quality Assurance Project Plans; USEPA QA/G-5 (6),

> "the process of data verification requires confirmation by examination or provision of objective evidence that the requirements of these specified QC acceptance criteria are met. In design and development, verification concerns the process of examining the result of a given activity to determine conformance to the stated requirements for that activity. For example, have the data been collected according to a specified method and have the collected data been faithfully recorded and transmitted? Do the data fulfill specified data format and metadata requirements?"

Regarding validation, G-5 states,

> "The process of data validation requires confirmation by examination and provision of objective evidence that the particular requirements for a specific intended use have been fulfilled: In design and development, validation concerns the process of examining a product or result to determine conformance to user needs."

Based on these definitions, verification is the responsibility of Westat; however, USEPA reserves the right to review the verification and will be responsible for validation.

6.1 Data Review, Verification, and Validation

All data were subjected to several layers of review and verification, which is described in the next section. The previously described assessments were also a key component of verification. Validation was primarily considered part of reconciliation with project objectives. General principles guiding acceptance/selection, verification, and validation of data are discussed immediately below.

All microbiological data was submitted to the USEPA (i.e., data from all sample volumes or dilutions, even if zero, uncountable or too numerous to count and all other forms of data, described above). The USEPA would decide whether or not data are acceptable, and chose which data were to be included in the final data set for the project. The guiding principles for microbial data acceptance/selection were:

- Legible data records.

- Dates and times correct.
- Sample IDs correct.
- Electronic and hard-copy data concur.
- Results are in an appropriate format.
- Results reasonable (i.e., not grossly wrong).
- CFU counts are in the ideal range, whenever possible.
- Dilutions with CFU counts outside ideal range are not grossly incompatible with those in the ideal range.
- Sample holding/analysis times were met.
- Associated QC sample results are acceptable.
- Specific acceptance criteria for the rapid methods adequate.

For ancillary data, the acceptance factors included:

- Legible data records.
- Dates and times correct.
- Electronic and hard-copy data concur.
- Results are in an appropriate format.
- Results reasonable (i.e., not grossly wrong).
- Associated QC sample results acceptable.
- Other factors support acceptance (or rejection).

For the remote chemical data the acceptance factors included:

- Legible ASR forms.
- Dates and times correct.
- Samples arrive in good condition at remote laboratory.

Verification and Validation Methods

Westat inspected forms to see that all appropriate data fields had values entered, and that entries were legible and reasonable. Westat also ensured that all planned samples had been collected. The verification of review was indicated by entering their initials on the field data sheets in provided spaces. Westat was responsible for seeing that all forms were present and that they were delivered to the laboratory.

Westat verified that all expected samples and field sheets were present upon arrival at the laboratory. Westat periodically inspected the record book and made a record of any such inspections.

The laboratory was responsible for verifying that all microbiological (and other laboratory) data fields were legibly filled out with apparently reasonable data. The verifier entered their initials and the date in appropriate fields on the data sheets to indicate their review and acceptance. Spaces for date and initials were provided on all data sheets. The laboratory's initials indicated their inspection and acceptance of data sheets prior to their delivery to USEPA.

Transmission of deliverables was the *de facto* indicator that the data were completely reviewed and believed to be accurate. The laboratory personnel responsible for reviewing the data was a person that was different than the person who originally keyed-in the data.

6.2 Making Corrections

On any hard copy data sheets, incorrect entries were singly lined-out *(i.e.,* not obliterated) and correct results entered. If the party making the correction was not the person who made the original entry, then the date and initials of the person modifying the entry was present next to the correction.

7. PERSONNEL

Westat proposed the following personnel for this work assignment. Dr. Robert Clickner was the Water Quality Project Director. Karen Della Torre was the Westat Project Leader, and Work Assignment Leader. In addition to Dr. Clickner, Amy Kominski, Sara Hader, Naa Adjei, and Rebecca Birch served as study support. All of the staff worked on the Beaches projects in previous years.

Robert Clickner, Ph.D.: Dr. Clickner is an Associate Director at Westat and a senior statistician with over 35 years of experience in the development, implementation, and management of statistical and environmental research projects, including two years experience directing the Beaches water quality studies for EPA**.** Dr. Clickner has also designed, conducted and analyzed biostatistical experiments involving pesticides and other environmental contaminants. His project management activities have included the development and maintenance of project completion plans, quality assurance plans, schedules, and budgets; management and coordination of multiple subcontractors, including numerous laboratories; staff assignments; review of deliverables; and client coordination and communication**.** He has developed and conducted international workshops on methodologies for human exposure assessment field studies.

Karen Della Torre, MS, MBA, PMP: Karen Della Torre is a Senior Study Director and systems manager with more than 15 years of experience in managing multiple research efforts in support of Federal government initiatives, including epidemiologic studies. She has managed large studies involving the collection of environmental data, questionnaire data, and biological measurements. She has designed and supervised the development of paperless data collection and transmission systems for environmental and health studies. Ms. Della Torre has managed field studies using hand-held devices to capture survey data, performed reliability testing of computer equipment in field conditions, and reviewed new technologies for application to field studies and other data collection efforts as they become commercially available. She supervises a staff of data collection specialists, systems analysts, programmers, web developers, database developers, survey designers, and subject-area specialists to produce systems to track environmental and biological specimens, collect data via the Internet, collect data using hand-held computers, administer computer-based surveys, and produce searchable environmental and medical literature databases. Ms. Della Torre holds a master's degree in biomedical engineering. She has performed research on diagnostic imaging procedures, computerized diagnostic

systems, and medical history data and image transmission techniques. She also holds an M.B.A. in Information Systems, specializing in database design and Internet applications.

Rebecca Jeffries, MPH: Rebecca Jeffries is an epidemiologist with 4 years of experience in study design, field data collection, data analysis, and program evaluation for environmental and occupational health studies. She has managed studies, data and sample collection, and the packaging and shipment of environmental samples. She has also designed data collection forms and study protocols, pilot tested survey instruments, supervised listers in preparing for sample selection, developed databases, analyzed data, and developed a manual of operating procedures. In addition, Ms. Jeffries has experience teaching at the high school and college levels, including instruction of non-native English speakers and international teaching experience in rural Kenya.

Ms. Amy Kominski, **BS:** Amy Kominski is an assistant study manager and research assistant at Westat. Ms. Kominski is a biologist, research assistant, with experience in collecting epidemiologic research data. Prior to working at Westat, she worked at The National Institutes of Health in Bethesda, Maryland. While at The NIH she worked in a clinical microbiology lab and has experience designing and managing public health studies, including; protocol development and implementation of large volume, multi-site research projects. Her specific experience includes infection control studies involving antibiotic resistant bacteria, specifically *Enterococci* and *Staphylococcus aureus*. Ms Kominski is proficient in general laboratory biochemical testing methods, quality assurance and control, antibiotic susceptibility testing and state-of-the-art molecular assays. Since joining Westat, Ms. Kominski has been involved with the work done at Fairhope Beach (2007) and Goddard Beach (2007) and has performed water sampling pilot studies and beach assessment studies.

Sara Hader, BA: Sara Hader is an assistant study manager and research assistant in Westat's Health Studies Sector. After graduating with a bachelor's degree in microbiology, she performed medical literature research for defense medical malpractice cases in trial law. Since joining Westat, Ms. Hader has supported studies for the Centers for Disease Control and Prevention and the National Cancer Institute. Ms. Hader has experience in coordinating forms and records request, receipt, and processing. She works with project directors, operations staff, programmers, and support staff in managing data collection and tracking systems on several environmental, occupational, and epidemiologic studies.

Ms. Naa Adjei BS. Naa Adjei has experience as quality assurance specialist for environmental sample collection. Ms. Adjei has 3 years of experience in scientific study design, site selection for field study, data collection, and data analysis. Naa is experienced in developing databases for data entry and reporting. She has performed beach site assessments and has worked as a quality assurance specialist to ensure that data collection and analysis adhere to strict protocols. Ms. Adjei holds a B.S. in neurobiology and physiology.

Field Staff. The field staff collected the water and sand samples, processed them and delivered them to the local laboratory, and performed related tasks. They worked under the supervision of a Westat person as well as the supervision of the subcontract laboratory. These staff had some post-secondary education in biology, environmental science, or a related discipline. Westat provided the necessary project-specific training.

8. REFERENCES

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9. Rapid Polymerase Chain Reaction (PCR)-Based Methods for Measuring Total *Enterococci and* Total *Bacteroides*, in Water Samples [revised March 2009]

Appendix E

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High Sensitivity of Children to Swimming-Associated Gastrointestinal Illness

Results Using a Rapid Assay of Recreational Water Quality

Timothy J. Wade,[a] *Rebecca L. Calderon,*[a] *Kristen P. Brenner,*[b] *Elizabeth Sams,*[a] *Michael Beach,*[c] *Richard Haugland,*[b] *Larry Wymer,*[b] *and Alfred P. Dufour*[b]

Background: Culture-based methods of monitoring fecal pollution in recreational waters require 24 to 48 hours to obtain results. This delay leads to potentially inaccurate management decisions regarding beach safety. We evaluated the quantitative polymerase chain reaction (QPCR) as a faster method to assess recreational water quality and predict swimming-associated illnesses.

Methods: We enrolled visitors at 4 freshwater Great Lakes beaches, and contacted them 10 to 12 days later to ask about health symptoms experienced since the visit. Water at the beaches was polluted by point sources that carried treated sewage. We tested water samples daily for *Enterococcus* using QPCR and membrane filtration (EPA Method 1600).

Results: We completed 21,015 interviews and tested 1359 water samples. *Enterococcus* QPCR cell equivalents (CEs) were positively associated with swimming-associated gastrointestinal (GI) illness (adjusted odds ratio per 1 $\log_{10}$ QPCR CE =1.26; 95% confidence interval = 1.06–1.51). The association between GI illness and QPCR CE was stronger among children aged 10 years and below (1.69; 1.24–2.30). Nonenteric illnesses were not consistently associated with *Enterococcus* QPCR CE exposure, although rash and earache occurred more frequently among swimmers. *Enterococcus* QPCR CE exposure was more strongly associated with GI illness than *Enterococcus* measured by membrane filtration.

Conclusions: Measurement of the indicator bacteria *Enterococci* in recreational water using a rapid QPCR method predicted swimming-associated GI illness at freshwater beaches polluted by sewage discharge. Children at 10 years or younger were at greater risk for GI illness following exposure.

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It would be useful to monitor recreational waters continuously for human pathogens as a way to prevent swimming-associated infections. However, there is considerable difficulty and expense associated with testing for the vast number of potentially pathogenic microorganisms. Instead, fecal indicator bacteria such as *Escherichia coli* or *Enterococcus* are used to assess the microbial safety of recreational waters. These indicator bacteria are generally not harmful but can be a marker for the presence of sewage and human feces, and the health risks that result from such exposures. Because currently used methods require 24 to 48 hours to obtain results, monitoring does not immediately detect changes in exposure, leading to delays in notifying beach-goers of possible risks.

A faster method of measuring water quality could improve protection of public health by reducing the time between exposure measurement and management decisions, potentially providing same-day results before most beach-goers enter the water. We previously reported that a faster method of measuring fecal indicator bacteria using quantitative polymerase chain reaction (QPCR) showed promise in its ability to predict swimming-associated gastrointestinal (GI) illness.[1] After sample collection and transport, the QPCR method can be performed in 3 hours or less. With further improvements this may be shortened to 2 hours or less.[2]

We expand on our previous analysis to include 2 additional freshwater beaches, an assessment of nonenteric illnesses (upper respiratory illness [URI], rash, eye irritations, and earaches), and separate analyses by age.

METHODS

Study Design

We conducted a prospective study of visitors to freshwater Great Lake beaches on Lake Michigan and Lake Erie during the summers of 2003 and 2004. The data collection

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methods have been described previously.[1] In brief, we attempted to enroll all beach-goers between 11:00 AM and 5:00 PM during summer weekends and holidays. We excluded unaccompanied minors (below 18 years) or those who could not speak English or Spanish. We interviewed volunteers as they were leaving the beach to ascertain information about swimming and other activities. Ten to 12 days later, one of the adults in the household was interviewed by telephone about health symptoms experienced by participating household members. All subjects provided oral consent. The study procedures were approved by the Institutional Review Board for the Centers for Disease Control and Prevention.

Beach Descriptions

Human-derived pollution sources generally cause the most health concern,[3] and beaches with such pollution were the focus of these studies. In 2003, we conducted studies at West Beach (on Lake Michigan in Indiana Dunes National Seashore in Indiana) and Huntington Beach (on Lake Erie near Cleveland, OH). In 2004, we studied 2 additional Lake Michigan Beaches: Silver Beach, near St. Joseph, Michigan, and Washington Park Beach in Michigan City, Indiana. The range of fecal indicator-bacteria concentration at these beaches is related to contamination by effluent from sewage treatment plants. Water quality at each beach was influenced by point-source tributaries that received combined treated sewage treatment discharges from communities with populations of at least 38,000 and with flow rates of over 10 million gallons per day (see Appendix A, available with the online version of this article, for additional details). These sewage plants provided secondary treatment as well as disinfection with chlorine or ultraviolet radiation during the summer.

Water Sample Collection and Sample Analysis

Water samples were tested for fecal indicator bacteria *Enterococcus* and *Bacteroides* using QPCR. Because of problems in the sensitivity of the *Bacteroides* QPCR assay, the data in 2004 were insufficient to assess this indicator in relation to health effects. Samples were also tested for *Enterococcus* using EPA Method 1600,[4] one of the culture-based methods currently recommended by the EPA for recreational freshwater monitoring.[5]

We collected water samples at 8:00 AM, 11:00 AM, and 3:00 PM, along 3 transects perpendicular to the shoreline—one sample in waist-high water (1 m deep) and one in shin-high water (0.3 m deep). Transects were located at least 60 m apart to encompass the swimming area. Because rock jetties at Huntington Beach prevented free circulation of water, we collected 4 additional samples at each sampling time to better characterize the water quality. Following collection, samples were placed in coolers and maintained on ice at 1 to 4°C. Analyses of samples by Method 1600 for *Enterococcus* were performed by local laboratories within 6 hours of collection. Samples were filtered for QPCR analysis within 6 hours of collection. To ensure consistency across the 4 beaches, the filters were frozen and sent on dry ice by overnight express for analysis by EMSL Analytical, Inc. Laboratory (Westmont, NJ).

The QPCR method used in this study has been previously described.[1,6] In brief, organisms in water samples were collected by membrane filters, total DNA was extracted, and polymerase chain reaction (PCR) amplification of a genus-specific DNA sequence of *Enterococcus* was carried out using the TaqMan PCR product detection system. The reactions were performed in a thermal cycling instrument (SmartCycler System, Cepheid, Sunnyvale, CA) that automated the detection and quantitative measurement of the fluorescent signals produced by probe degradation during each cycle of amplification. Ratios of the target sequences in a test sample were compared with a calibrator sample using an arithmetic formula, referred to as the Comparative Cycle Threshold Method.[7] These ratios were converted to measurements of calibrator cell equivalents in test samples through the use of calibrator samples processed in the same manner as the test samples and containing a known quantity of the target organism cells. Results are reported in QPCR cell equivalents (QPCR CE) per 100 mL of original sample.

At each sampling time we recorded environmental conditions, including air and water temperature, cloud cover, rainfall, wind speed and direction, wave height, beach population density, boats, animals (number and type), and debris.

Health Assessments

We assessed 5 endpoints, defined a priori, and similar to those previously studied.[8–13]

1. "Gastrointestinal illness" (GI illness) was defined as any of the following: diarrhea (three or more loose stools in a 24-hour period); vomiting; nausea and stomach ache; nausea or stomach ache, and interference with regular activities (missed time from work or school, or missed other regular activities as a result of the illness).
2. "Upper respiratory illness" (URI) was defined as any 2 of the following: sore throat, cough, runny nose, cold, or fever.
3. "Rash" was defined as a rash or itchy skin.
4. "Eye ailments" were defined as either eye infection or watery eye.
5. "Earache" was defined as earache, ear infection, or runny ears.

Consistent with some other studies,[8,10,11] URI and GI illness were not restricted to persons with fever, since infections can produce these illnesses without fever (eg, *E. coli* 0157:H7 and norovirus infections). We were also concerned about the accuracy of self-reported low-grade fever.

People who were ill within 3 days before their beach visit were excluded for the outcome with which they had been afflicted. We examined various definitions of GI illness

including diarrhea (three or more loose stools in a 24-hour period) alone and GI illness with complications (defined as missing regular activities, using medications, or visiting a health provider as a result of a GI symptom).

Definition of Swimming

"Swimmers" were those who reported immersing their body to their waist or higher. In our previous analysis, immersion to the waist showed a pattern of risk similar to head immersion.[1] Nonswimmers were defined as those who reported no contact with water. Those entering the water not up to their waist were classified as "waders."

Statistical Analysis

Because QPCR CE were highly skewed, raw data were log-transformed (base 10). The arithmetic mean of the log-transformed values was used to summarize water quality at a given day, time, or location. We previously used a maximum-likelihood method to impute results for samples below the limit of detection.[1] However, QPCR CE from 2004 beaches were not as well approximated by a log-normal distribution, making imputation based on this exact distributional assumption questionable. Furthermore, we had concern that partial inhibition may have been responsible for some nondetected results, making the imputed detection limits incorrect. We therefore excluded samples below the limit of detection from the calculation of averages. However, this choice of method for dealing with the limit of detection did not affect the results (see Appendix B, available with the online version of this article). We focused the analyses primarily on 2 summary measures: the daily average of all samples and the average of the 8:00 AM samples. The daily average represented average water quality at a beach on a particular day. The 8:00 AM average was used to determine if morning water quality was predictive of illness among swimmers exposed later that day—an important consideration for assessing the utility of a faster method such as QPCR for water quality evaluation. Analyses using depth-specific averages were also conducted and results are shown in Appendix B. Analysis of variance models were used to explore the relationship between $\log_{10}$ QPCR CE with beach, collection date, time, and sample depth.

To account for correlated environmental measurements as potential confounders of the swimming and health effects relationships, we used principal-components analysis to produce summary components. The 5 principal components that accounted for the majority of the variability (54%) were included in health effects regression models. These 5 components were beach-goer density, temperature (water and air), rainfall, wind direction and debris, and wind speed and wave height. To avoid data loss when one or more of the environmental observations were missing (18 of 85 days), principal components were imputed using best-subset regression.[14]

We used generalized linear-regression models to evaluate the association between water quality and health effects. Logistic regression models were used to describe the strength of the association between the QPCR CE measures and incidence of illness among swimmers. Models using an identity link and a binomial error structure (linear model) were used to directly estimate the attributable risk[15] (swimmer risk minus nonswimmer risk), which we refer to as "swimming-associated illness." Although the linear and logistic models produced similar results, the linear models allowed direct estimation of the attributable risk, which is often considered a more meaningful and direct statement of risk.[5] Nonswimmers were included in models and were assigned water quality exposures of zero. Indicators for "swimming" and "beach" were included in all models. Log-linear models were used to estimate the adjusted cumulative incidence ratio associated with swimming (without regard to water quality).[15] Robust estimates of variance were used to account for the nonindependence of observations within household.[16–18]

Covariates strongly associated with swimming, water quality or illness, or those considered by investigators to be potential confounding factors were considered for inclusion in regression models. These factors included age, sex, race, contact with animals, contact with other persons with diarrhea, number of other visits to the beach, any other chronic illnesses (GI, skin, asthma), digging in sand, and the first 5 principal components of the environmental/meteorological factors (described above). An indicator was also created for a festival that took place at Silver Beach, drawing 17,000 visitors to an area adjacent to the beach. For URI, rash, and eye outcomes, use of insect repellent and sun block were also considered. For each analysis, the set of covariates was reduced through a change-in-estimate procedure.[19] A criterion of a 5% change was used, although this was occasionally relaxed to obtain a parsimonious model. The selection procedure generally reduced the numbers of covariates to 7 or fewer.

To evaluate heterogeneity in the indicator/illness relationship across the beaches, we graphically examined the relationship at each beach and conducted likelihood ratio tests. These tests compared models with interaction terms between beach and water quality (which allowed slopes to differ across beaches) with restricted models constrained to a single slope across the 4 beaches.

We conducted separate analysis for the age categories 0 to 10 years, 11 to 54 years, and 55 years and older. The age groups were selected a priori based on sample size and investigators' judgment.

RESULTS

A total of 21,015 interviews from 10,093 household groups were completed (Appendix B). Respondents at the 4 beaches differed by age, race, miles traveled to the beach and proportion of swimmers (Appendix B). Respondents were

85% white and 56% female, with a median age of 27 years. Swimmers were younger than nonswimmers (median age 19 and 35 years, respectively) but were equally likely to report rash, sore throat, vomiting, and eye irritations in the 3 days prior to the beach visit, chronic respiratory illness (eg, asthma), and chronic skin problems (Table 1). Slightly fewer swimmers compared with nonswimmers reported chronic GI conditions (2% vs. 3%), GI symptoms (other than vomiting) in the 3 days prior to the beach visit (2% vs. 3%), chronic allergies (18% vs. 21%), and consumption of red or raw meat prior to or immediately after the beach visit (8% vs. 10%). There were more female beach-goers than male in all 3 water-use groups, with the largest discrepancy among the waders (63% vs. 37%) and the smallest among the swimmers (52% vs. 48%). Most were white. The percentages of other races were similar across water-use groups, except that the percentage of Hispanic/Latino respondents was highest among the swimmers. More swimmers than nonswimmers reported using sunblock (61% vs. 40%), insect repellant (3% vs. 2%), and having had contact with animals (79% vs. 75%).

Water Quality

Enterococcus QPCR CE differed by beach (Table 2) and sample depth. Median QPCR CEs at shin depth were higher than waist depth (93 and 65 QPCR CE/100 mL, respectively). Collection time was not an important factor in the variability of QPCR CE, although QPCR CE levels measured at 3:00 PM were slightly higher than at 8:00 AM (median QPCR CE/100 mL was 74, 78, and 80 at 8:00 AM, 11:00 AM, and 3:00 PM, respectively).

Twenty-five of 78 days (32%) exceeded the current geometric mean guideline value of 33 colony forming units (CFU)/100 mL *Enterococcus* measured by Method 1600.[5]

TABLE 1. Characteristics of Nonswimmers, Waders, and Swimmers

	Nonswimmers (n = 6888) No. (%)	Waders (n = 3597) No. (%)	Swimmers (n = 10,436) No. (%)
Age (yrs)			
0–4	365 (5)	303 (9)	975 (10)
5–10	242 (4)	231 (7)	2156 (21)
11–19	815 (12)	360 (10)	2007 (20)
20–54	4574 (68)	2304 (66)	4599 (45)
55+	776 (11)	319 (9)	386 (4)
Sex			
Male	2786 (40)	1332 (37)	5049 (48)
Female	4098 (60)	2260 (63)	5366 (52)
Race			
White	5848 (86)	3143 (89)	8617 (84)
Black	231 (3)	102 (3)	260 (3)
Asian	122 (2)	64 (2)	118 (1)
American Indian	21 (<1)	16 (1)	27 (<1)
Hispanic/Latino	554 (8)	197 (6)	1138 (11)
Multiethnic/other	51 (1)	26 (1)	129 (1)
Conditions in the 3 d prior to the beach visit			
Vomiting	59 (1)	41 (1)	95 (1)
Other GI symptoms	178 (3)	79 (2)	179 (2)
Sore throat	397 (6)	201 (6)	605 (6)
Rash	155 (2)	74 (2)	227 (2)
Eye irritations	35 (1)	16 (<1)	47 (<1)
Earache	84 (1)	35 (1)	147 (1)
History of allergies	1467 (21)	781 (22)	1879 (18)
History of chronic GI illness	208 (3)	106 (3)	218 (2)
Any history of chronic GI illness, asthma, or allergies	1980 (29)	1061 (30)	2707 (26)
Contact with animals 48 h prior to or after beach visit, or between beach visit and phone interview	5178 (75)	2821 (78)	8221 (79)
Consumption of raw meat 48 h prior to beach visit or between beach visit and phone interview	701 (10)	333 (9)	881 (8)

TABLE 2. *Enterococcus* QPCR CE by Beach (QPCR CE/100 mL)

	No.	Mean (SD)	Median	Min	25th Percentile	75th Percentile	Max
All beaches	1359	770 (10,800)	76.4	0.050	27.5	267	376,000
West Beach	320	572 (1280)	134	0.080	41.7	486	15,800
Huntington Beach	339	450 (1300)	127	0.050	32.8	327	14,800
Silver Beach	352	553 (6260)	56.8	0.14	15.1	143	117,000
Washington Park Beach	348	1480[a] (20,300)	60.0	0.080	23.1	165	376,000

[a]Mean influenced by outlying maximum value.

Twenty-two percent (333 of 1482) of individual samples exceeded the single sample maximum of 61 CFU/100 mL.[5]

Health Effects

The incidence of new GI illness was 7.3% (1497 of 20,414) during the 10 to 12 day follow-up period. GI illness incidence was highest among children younger than 5 years (9.0%) and lowest among those aged 55 and older (4.9%). The adjusted risk of GI illness was 1.44 times higher in swimmers than nonswimmers (95% CI = 1.27–1.64; Table 3). The risks among children aged 10 and younger, and children and adults aged 11 to 54, were similar to the pooled risk. Among those aged 55 and older, swimmers reported 2.3 times as many illnesses as nonswimmers of the same age (1.33–3.99; Table 4). Children aged 5 and younger showed a similar pattern of risk as those aged 10 years and younger, but with the exception of GI illness (1.67 [CI = 1.03–2.69]), small sample sizes prohibited making conclusions about this age group.

Approximately 5.7% of respondents reported URI. Incidence was highest in children younger than 5 (10.6%) and lowest in those aged 55 and older (2.5%). The crude incidence of URI was higher among swimmers than nonswimmers, but after adjustment there was little difference in risk (1.06 [0.90–1.24]; Table3]. Age was a strong confounder because young respondents were both more likely both to swim and report URI. Among children aged 10 years and younger, URI risk was not elevated among swimmers (0.95 [0.66–1.38]; Table 4).

Approximately 2.7% of all respondents reported rash, with the highest incidence in children younger than 5 years (4.1%), and the lowest in those aged 55 and older (2.1%). Swimmers reported more rash than nonswimmers (1.38 [CI = 1.12–1.72]; Table 3). Rashes occurred more frequently on the upper and lower back (26%) of swimmers reporting rash than of nonswimmers reporting rash (12%).

The incidence of eye irritations and infections was 2.9%; these were reported with equal frequency by swimmers and nonswimmers (1.00 [0.81–1.24]; Table 3).

TABLE 3. Illness Incidence and Adjusted Cumulative Incidence Ratios (aCIR) Comparing Swimmers With Nonswimmers (Excluding Waders)

	Incidence		
Illness	Nonswimmers No. (%)	Swimmers No. (%)	aCIR (95% CI)
GI	397 (6.0)	849 (8.3)	1.44 (1.27–1.64)
URI	321 (5.0)	589 (6.0)	1.06 (0.90–1.24)
Rash	144 (2.1)	305 (3.0)	1.38 (1.12–1.72)
Eye ailments	219 (3.2)	280 (2.7)	1.00 (0.81–1.24)
Earache	78 (1.2)	190 (1.9)	1.63 (1.23–2.17)

Numbers are those reporting new symptoms, among those without baseline symptoms. For GI illness, subjects reporting vomiting or other GI symptoms in the past 3 d shown in Table 1 were excluded. Fourteen nonswimmers and 15 swimmers reported both vomiting and other GI symptoms at baseline. aCIR estimated from log–linear regression model.

Relationships Between Water Quality and Health

The incidence of GI illness was consistently associated with *Enterococcus* QPCR CE exposure (Table 5, Figs. 1 and 2). Among all subjects, a 1 $\log_{10}$ increase in the daily QPCR CE average resulted in a 1.26 increase in the risk (odds) of GI illness (95% CI = 1.06–1.51). The relationship was stronger among children, with a similar association for those aged 10 years and younger (1.69 [1.24–2.30]), 5 and younger (1.67 [1.08–2.57]), and 2 and younger (1.65 [0.81–3.36]). The association between the 8:00 AM *Enterococcus* QPCR CE average and GI illness was nearly identical to that of the daily average. As illustrated in Figure 1, 1000 swimmers exposed to 100 *Enterococcus* QPCR CE would experience an average of 34 more episodes of GI illness than nonswimmers. One thousand swimming children aged 10 and younger would experience an average of 49 more episodes than nonswimming children (Fig. 2). The associations between *Enterococcus* QPCR CE and GI illness were positive at each of the 4 beaches, and tests for heterogeneity indicated no difference in these relationships across the 4 beaches among all subjects ($P = 0.84$), or among children aged 10 and younger ($P = 0.65$). Crude rates of GI illness and numbers exposed are presented in Appendix C (available with the online version of this article).

TABLE 4. Adjusted Cumulative Incidence Ratios (aCIR) Comparing Swimmers With Nonswimmers by Age and Beach

	GI Illness aCIR (95% CI)	URI aCIR (95% CI)	Rash aCIR (95% CI)	Eye aCIR (95% CI)	Earache aCIR (95% CI)
Age (yrs)					
≤10	1.42 (0.99–2.11)	0.95 (0.66–1.38)	1.38 (0.81–2.36)	1.65 (0.78–3.52)	1.56 (0.78–3.12)
11–54	1.40 (1.22–1.61)	1.12 (0.93–1.35)	1.40 (1.10–1.79)	1.01 (0.80–1.27)	1.77 (1.28–2.45)
55+	2.30 (1.33–3.99)	0.89 (0.38–2.06)	0.86 (0.31–2.38)	0.63 (0.30–1.33)	0.62 (0.09–4.47)
Beach					
West Beach	1.90 (1.23–2.92)	1.29 (0.74–2.24)	2.31 (1.23–4.32)	1.32 (0.77–2.27)	1.83 (0.71–4.71)
Huntington Beach	1.39 (1.03–1.86)	1.08 (0.70–1.68)	0.81 (0.44–1.51)	0.63 (0.36–1.10)	1.97 (0.78–4.98)
Washington Park	1.32 (0.99–1.74)	0.98 (0.64–1.50)	1.33 (0.85–2.08)	1.41 (0.87–2.27)	1.32 (0.71–2.46)
Silver Beach	1.43 (1.18–1.74)	1.03 (0.83–1.29)	1.39 (1.02–1.88)	0.94 (0.70–1.27)	1.60 (1.05–2.45)

aCIR estimated from log-linear regression model.

TABLE 5. Adjusted Odds Ratios (aOR) for Illness Associated With a 1-log Increase in *Enterococcus* QPCR CE Exposure (Daily Average and 8:00 AM Average)

Age (yrs)	Average	GI Illness aOR (95% CI)	Diarrhea aOR (95% CI)	GI Symptom With Complications aOR (95% CI)	URI aOR (95% CI)	Rash aOR (95% CI)	Eye aOR (95% CI)	Earache aOR (95% CI)
All	Daily	1.26 (1.06–1.51)	1.31 (1.06–1.61)	1.27 (1.04–1.56)	0.87 (0.69–1.09)	1.21 (0.92–1.58)	0.80 (0.59–1.07)	1.01 (0.69–1.48)
	8:00 AM	1.29 (1.10–1.52)	1.36 (1.13–1.63)	1.19 (0.98–1.46)	0.95 (0.76–1.17)	1.03 (0.80–1.32)	0.91 (0.70–1.18)	0.96 (0.69–1.33)
≤10	Daily	1.69 (1.24–2.30)	2.02 (1.39–2.93)	1.60 (1.04–2.45)	0.83 (0.54–1.27)	1.58 (0.90–2.76)	0.80 (0.45–1.42)	0.73 (0.41–1.28)
	8:00 AM	1.67 (1.25–2.22)	1.98 (1.36–2.88)	1.56 (1.05–2.31)	1.05 (0.71–1.54)	1.18 (0.68–2.06)	1.14 (0.67–1.93)	0.61 (0.34–1.09)
11–54	Daily	1.13 (0.93–1.39)	1.17 (0.92–1.49)	1.19 (0.95–1.50)	0.91 (0.72–1.15)	1.17 (0.87–1.59)	0.73 (0.54–0.99)	1.19 (0.77–1.84)
	8:00 AM	1.16 (0.96–1.39)	1.21 (0.97–1.51)	1.09 (0.86–1.37)	0.93 (0.74–1.16)	1.02 (0.77–1.35)	0.89 (0.68–1.15)	1.16 (0.80–1.68)
55+	Daily	1.21 (0.47–3.09)	0.68 (0.23–2.02)	1.62 (0.54–4.83)	0.42 (0.05–3.28)	0.82 (0.07–10.13)	0.40 (0.06–2.47)	NA
	8:00 AM	1.17 (0.53–2.54)	1.02 (0.43–2.43)	1.63 (0.54–4.83)	0.34 (0.05–2.19)	0.55 (0.03–9.57)	0.46 (0.06–3.38)	NA

aOR estimated from logistic regression model.
NA indicates not applicable (only 8 subjects aged 55 and older reported earache).

As time spent in the water increased beyond 1.5 hours, the association between *Enterococcus* QPCR CE and GI illness also increased. Among subjects exposed at least 2 hours, the risk of GI illness associated with *Enterococcus* QPCR CE exposure increased (1.89 [1.07–3.35]). Children aged 5 to 10 years spent the most time in the water, an average of 1.5 hours compared with 1.2 hours for those younger than 5 years, 1.2 hours for those aged 11 to 20, and less than an hour for those older than 20.

Other illnesses did not show strong or consistent associations with *Enterococcus* QPCR CE. For example, rash was positively associated overall with *Enterococcus* QPCR CE exposure among all subjects (aOR = 1.21 [CI = 0.92–1.58]; Table 5) and particularly among children (1.58 [0.90–2.76]; Table 5). However, there was significant ($P = 0.02$) variation in the association across the 4 beaches, with strong positive associations at 2 of the beaches (at Silver Beach, aOR = 1.78 [CI = 1.08–2.94] and at Washington Park Beach aOR = 1.60 [0.59–4.31]. At the other 2 beaches there was no evidence of an association (Huntington Beach 0.87 [0.27–2.85], and West Beach 0.92 [0.57–1.48]). The data were too sparse to reliably assess heterogeneity on the association of beach-specific rash with *Enterococcus* QPCR CE among children.

Enterococcus Measured by Method 1600 and GI Illness

Swimmers exposed above the guideline value of 33 CFU/100 mL had higher risks than nonswimmers or swimmers exposed below this value (Table 6). As with QPCR CE, the risks associated with *Enterococcus* CFU exposure were more pronounced among children aged 10 and younger.

Enterococcus QPCR CE levels were a stronger predictor of GI illness than the CFU measure. Among all subjects, a quartile increase in *Enterococcus* QPCR CE was associated with a 1.44 (95% CI = 1.10–1.90) increase in the odds of illness for all subjects, whereas a quartile increase *Enterococcus* CFU was associated with only a 1.04 (0.90–1.21) increase. Among children, a quartile increase in *Enterococcus*

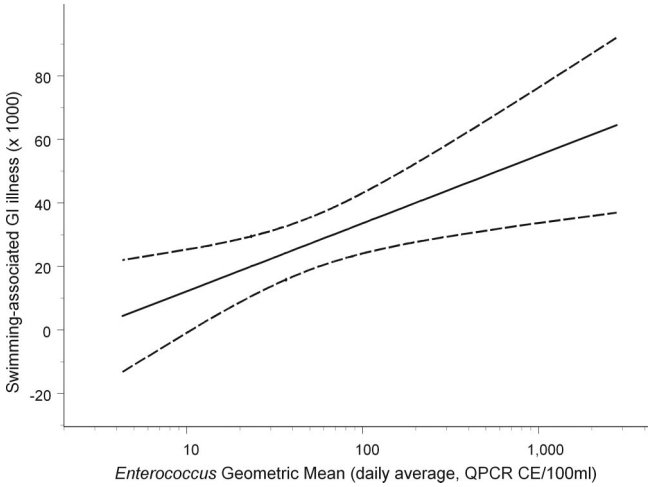

FIGURE 1. Swimming-associated GI illness rate (rate in swimmers minus rate in nonswimmers) among all subjects as a function of daily average Enterococcus QPCR Cell Equivalent exposure. Swimming-associated illness rate estimated from linear regression model, adjusting for factors described in Table 5. Swimming-associated GI illness = −0.0091816 + log 10 Enterococcus QPCR CE × 0.0213998. Solid line indicates rate; dashed line indicates 95% confidence interval.

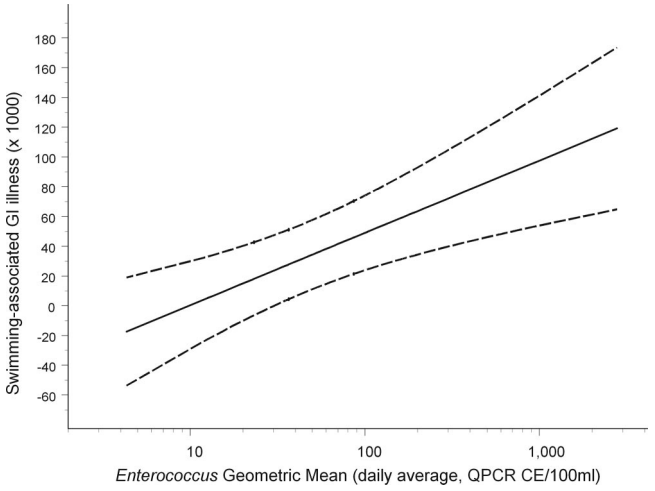

FIGURE 2. Swimming-associated GI illness rate (rate in swimmers minus rate in nonswimmers) among children aged 10 years and below as a function of daily average Enterococcus QPCR Cell Equivalent exposure. Swimming-associated illness rate estimated from linear regression model, adjusting for factors described in Table 5. Swimming-associated illness = −0.04821 + log 10 Enterococcus QPCR CE × 0.0486077. Solid line indicates rate; dashed line indicates 95% confidence interval.

QPCR CE was associated with a 2.27 increase in the odds of illness (1.34–1.68) compared with a 1.21 (95% CI = 0.94–1.55) increase for a quartile increase in *Enterococcus* CFU. In models including both *Enterococcus* measurements, a quartile increase in the daily QPCR average and illness was strengthened (1.56 [1.14–2.12]), while the relationship between a quartile increase in CFU and GI illness was weakened (0.95 [0.79–1.13]).

DISCUSSION

A molecular method for rapid measurement of water quality (*Enterococcus* QPCR CE) was consistently associated with swimming-associated GI illness at 4 freshwater beaches. Furthermore, the *Enterococcus* QPCR CE showed that children up to age 10 years were especially susceptible to GI illness following swimming exposure. While a sensitivity among children to illness following recreational water exposure has often been hypothesized,[3,20–23] this is the first study to demonstrate this sensitivity as a function of microbial water quality. At least one previous study has observed higher rates of swimming-associated illness among children, but the authors did not attribute the increased illnesses to measures of water quality.[24] Children may be more likely to swallow water,[25] transfer water to their mouth after exposure, or, as we observed, spend a longer time in water, resulting in a greater likelihood of contact with pathogens. Children are at increased susceptibility to infection and illness caused by several enteric pathogens.[26,27] Such susceptibility may be due to differences in immune system function, hygiene, and other physiological and behavioral differences.[27]

We saw no evidence of increased susceptibility among those aged 55 and older, but our ability to make valid conclusions among this group was limited because they swam infrequently and reported the lowest incidence of illness. Swimmers in this age group did have a higher overall risk for GI illness compared with nonswimmers, but the relative risk may have been skewed by the low incidence of GI illness among nonswimmers.

Some of the health endpoints were nonspecific, and may have been affected by recall bias. Broad endpoints accounted for the diverse range of symptoms potentially associated with recreational water exposure but such broad symptoms may obscure more specific effects of water quality and swimming exposure. The association between *Enterococcus* QPCR CE and GI illness, however, was robust to different definitions (diarrhea, GI illness with complications). While swimmers may have been more likely to recall illness than nonswimmers, it is unlikely such a recall bias would occur among swimmers at varying levels of water quality. As with our previous analysis, a more stringent definition of swimming with head immersion did not substantially alter the results (data not shown).

Numerous studies have considered associations between fecal indicator bacteria and symptoms of illness. The majority of these studies have observed some association with GI illness.[22,28,29] Associations between fecal indicator

TABLE 6. Adjusted Cumulative Incidence Ratios (aCIR) for Gastrointestinal Illness and Exposure to Current Freshwater *Enterococcus* Method 1600 Guideline Value (33 CFU/100 mL)

	Reference Group							
	Nonswimmers				Swimmers			
	All Subjects		Children Aged 10 and Younger		All Subjects		Children Aged 10 and Younger	
	aCIR	(95% CI)	aCIR	(95% CI)	aCIR	(95% CI)	aCIR	(95% CI)
Nonswimmers	1.00[a]		1.00[a]		NA		NA	
Swimmers								
<33 CFU/100 mL	1.43	(1.24–1.64)	1.30	(0.89–1.91)	1.00[a]		1.00[a]	
≥33 CFU/100 mL	1.61	(1.36–1.91)	1.72	(1.13–2.60)	1.13	(0.96–1.32)	1.32	(1.00–1.73)

[a]Reference category.

bacteria and nongastrointestinal (nonenteric) health conditions appear to be less consistent. Several studies[9–11,13] observed associations with respiratory illness, although not all.[8,12,30–33] Similar inconsistencies have been observed for skin, ear, and eye ailments.[8–10,12,30,31,34] Earaches and ear infections are often associated with swimming and water exposure, but associations with specific indicator organisms have been inconsistent.[8–10,30,35]

Enterococcus QPCR CE was more strongly associated with illness than the currently recommended culture-based method of measuring *Enterococcus*. The QPCR measure may be a truer representation of fecal contamination, because it measures all *Enterococcus* associated with feces, not just viable cells. The molecular measurement of *Enterococcus* DNA provides a stable, conservative means of quantifying the level of fecal contamination, which is not subject to die-off but may mirror the dilution and dispersion of fecal material. Studies have demonstrated that pathogenic microorganisms (especially viruses and certain protists) are capable of surviving the sewage treatment process. Levels of such pathogens in treated effluent are often poorly correlated with indicator bacteria measured by cultural methods.[36] Whereas fecal indicator bacteria are often nondetectable by culture methods following sewage treatment, these same bacteria can be detected by QPCR.[37] A recent study found human adenoviruses at both Silver Beach and Washington Park Beach, with municipal discharges as the likely source.[38]

The water quality at the beaches we studied was influenced by human sources of pollution. We do not know if the relationships we observed between *Enterococcus* QPCR CE and GI illness can be extended to marine beaches, or to recreational waters affected by different sources of fecal contamination. Our failure to observe consistent associations between nonenteric illness and fecal indicator bacteria suggests a continuing need to investigate the causes of excess nonenteric illnesses commonly observed among swimmers.

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Appendix F

Report on additional monitoring for Urban Runoff Sites

DRAFT Final Report

Monitoring of Marine Beaches Impacted by Urban Runoff

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1.0 Introduction

The United States Environmental Protection Agency's (EPA's) recreational water criteria are based on epidemiology studies of publicly owned treatment works (POTW) impacted waters. The Critical Path Science Plan (CPSP), an effort by the EPA Office of Water (OW) and the EPA Office of Research and Development (ORD) to assess recreational criteria, includes the investigation of non-POTW impacted recreational waters to determine whether potential health risks are different than those in recreational waters associated with POTWs. To assist with this investigation, EPA's Standards and Health Protection Division (SHPD) contracted Great Lakes Environmental Center (GLEC) to provide support for a study designed to monitor recreational waters impacted primarily by urban/suburban runoff, which may carry a variety of pollutants including bacterial pathogens and indicators of fecal contamination.

The primary objective of this study was to perform preliminary microbial monitoring (i.e., enumeration of enterococci and *Pseudomonas aeruginosa*, and qPCR analysis) in support of EPA's search for a marine, non-POTW impacted beach affected by urban runoff. Urban runoff is defined as storm water from rain, snowmelt or irrigation that flows over the land surface and is not absorbed into the ground, instead flowing into streams or other surface waters or land depressions, including the possible discharges of storm water or storm water runoff. The marine waters selected for this study are not known by EPA to be impacted by: (1) discharges from POTWs or combined sewer overflows (CSO) or (2) identified discharges of untreated human waste from sanitary sewer systems. Therefore, the study was designed to collect data that will allow for the determination of a relationship between human illness and fecal indicators that originate from urban runoff in the absence of POTW and CSO discharges and untreated human wastes.

2.0 Materials and Methods

The following section briefly outlines the methods by which samples were collected, processed and analyzed for this monitoring effort. Extensive details concerning the collection protocols and analytical methods utilized for this project are provided in the project's Quality Assurance Project Plan (QAPP) and Sampling and Analysis Plan (SAP) (Appendix A and Appendix B, respectively).

Occasionally there were instances when the procedures outlined in the QAPP and SAP were not followed in entirety. Deviations from the QAPP and SAP are explained throughout the text of this report and are summarized in Table 1.

2.1 Sample Locations

Through rigorous selection criteria based on Westat and Molina (2008), five marine beaches were identified by the EPA for monitoring. Three of these beaches are located in South Carolina and two are located in Florida. The South Carolina beaches include: Canes Patch Swash in Myrtle Beach, Surfside Swash in Surfside and Withers Swash in Myrtle Beach. All of these beaches are located in Horry County. The Florida beaches include: Silver Beach and Florida Shores. Both of these beaches are located in Volusia County, north of Daytona Beach. Maps of the sampling locations at each of the five beaches monitored during this study are provided in Figures 1 through 5. Digital pictures collected by GLEC at each beach and swash (where appropriate, see Sampling Procedures below) are provided in Figures 6 through 9 (Canes Patch Swash), Figures 10 through 13 (Surfside Swash), Figures 14 through 17 (Withers Swash), Figure 18 (Florida Shores) and Figure 19 (Silver Beach). Global positioning system (GPS) coordinates for each sampling location are provided in Table 2.

2.2 Sampling Procedures

2.2.1 South Carolina

2.2.1.1 Baseline Sampling

At each of the three South Carolina beaches (Figures 1 through 3), the sampling area was divided into five transects, each located perpendicular to the shoreline, with approximately 100 m between each transect. The sampling area was selected by identifying the main ditch or storm drain affecting the bathing zone. The middle transect was delineated at the main ditch or storm drain. Samples were collected from this transect, and also from two transects to the right (north) of the drain and from two transects to the left (south) of the drain. Therefore, a total of five transects, each located 100 m apart, were sampled at each of the three South Carolina beaches. Three water samples were collected from one location per transect at waist deep (approximately 1.0 m deep, 0.3 m below the surface) in 500 mL, pre-sterilized polycarbonate bottles and then composited into a 2 L pre-sterilized polycarbonate bottle. This composite sample was used for enterococci, *Pseudomonas* and qPCR sample analyses.

In addition to sample collection at each of the five beach transects, sampling was also conducted in the ditch or storm drain stream used to delineate the beach transects (Figures 1 through 3). The open ditch or stream was divided into three segments, each between 100 and 300 m apart. Three water samples were collected per segment at a depth of 0.3 m, if water depth allowed for such an interval, in 500 mL, pre-sterilized polycarbonate bottles. If water depth was inadequate to collect a sample from a 0.3 m depth, samples were collected to avoid re-suspension of bottom sediments to the greatest degree possible.

With two exceptions, baseline sampling at each of the three South Carolina beaches was conducted by GLEC three times per week (Sundays, Tuesdays and Thursdays), over a period of five weeks from December 16, 2008 through January 18, 2009. Because this sampling effort coincided with the Christmas and New Year holidays, sampling originally scheduled to be

completed on these days was moved to the Friday immediately after the holidays. GLEC attempted to collect the samples within a two hour time frame during the morning hours (9:00 to 11:00 AM) at each beach and ditch/storm drain. This was accomplished for most sampling dates. However, there were instances when GLEC was unable to complete sample collection within the two hour window because of weather, beach conditions and/or access (see Table 1). Excluding day one of the sampling effort, the longest window during which all samples were collected on a given day in South Carolina was approximately 2.5 hours (December 23, 2008).

On Tuesdays only, one additional water sample was collected for dissolved organic carbon (DOC) analysis from each of the five beach transects and three storm drain/ditch locations (for each of the three South Carolina beaches). This sample was collected in an ashed (at ≥ 400°C for two hours) 250 mL glass bottle, at the same location and depth as the composite samples used for enterococci, *Pseudomonas* and qPCR analyses.

2.2.1.2 Rain Event Sampling

When there was an appreciable rain event (≥ 0.25 inches of rain) that was not captured during the scheduled baseline sampling days, additional sampling was conducted within two to four hours after accumulation of 0.25 inches or more of rainfall. In South Carolina, rain event samples were collected on January 13 and January 29, 2009. For each of these two rain events, GLEC followed the sampling procedures, and sampled at the same locations, as those outlined above.

2.2.1.3 Duplicate and Field Blank Sample Collection

For enterococci, *Pseudomonas* and qPCR samples collected during the baseline monitoring period, field duplicates were collected at a rate of ≥ 10%. These samples were equally divided across the three beaches and ditch/storm drain locations. Duplicate samples were collected in the same manner in which investigative samples were collected. Field blank samples for enterococci, *Pseudomonas* and qPCR collected during the baseline monitoring period were collected at a rate of ≥ 5%. These samples were also equally divided across the three beaches and ditch/storm drain locations. Field blanks for the bacterial analyses were collected by filling three of the 500 mL, pre-sterilized polycarbonate sample bottles with sterile phosphate buffer and compositing them into a 2 L pre-sterilized polycarbonate bottle. The field blanks were then handled in the same manner as investigative samples.

For DOC, field duplicate samples were collected in the same manner in which investigative samples were collected: every Tuesday, in conjunction with collection of the investigative DOC samples. One DOC field duplicate sample was collected at each beach or ditch/storm drain location every Tuesday. Field blank samples for DOC were collected every Tuesday in conjunction with the collection of the investigative DOC samples by filling a 250 mL ashed glass bottle with 250 mL of laboratory de-ionized (DI) water. The field blank samples were then processed in the same manner as the investigative DOC samples.

Rain event field blank and field duplicate samples were collected at a rate of one blank and one duplicate for each rain event at each beach or dith/storm drain location.

2.2.2 Florida

2.2.2.1 Baseline Sampling

At each of the two Florida beaches (Figures 4 and 5), the sampling area was divided into three transects, with approximately 200 m between each transect. Each transect was situated perpendicular to the shoreline. Three water samples were collected from one location per transect at waist deep (approximately 1.0 m deep, 0.3 m below the surface) in 500 mL, pre-sterilized polycarbonate bottles. The three samples were then composited into a single 2 L pre-sterilized polycarbonate bottle.

With three exceptions, baseline sampling at each of the two Florida beaches was conducted three times per week (Sundays, Tuesdays and Thursdays), over a period of five weeks from December 16, 2008 through January 18, 2009. Because this sampling effort coincided with the Christmas and New Year holidays, sampling originally scheduled to be completed on these days was moved to the Friday immediately after the holidays. In addition, samples were not collected on December 28 (Sunday) because of a flight cancellation for the GLEC field crew leader (Table 1). Therefore, this sample was collected the next day (December 29, 2008). With few exceptions, GLEC collected the samples within a two hour time frame during the morning hours (9:00 to 11:00 AM) at each beach.

On Tuesdays only, one additional water sample was collected for DOC analysis from the three transects at each of the two beach locations. This sample was collected in an ashed (at ≥ 400°C for two hours) 250 mL glass bottle at each transect, at the same location and depth as the composite samples used for enterococci, *Pseudomonas* and qPCR analyses.

2.2.2.2 Rain Event Sampling

When there was an appreciable rain event (≥ 0.25 inches of rain) that was not captured during the baseline sampling period, additional sampling was conducted within two to four hours after accumulation of 0.25 inches of rainfall. In Florida, samples were collected during one rain event on January 30, 2009. During this rain event GLEC followed the sampling procedures, and sampled at the same locations, as those outlined above.

2.2.2.3 Duplicate and Field Blank Sample Collection

For enterococci, *Pseudomonas* and qPCR samples collected during the baseline sampling period, field duplicates were collected at a rate of ≥ 10%. These samples were equally divided across the two beaches. Duplicate samples were collected in the same manner in which investigative samples were collected. Field blank samples for enterococci, *Pseudomonas* and qPCR collected during the baseline monitoring period were collected at a rate of ≥ 5%. These samples were also equally divided across the two beaches. Field blanks for the bacterial analyses were collected by filling three of the 500 mL, pre-sterilized polycarbonate sample bottles with sterile phosphate buffer and compositing them into a 2 L pre-sterilized polycarbonate bottle. The field blanks were then handled in the same manner as investigative samples.

For DOC, field duplicate samples were collected in the same manner in which investigative samples were collected: every Tuesday, in conjunction with collection of the investigative DOC samples. One DOC field duplicate sample was collected at each beach every Tuesday. Field blank samples for DOC were collected every Tuesday in conjunction with the collection of the investigative DOC samples by filling a 250 mL ashed glass bottle with 250 mL of DI water. The field blank samples were then processed in the same manner as the investigative DOC samples.

Rain event field blank and field duplicate samples were collected at a rate of one blank and one duplicate for the rain event at each beach location.

2.3 Sample Collection

Samples were collected at each location using the methods outlined in Section 9060 of APHA et al. (1998). A brief summary of this method is outlined below. Extensive details concerning the methods by which samples were collected are also provided in the QAPP and SAP (Appendix A and B, respectively).

Using aseptic techniques (i.e. latex or nitrile gloves and, where necessary, shoulder length polyethylene gloves, and waders), three water samples were collected from one location per transect at waist deep (approximately 1.0 m) in 500 mL, pre-sterilized polycarbonate bottles. To collect these samples, the un-capped, face down 500 mL sample bottle was lowered to a depth of

approximately 0.3 m below the water surface, taking care to avoid surface scum, vegetation and substrates. The mouth of the container was pointed away from the sampler. The bottle was then righted, with the opening facing away from the body, and raised through the water column, allowing the bottle to fill completely. This process was repeated three times (once per bottle). Bottles were capped immediately after sample collection.

The three water samples were then composited into a 2 L, pre-sterilized polycarbonate bottle, and a reducing agent (three tablets of sodium thiosulfate ($Na_2S_2O_3$)) was added to prevent the continuation of bactericidal action and to reduce any strong oxidants that may have been present in the sample and interfered with the analysis. Samples were placed in a cooler immediately after collection and maintained at < 4°C on wet ice.

A 500 mL portion of each of the composited water samples was removed from the 2 L polycarbonate bottle and transferred to a 500 mL pre-sterilized polycarbonate bottle and stored on wet ice for later filtration (within 6 hours) for qPCR analysis. There were three individual filters processed for each qPCR sample collected in South Carolina and five individual filters were processed for each qPCR sample collected in Florida. Each of these filters was maintained in a cooler on dry ice. The remaining portion of the composite sample (1,000 mL) was delivered to the local analytical laboratory on wet ice where it was filtered for enumeration of culturable enterococci and *Pseudomonas aeruginosa*.

2.4 Collection of Ancillary Data

Site-specific ancillary data were collected during sampling visits at each of the five beaches and each of the nine ditch/storm drain locations. The parameters that were measured in the field are shown in Table 3, along with descriptions of their measurement. The ancillary data collected for this effort are provided on the accompanying project DVD and are not discussed directly in this report.

2.5 Sample Filtration and Processing

2.5.1 qPCR Samples

With few exceptions, filtration of the composite water samples for qPCR analysis was performed by GLEC within six hours of sample collection. However, there were instances (particularly in South Carolina) when, because of the exceptional level of effort required for sample filtration, the field crew exceeded the six hour filtration threshold. Instances when the six hour threshold was exceeded are highlighted in the final data files (see project DVD) and Table 1.

In addition to occasionally exceeding the recommended holding time, no qPCR filters were collected in South Carolina on December 16, 2008 because of the extraordinary time required to collect and filter the samples (filtration of the first of six batches of samples on this date was not completed until approximately six hours after the recommended holding time). Because of the holding time exceedance for qPCR filters on December 16, 2008 (the first day of sample collection), GLEC reduced the filtration effort by only collecting two filters per sample on December 18 and 21, 2008. Per EPA's guidance, three qPCR filters were collected per sample in South Carolina beginning on December 23, 2008 (and continuing for the remainder of the study). However, only one qPCR filter was collected per sample during the rain event on January 29, 2009 in South Carolina because of the concern of exceeding the holding time for all samples if three filters were collected per sample. Exceptions to the QAPP in regards to the number of qPCR filters collected in South Carolina are summarized in Table 1.

Specific details regarding the procedure by which qPCR samples were filtered and preserved are provided in the project's SAP (Appendix B). Briefly, five 100 mL aliquots of the composited water sample were filtered using separate 47 mm, 0.4 µm polycarbonate filters. These filters were then placed in microcentrifuge tubes and stored on dry ice (approximately -80°C) until shipment. In

Florida two of the five qPCR filters for each sample were shipped on dry ice to NRMRL/WSWRD, Cincinnati, OH, and the other three filters were shipped to NERL/ERD, Athens, GA (see full addresses below). In South Carolina one of the three qPCR filters for each sample was shipped on dry ice to NRMRL/WSWRD, Cincinnati, OH, and the other two filters were shipped to NERL/ERD, Athens, GA. The filters were shipped to each laboratory after the first two weeks of sample collection, and then after the last three weeks of sample collection. Additional samples (rain events) were shipped separately to the EPA laboratories. All qPCR sample filters were shipped packed in dry ice (approximately -80°C) via overnight courier.

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In addition to investigative samples, negative controls for the rinse procedure (i.e. equipment blanks) using PCR grade water were collected twice in both South Carolina and Florida for each laboratory (i.e. two blanks per state per EPA lab for a total of eight equipment blanks). In addition, a suspension with a known concentration of *Enterococcus faecalis* was filtered to provide a positive control. The positive control samples were provided to each EPA laboratory receiving qPCR samples at a rate of two positive controls per state (total of eight samples).

The analytical results for qPCR are available from EPA and are not discussed directly in this report.

2.5.2 Dissolved Organic Carbon Samples

The DOC samples were kept on wet ice or in a refrigerator and stored at approximately 4 °C until sample filtration. With several exceptions, each DOC sample was filtered within 24 hours of sample collection (see project DVD and Table 1). The method by which samples were filtered is provided in the project's SAP (Appendix B). Once filtered, the DOC samples were shipped to NERL/ERD, Athens, GA (see address, above) after the first two weeks of sample collection, and after the last three weeks of sample collection. The samples were shipped packed in wet ice via overnight courier.

The analytical results for DOC are available from EPA and are not discussed directly in this report.

2.5.3 Bacteriological Samples

The remaining portion of the 2 L composite samples (after the removal of the 500 mL sample for qPCR processing) was delivered to local analytical laboratories on wet ice (approximately 4°C)

where they were filtered for enumeration of culturable enterococci and *Pseudomonas aeruginosa*. With few exceptions, they were processed within six hours of sample collection (see project DVD and Table 1). In Florida, laboratory analyses were completed by the Volusia County Environmental Health Laboratory. In South Carolina, laboratory analyses were completed by Environmental Systems Testing Services. Contact information for these laboratories is provided below.

1. Volusia County Environmental Health Laboratory
   1250 Indian Lake Road
   Daytona Beach, FL 32124-3518
   Contact: Jack Towle
   Phone: 386-248-1781
   Fax: 386-248-1785
   Email: jack_towle@doh.state.fl.us

2. Environmental Systems Testing Services
   Post Office Box 1615
   Conway, SC 29528-1615
   Contact: Kellah Webster
   Phone: 843-347-7688
   Fax: 843-347-6739
   Email: kellahwebster@msn.com

2.5.3.1 Enterococci Samples

Enterococci enumeration followed EPA Method 1600 on mEI agar plates (EPA 2006; Haugland et al. 2005). For the culturable enterococci analyses, volumes of 100 mL, 50 mL and 10 mL of the water sample will be filtered, if necessary. These dilutions were adjusted for each beach depending on historical or previous samples. Colony counts from the 100 mL sample volumes were reported unless they exceeded 150, in which case counts from one or the other of the smaller volumes were used after multiplying by an appropriate correction factor to express the enterococci counts in CFU/100 mL.

As described in the method, verification tests on the identities of five colonies per sample were performed for all water samples collected during the first day of the study at each beach site. This verification step was completed at GLEC by a GLEC microbiologist. GLEC coordinated the shipment of processed (counted and marked) filters with the laboratories to avoid weekend delivery (for temperature maintenance concerns) to GLEC.

Each new batch of mEI agar was tested for positive performance using pure cultures of *Enterococcus faecalis*, and for negative performance using a pure culture of a non-target organism, e.g. *E. coli*. The sterility of the filters and phosphate-buffered water used for rinsing the filtration apparatus was also tested with each batch of samples arriving together at the laboratory.

2.5.3.2 *Pseudomonas* Samples

For enumeration of Pseudomonas aeruginosa, the laboratories followed ASTM Method D5246-92 (2004). A volume of 100 to 200 mL was filtered, with appropriate dilutions when bacterial concentrations were high. Colony counts were reported on a per 100 mL basis.

As described in the method, verification tests on the identities of ten colonies per sample were performed in skim milk agar, and were performed for all water samples collected on one day during the first week of the study at each beach site. This verification was completed at GLEC by a GLEC microbiologist. GLEC coordinated the shipment of processed (counted and marked) filters with the laboratories to avoid weekend delivery (for temperature maintenance concerns) to GLEC.

Each new batch of agar was tested for positive performance using pure cultures of *Pseudomonas aeruginosa*, and for negative performance using a pure culture of non-target organisms, e.g. *E. feacalis*. The laboratories reported results from plates producing between 20 and 80 colonies, and not more than 150 colonies total per plate.

3.0 Results and Discussion

3.1 Verifications Tests and Laboratory Audits

The Volusia County Environmental Health Laboratory and Environmental Systems Testing Services provided final microbiological plates to GLEC for verification of the procedures for isolating enterococcus and *Pseudomonas aeruginosa*. Plates from early sample events were sent on ice via FedEx Priority Overnight service from the laboratories to GLEC. Colonies which were counted for data were identified on the plates and subjected to verification procedures as outlined in Method 1600 for enterococcus and Method D5246-92 for *Pseudomonas* (see QAPP in Appendix A). Eight plates were selected at random from each laboratory for evaluation of enterococcus. In addition, eight plates were selected at random for evaluation of *Pseudomonas* from the South Carolina laboratory, but due to lack of growth, only a single plate was available from the Florida laboratory.

Verification results for enterococcus from both the Florida and South Carolina laboratories indicated that all selected counted colonies were correctly verified as enterococcus using the multi-stage process outlined in the Method. Isolation of enterococci in water using mEI media is quite good, with fairly low false positive and false negative results in a variety of environmental water samples.

Verification results for *Pseudomonas* from the Florida laboratory for the single plate containing colony growth were verified as *Pseudomonas aeruginosa*. Seven of the eight plates selected at random for the South Carolina laboratory were also positively identified for *Pseudomonas aeruginosa.* One plate contained colonies thought to be *Pseudomo*nas, but did not produce the correct clearing of the medium or fluorescent green-yellow pigment as described in the Method verification procedure. These colonies had a more “yellow to white” color than the “pink to brown” color specified in the Method. The laboratory was provided with this information to correct future plate counts.

The Florida and South Carolina laboratories were also subjected to audits in order to ensure that the data generated by each laboratory were of the quality necessary to meet the goals of the study.

An initial site visit for each laboratory was conducted by GLEC research staff in order to assess general laboratory operations and to confirm that each microbiology laboratory was clean and prepared, and all necessary equipment was present and calibrated. Observations were made of sample receipt and sample tracking using customary chain-of-custody reports. In addition to the onsite visit, GLEC participated in several pre-implementation phone calls with each laboratory to provide technical support for the laboratory for the selected methods. During the project, GLEC was also in contact with the laboratories to answer questions, and to provide additional technical support as required.

GLEC required that each laboratory send Enterococci and *Pseudomonas* plates from the final project sample event for an assessment and quality control (QC) check of colony counts. Plates were counted by the laboratories, sealed and sent on ice via FedEx Priority Overnight. Upon receipt, GLEC staff independently assessed colony numbers for 10 and 6 randomly selected plates from the South Carolina and Florida laboratories, respectively. Colony numbers were assessed for both enterococcus and *Pseudomonas*. Colony counts were within ± 5% for the Florida laboratory for both bacteria assessed. The South Carolina laboratory was ± 10% for 8 of 10 samples of enterococcus, and 7 of 10 for *Pseudomonas*. Colony numbers for both bacterial types were much higher in South Carolina than Florida (see results below).

At the conclusion of the project, GLEC solicited additional laboratory documentation for quality assurance (QA)/QC data, equipment calibration logs, laboratory supplies and consumables to verify this documentation was in place and up-to-date. No deficiencies for either lab were noted.

Data sheets for the verification and plate counting audits are provided on the project DVD.

3.2 Manipulation of Laboratory Data

The enterococci and *Pseudomonas* concentration data reported by the Florida and South Carolina microbiological laboratories are provided in Appendix C. After reviewing these data, GLEC determined that there were inconsistencies in the method by which the South Carolina laboratory determined plate counts for the samples. On occasion the laboratory ceased counting colonies of *Pseudomonas* or enterococci bacteria once 60 colonies were identified in a particular dilution. In these instances the laboratory reported values as >60 CFU/100 mL (for a 100 mL sample), or another value appropriate for the dilution factor (>240, >600 or >6,000 CFU/100 mL for 25, 10 and 1 mL dilutions, respectively). However, there were also instances when the laboratory counted more than 60 colonies on a plate for any of a number of dilutions. For example, the laboratory may have counted 120 colonies in 25 mL of sample. In this case the South Carolina laboratory may have reported a value of 480 CFU/100 mL.

In addition to the inconsistencies reported above, the South Carolina microbiological laboratory also (on occasion) reported values for all dilutions, regardless of which data were most appropriate (based on the method) for reporting purposes.

To address these inconsistencies, GLEC altered some of the South Carolina laboratory data based on the following rules:

1) If a direct count was reported by the laboratory (i.e., no > sign associated with the data), this value was used without alteration by GLEC.

2) If two direct counts were reported by the laboratory for two separate dilutions of the same sample, GLEC used the count between 20 and 60 for a particular dilution to determine the reported value. If colony counts for a particular dilution were not between 20 and 60, the dilution volume with a count closest to the 20 to 60 range was used to determine the value reported by GLEC.

3) When >60 colonies were reported for all dilutions for the same sample, the smallest dilution was used to determine the reported value.

The GLEC altered data (with original data as reported by the South Carolina laboratory) are provided in Appendix D; these altered data were used in this report. Please note that no data from the Florida microbiological laboratory were altered by GLEC; these data are provided in Appendix C.

Because the inclusion of > or < symbols during the calculation of data means, figure generation, etc. is problematic, GLEC chose to remove the > symbol from data, where appropriate. For example, data with a reported value of >60 were assigned a value of 60 to allow for the generation of data means, figures, etc. in this report. In addition, values less than the reporting limit (e.x. <2) were assigned a value of 0 for the generation of data means, figures, etc. in this report.

3.3 *Pseudomonas* Data

Tables 4 through 6 present *Pseudomonas* concentration data for beaches sampled in South Carolina and Tables 7 and 8 present *Pseudomonas* concentration data for beaches sampled in Florida.

3.3.1 Baseline Data

In South Carolina, concentrations of *Pseudomonas* bacteria collected during the baseline monitoring period ranged between <2 and >600 CFU/100 mL at sites associated with Canes Patch Swash (Table 4), between <2 and 144 CFU/100 mL at sites associated with Withers Swash (Table 5) and between <2 and 176 CFU/100 mL at sites associated with Surfside Swash (Table 6). Mean concentrations at each of the three sampling areas, averaged across the entire baseline sampling period, ranged from 17 to 35 CFU/100 mL (Table 7).

Across the entire baseline sampling period, *Pseudomonas* concentrations at the South Carolina beach transects ranged between 1.7 (Surfside Swash BT-3) and 14 CFU/100 mL (Canes Patch Swash BT-2) (Table 7). When averaging across beach transects, concentrations of *Pseudomonas* bacteria collected during the baseline period were most elevated at Canes Patch Swash (11 CFU/100 mL) and least at Surfside Swash (3.7 CFU/100mL).

Across the entire baseline sampling period, *Pseudomonas* concentrations at the South Carolina ditch/storm drain sampling locations ranged between 18 (Canes Patch Swash DT-3) and 123 CFU/100 mL (Canes Patch Swash DT-2) (Table 7). When averaging across ditch/storm drain sampling locations in South Carolina, concentrations of *Pseudomonas* bacteria collected during the baseline period were most elevated at Canes Patch Swash (74 CFU/100 mL) and least at Withers Swash (43 CFU/100mL). The relatively high *Pseudomonas* bacteria counts associated with the ditch/storm drain at Canes Patch Swash corresponded to elevated concentrations of *Pseudomonas* bacteria observed at the beach transects (Table 4).

Overall, mean concentrations of *Pseudomonas* bacteria in South Carolina were more elevated and variable at the ditch/storm drain sampling locations than at associated beach locations (Figures 20 through 23 and Table 7).

In Florida, *Pseudomonas* bacteria collected during the baseline monitoring period were only measured above the method detection limit in two samples collected at Silver Beach (Table 8) and two samples collected at Florida Shores (Table 9). These four samples had reported *Pseudomonas* concentrations of 1 CFU/100 mL. Three of these four samples were collected on December 18, 2008.

3.3.2 Rain Event Data

In South Carolina, concentrations of *Pseudomonas* bacteria collected during the rain events (January 13 and 29, 2009) ranged between <2 and 168 CFU/100 mL at sites associated with Canes Patch Swash (Table 4), between 4 and >120 CFU/100 mL at sites associated with Withers Swash (Table 5) and between <2 and >120 CFU/100 mL at sites associated with Surfside Swash (Table 6). Mean concentrations at each of the three sampling areas, averaged across the two rain events, ranged from 41 to 60 CFU/100 mL (Table 7). With the exception of the ditch/storm drain sampling locations at Canes Patch Swash, concentrations of *Pseudomonas* bacteria in South Carolina were always more elevated during rain events than during the baseline monitoring period (Tables 4 through 7).

When considering the two rain events in South Carolina, *Pseudomonas* concentrations at the beach transects ranged between 5.0 (Surfside Swash BT-2 and BT-3) and 66 CFU/100 mL (Withers Swash BT-3) (Table 7). When averaging across beach transects, concentrations of *Pseudomonas* bacteria collected during the rain events were most elevated at Canes Patch Swash (52 CFU/100 mL) and least at Surfside Swash (17 CFU/100mL). This pattern was also observed for *Pseudomonas* concentrations observed at the beach transects during the baseline monitoring period.

Across the two rain events, *Pseudomonas* concentrations at the South Carolina ditch/storm drain sampling locations ranged between <2 (Canes Patch Swash DT-2) and 168 CFU/100 mL (Canes

Patch Swash DT-1) (Table 7). When averaging across ditch/storm drain sampling locations in South Carolina, concentrations of *Pseudomonas* bacteria collected during the rain events were most elevated at Withers Swash (87 CFU/100 mL) and least at Canes Patch Swash (72 CFU/100mL). Contrary to the pattern observed during the baseline monitoring period, the relatively high *Pseudomonas* bacteria counts associated with the ditch/storm drains during the rain events did not necessarily translate to elevated concentrations of *Pseudomonas* bacteria observed at the beach transects (Table 7).

In Florida, *Pseudomonas* bacteria collected during the rain event (January 30, 2009) were never measured above the method detection limit.

3.4 Enterococci Data

Tables 4 through 6 present enterococci concentration data for beaches sampled in South Carolina and Tables 7 and 8 present enterococci concentration data for beaches sampled in Florida.

3.4.1 Baseline Data

In South Carolina, concentrations of enterococci bacteria collected during the baseline monitoring period ranged between <2 and 876 CFU/100 mL at sites associated with Canes Patch Swash (Table 4), between <4 and 2,760 CFU/100 mL at sites associated with Withers Swash (Table 5) and between <4 and 2,470 CFU/100 mL at sites associated with Surfside Swash (Table 6). Mean concentrations at each of the three sampling areas, averaged across the entire baseline sampling period, ranged from 86 to 231 CFU/100 mL (Table 7).

Across the entire baseline sampling period, enterococci concentrations at the South Carolina beach transects ranged between 23 (Surfside Swash BT-1) and 177 CFU/100 mL (Canes Patch Swash BT-5) (Table 7). When averaging across beach transects, concentrations of enterococci bacteria collected during the baseline period were most elevated at Canes Patch Swash (163 CFU/100 mL) and least at Surfside Swash (25 CFU/100mL).

Across the entire baseline sampling period, enterococci concentrations at the South Carolina ditch/storm drain sampling locations ranged between 19 (Canes Patch Swash DT-3) and 992 CFU/100 mL (Withers Swash DT-2) (Table 7). When averaging across ditch/storm drain sampling locations in South Carolina, concentrations of enterococci bacteria collected during the baseline period were most elevated at Withers Swash (654 CFU/100 mL) and least at Canes Patch Swash (189 CFU/100mL). Elevated enterococci bacteria counts associated with the ditch/storm drains did not necessarily translate to elevated concentrations of enterococci bacteria observed at the associated beach transects (Table 7).

Overall, mean concentrations of enterococci bacteria in South Carolina were more elevated and variable at the ditch/storm drain sampling locations than at associated beach transects (Figures 24 through 26 and Table 7).

In Florida, concentrations of enterococci bacteria collected during the baseline monitoring period ranged between <1 and 47 CFU/100 mL at sites associated with Silver Beach (Table 8) and between <1 and 108 CFU/100 mL at sites associated with Florida Shores (Table 9). Mean concentrations at each of the two sampling areas, averaged across the entire baseline sampling period, were 12 and 20 CFU/100 mL for Silver Beach and Florida Shores, respectively (Table 7). Concentrations of enterococci measured during the baseline monitoring period were also more variable at Florida Shores than at Silver Beach (Figure 27). Across the entire baseline sampling period, enterococci concentrations at the Florida beach transects ranged between 10 (Silver Beach transect 2) and 26 CFU/100 mL (Florida Shores transect 3) (Table 7).

Enterococci concentrations measured during the baseline monitoring period in South Carolina were more elevated than those measured during the same time period in Florida (Tables 4 through 6, Tables 8 and 9 and Figure 28). Mean concentrations of enterococci bacteria, averaged across the entire baseline sampling period for each of the five beach locations, never exceeded 20 CFU/100 mL in Florida and were never less than 86 CFU/100mL in South Carolina (Table 7).

3.4.2 Rain Event Data

In South Carolina, concentrations of enterococci bacteria collected during the rain events (January 13 and 29, 2009) ranged between <4 and 1,120 CFU/100 mL at sites associated with Canes Patch Swash (Table 4), between <4 and 2,740 CFU/100 mL at sites associated with Withers Swash (Table 5) and between <4 and >2,000 CFU/100 mL at sites associated with Surfside Swash (Table 6). Mean concentrations at each of the three sampling areas, averaged across the two rain events, ranged from 279 to 816 CFU/100 mL (Table 7).

When considering the two rain events in South Carolina, enterococci concentrations at the beach transects ranged between 112 (Canes Patch Swash BT-4) and 446 CFU/100 mL (Withers Swash BT-4) (Table 7). When averaging across beach transects, concentrations of enterococci bacteria collected during the rain events were most elevated at Withers Swash (415 CFU/100 mL) and least at Canes Patch Swash (129 CFU/100mL). This pattern was different than that observed for enterococci concentrations at the beach transects during the baseline monitoring period; Canes Patch Swash had the most elevated enterococci concentration during the rain events (when averaged across beach transects).

Across the two rain events, enterococci concentrations at the South Carolina ditch/storm drain sampling locations ranged between 64 (Canes Patch Swash DT-3) and 1,750 CFU/100 mL (Withers Swash DT-2) (Table 7). When averaging across ditch/storm drain sampling locations in South Carolina, concentrations of enterococci bacteria collected during the rain events were most elevated at Withers Swash (1,485 CFU/100 mL) and least at Canes Patch Swash (530 CFU/100mL). The relatively high enterococci bacteria counts associated with the ditch/storm drains during the rain events translated directly to elevated concentrations of enterococci bacteria observed at the beach transects (Table 7).

With the exception of the beach sampling locations at Canes Patch Swash, mean concentrations of enterococci bacteria in South Carolina were always more elevated during rain events than during the baseline monitoring period (Tables 4 through 7).

In Florida, concentrations of enterococci bacteria collected during the one rain event (January 30, 2009) ranged between 3 and 13 CFU/100 mL at sites associated with Silver Beach (Table 8) and between 21 and 24 CFU/100 mL at sites associated with Florida Shores (Table 9). Mean concentrations at each of the two sampling areas, averaged across the entire baseline sampling period, were 8.3 and 22 CFU/100 mL for Silver Beach and Florida Shores, respectively (Table 7). During the one rain event, enterococci concentrations at the Florida beach transects ranged between 3 (Silver Beach transect 2) and 24 CFU/100 mL (Florida Shores transect 1) (Table 7).

Enterococci concentrations measured during the rain events in South Carolina were more elevated than those measured during the rain event in Florida (Tables 4 through 6, Tables 8 and 9). Mean concentrations of enterococci bacteria, averaged across the rain events and sites associated with the five beaches, never exceeded 22 CFU/100 mL in Florida and were never less than 279 CFU/100mL in South Carolina (Table 7).

3.5 Field Blanks and Duplicates

In general there were relatively few (if any) *Pseudomonas* or enterococci bacteria detected in field blank samples collected as part of the quality assurance/quality control QA/QC program (Table 10). Concentrations of enterococci bacteria were always less than the method detection limit with

three exceptions: two field blanks collected on January 29 (Canes Patch Swash BT1 and Withers Swash BT5) had concentrations of 36 and 4 CFU/100 mL, respectively. These samples were associated with the second rain event in South Carolina. One additional enterococci field blank sample collected on January 15, 2009 at Wither Swash (BT3) also had a measurable concentration of 24 CFU/100 mL. When reviewing the *Pseudomonas* field blank data, there was only one sample that was measured above the method detection limit. This sample was collected on December 28, 2008 at Withers Swash (BT3) (measured concentration of 4 CFU/100 mL).

Duplicate samples, collected as part of the QA/QC program, showed considerable agreement with investigative samples (Table 11). With few exceptions, the relative percent difference between the investigative sample and the field duplicate were within acceptable limits as defined in the project's QAPP (Appendix A).

3.6 Sources of Urban Runoff

As depicted in Figures 29 and 30, there are a considerable number of storm water outfalls in Myrtle Beach, South Carolina in the vicinity of the sampling sites that were monitored by GLEC. (Please note that there were no data available for outfalls in the vicinity of Surfside Swash in Surfside, South Carolina. However, we can reasonably assume that a similar configuration exists in this area). Each of these outfalls has the capacity to carry storm water runoff from rain, snowmelt or irrigation and may potentially affect the data that were collected for this project (particularly those data collected during the two rain events).

Volusia County, Florida, which includes the city of Daytona Beach, has no direct storm sewer outfalls to the ocean; storm drains empty directly into the Halifax River (Towle 2009). The outlet of this river is located approximately eight miles south of the closer of the two Florida beaches monitored during this study (Florida Shores). However, there are sites at the beach by which stormwater could directly enter the ocean; these sites are primarily associated with condominium/apartment complexes, parking lots or beach approaches (streets that allow cars direct access to the beach) rather than from an outfall (Winters 2009).

4.0 Literature Cited

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Westat. Technical Support for EPA's Search for Potential Non-POTW Impacted Beaches Affected by Runoff from Non-point Sources. Revision 1. Prepared for USEPA under Contract EP-D-04-064.

Winters, Jennifer. 2009. Email to Jamie Saxton. February 4. jwinters@co.volusia.fl.us.

FIGURES

Figure 1. Locations of beach and ditch sampling locations at Canes Patch Swash in Myrtle Beach, South Carolina.

Figure 2. Locations of beach and ditch sampling locations at Surfside Swash in Surfside, South Carolina.

Figure 3. Locations of beach and ditch sampling locations at Withers Swash in Myrtle Beach, South Carolina.

Figure 4. Locations of beach sampling locations at Florida Shores in Daytona Beach, Florida.

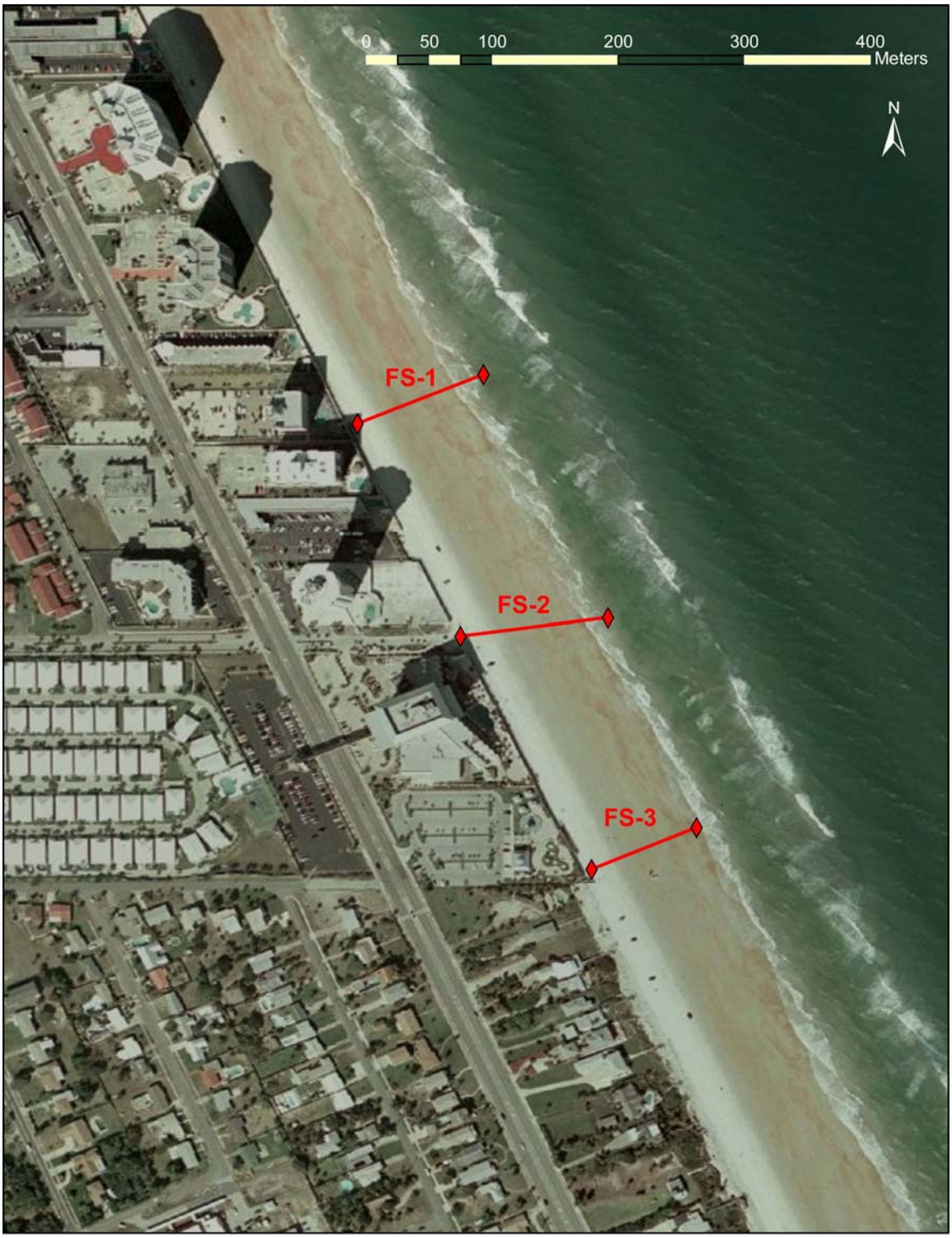


Figure 5. Locations of beach sampling locations at Silver Beach in Daytona Beach, Florida.

Figure 6. Digital picture of Canes Patch Swash at conjunction with the Atlantic Ocean looking north in Myrtle Beach, South Carolina.

Figure 7. Digital picture of Canes Patch Swash Transect 1 (CPS-DT1) looking upstream, Myrtle Beach, South Carolina.

Figure 8. Digital picture of Canes Patch Swash Transect 2 (CPS-DT2) looking downstream, Myrtle Beach, South Carolina.

Figure 9. Digital picture of Canes Patch Swash Transect 3 (CPS-DT3) looking downstream, Myrtle Beach, South Carolina.

Figure 10. Digital picture of Surfside Swash at conjunction with the Atlantic Ocean looking north in Myrtle Beach, South Carolina.

Figure 11. Digital picture of Surfside Swash Transect 1 (SS-DT1) looking downstream, Myrtle Beach, South Carolina.

Figure 12. Digital picture of Surfside Swash Transect 2 (SS-DT2) looking upstream, Myrtle Beach, South Carolina.

Figure 13. Digital picture of Surfside Swash Transect 3 (SS-DT3) looking downstream, Myrtle Beach, South Carolina.

Figure 14. Digital picture of Withers Swash at conjunction with the Atlantic Ocean looking south in Myrtle Beach, South Carolina.

Figure 15. Digital picture of Withers Swash Transect 1 (WS-DT1) looking upstream, Myrtle Beach, South Carolina.

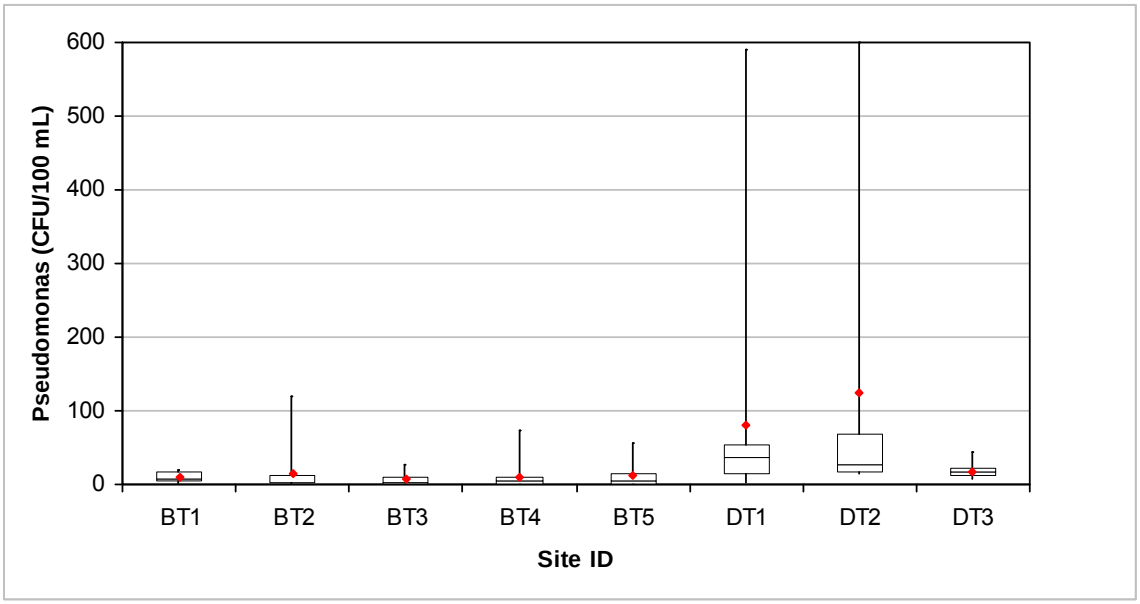
Figure 16. Digital picture of Withers Swash Transect 2 (WS-DT2) looking upstream, Myrtle Beach, South Carolina.

Figure 17. Digital picture of Withers Swash Transect 3 (WS-DT3) looking upstream, Myrtle Beach, South Carolina.

Figure 18. Digital picture of Florida Shores Beach looking south in Daytona Beach, Florida.

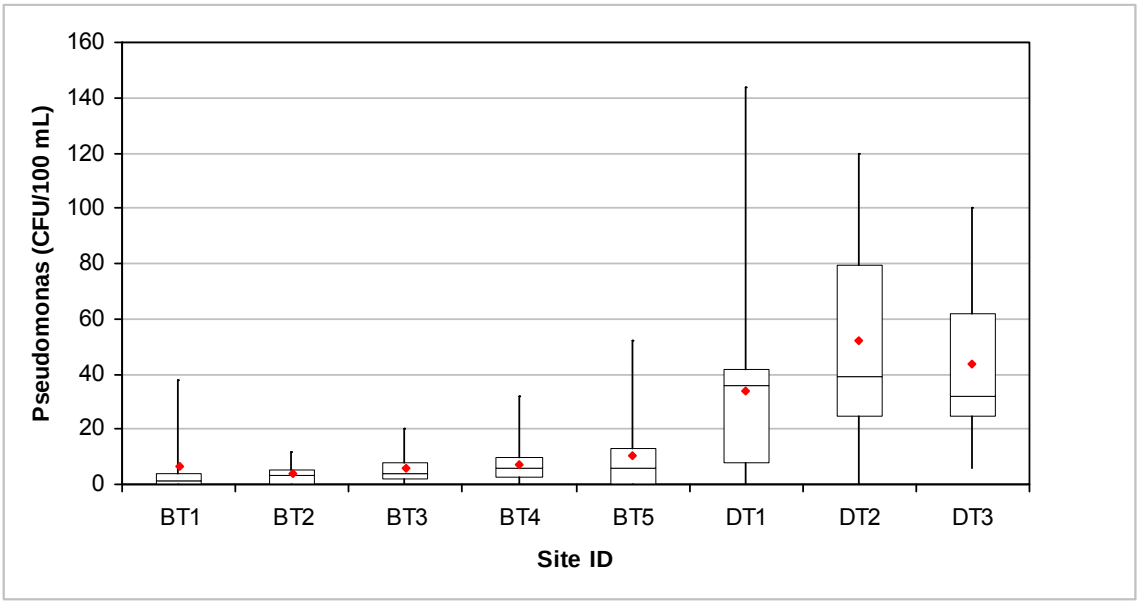
Figure 19. Digital picture of Silver Beach looking south in Daytona Beach, Florida.

Figure 20. Box and whisker plot* depicting baseline *Pseudomonas* concentrations at each of eight sites sampled in the vicinity of Canes Patch Swash, Myrtle Beach, South Carolina.



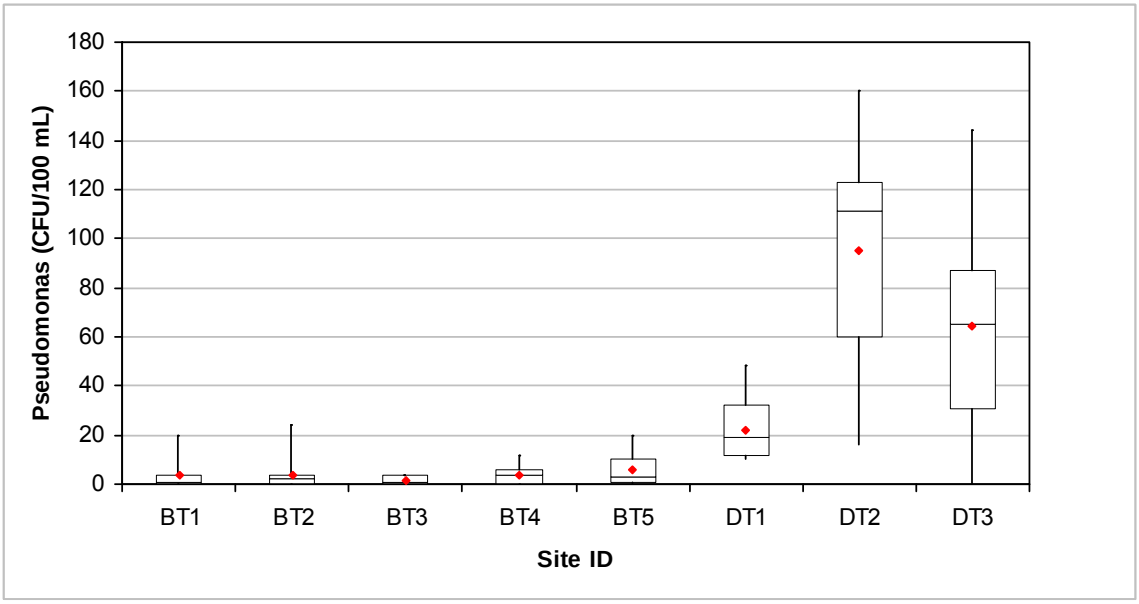
* Each box exhibits the inner quartiles, the whiskers represent the outer quartiles, the median is represented by a solid line and the mean is presented as a red diamond.

Figure 21. Box and whisker plot* depicting baseline *Pseudomonas* concentrations at each of eight sites sampled in the vicinity of Withers Swash, Myrtle Beach, South Carolina.



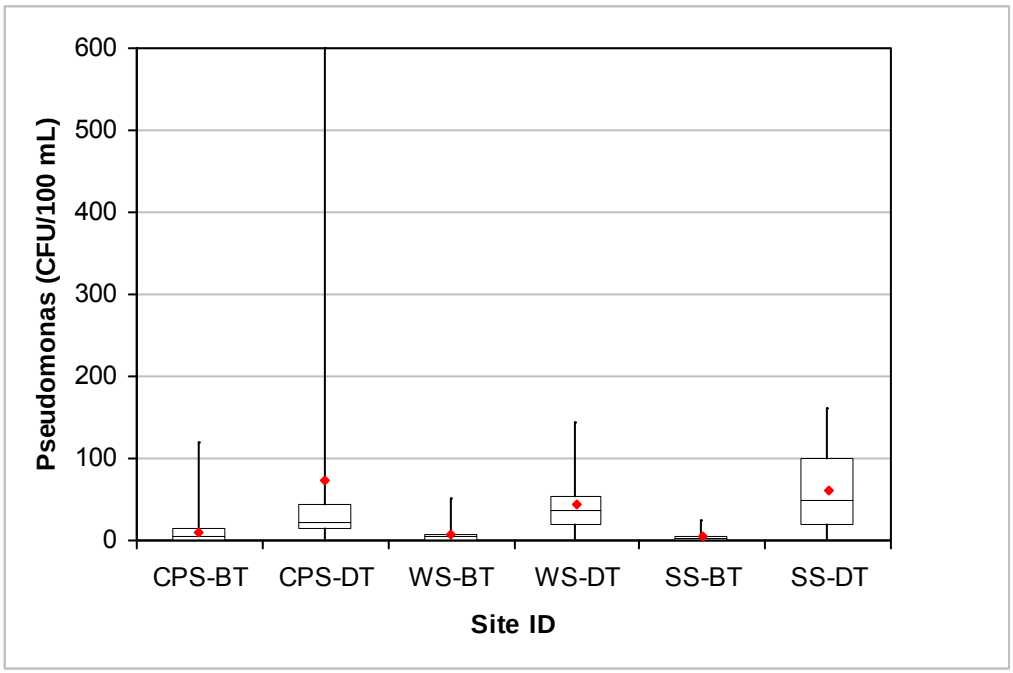
* Each box exhibits the inner quartiles, the whiskers represent the outer quartiles, the median is represented by a solid line and the mean is presented as a red diamond.

Figure 22. Box and whisker plot* depicting baseline *Pseudomonas* concentrations at each of eight sites sampled in the vicinity of Surfside Swash, Surfside, South Carolina.



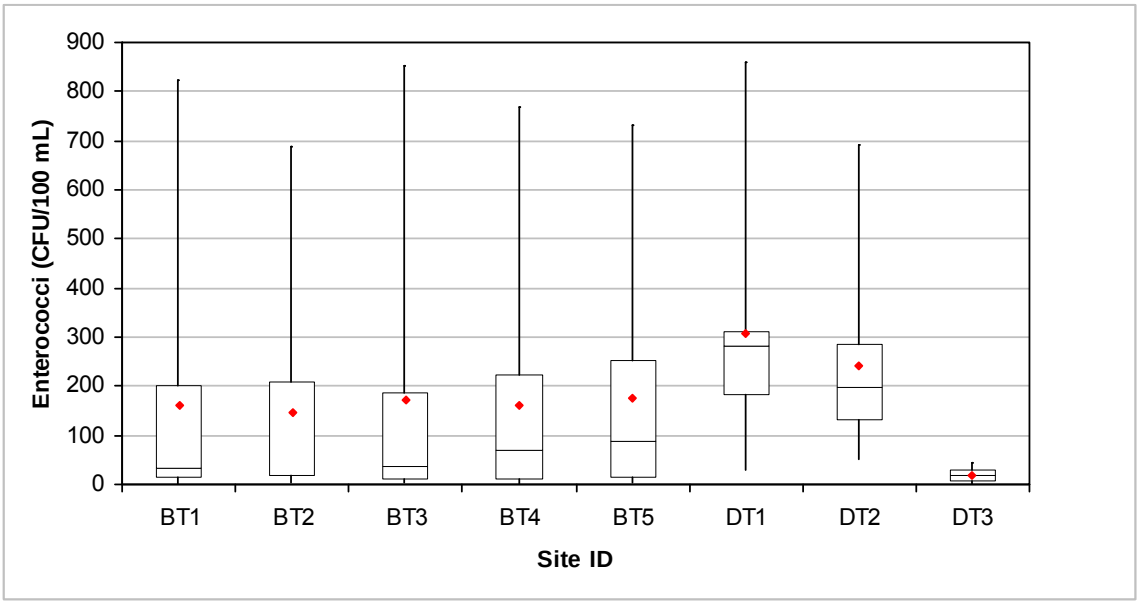
* Each box exhibits the inner quartiles, the whiskers represent the outer quartiles, the median is represented by a solid line and the mean is presented as a red diamond.

Figure 23. Box and whisker plot* depicting baseline *Pseudomonas* concentrations at each of six sites sampled in the vicinity of Myrtle Beach, South Carolina.



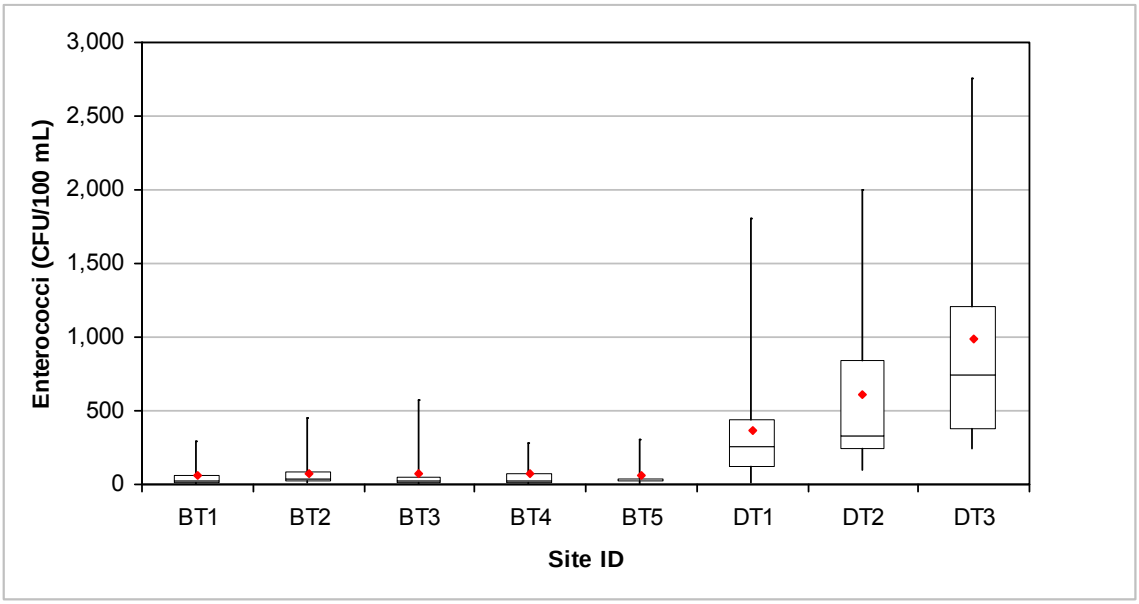
* Each box exhibits the inner quartiles, the whiskers represent the outer quartiles, the median is represented by a solid line and the mean is presented as a red diamond.

Figure 24. Box and whisker plot* depicting baseline Enterococci concentrations at each of eight sites sampled in the vicinity of Canes Patch Swash, Myrtle Beach, South Carolina.



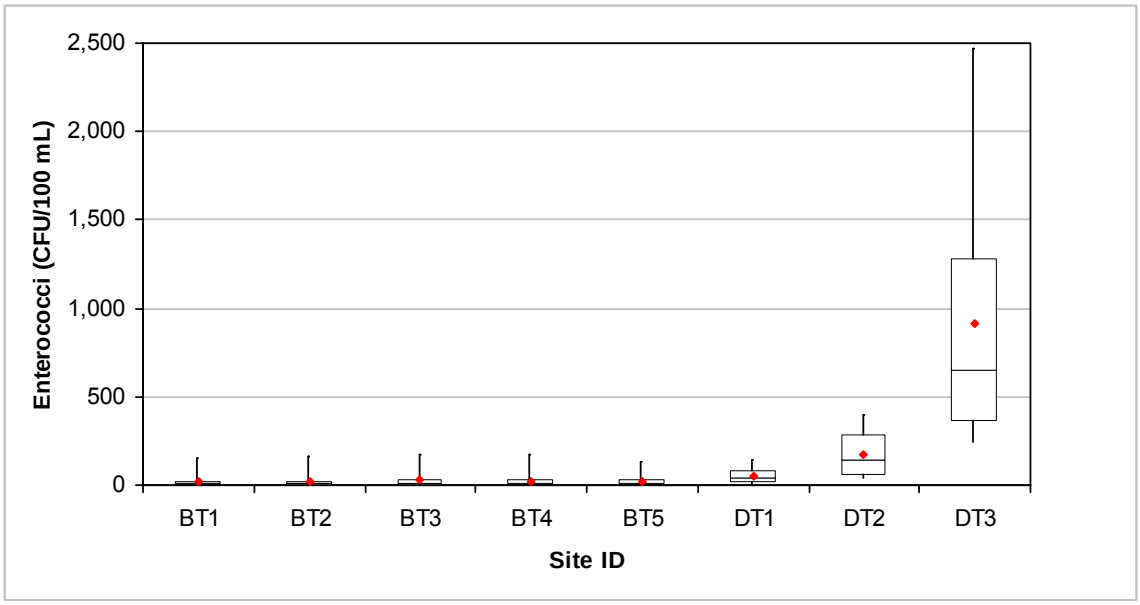
* Each box exhibits the inner quartiles, the whiskers represent the outer quartiles, the median is represented by a solid line and the mean is presented as a red diamond.

Figure 25. Box and whisker plot* depicting baseline Enterococci concentrations at each of eight sites sampled in the vicinity of Withers Swash, Myrtle Beach, South Carolina.



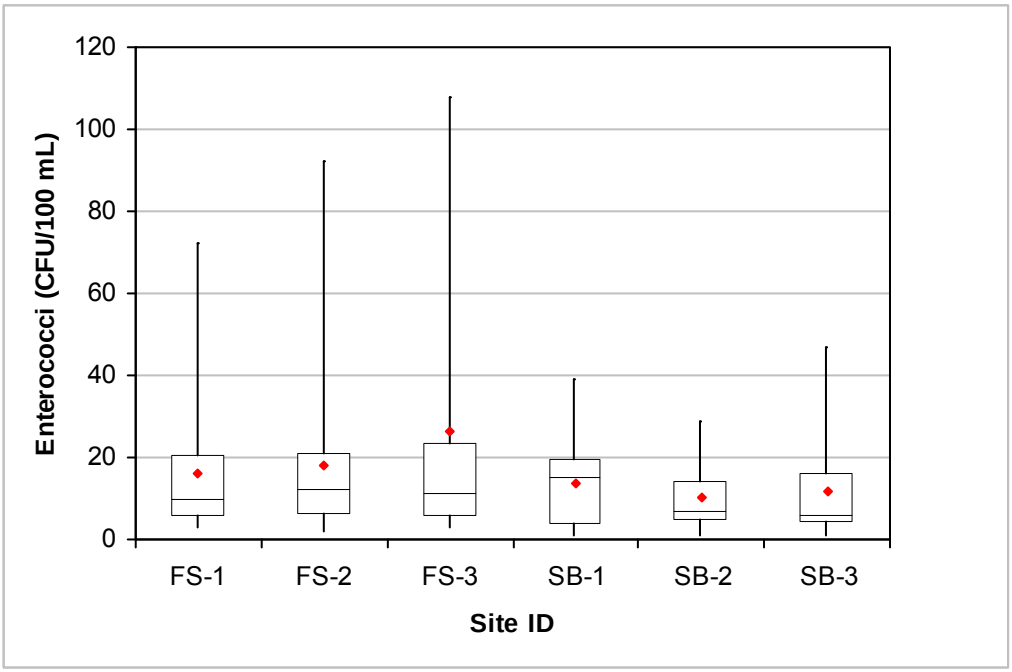
* Each box exhibits the inner quartiles, the whiskers represent the outer quartiles, the median is represented by a solid line and the mean is presented as a red diamond.

Figure 26. Box and whisker plot* depicting baseline Enterococci concentrations at each of eight sites sampled in the vicinity of Surfside Swash, Surfside, South Carolina.



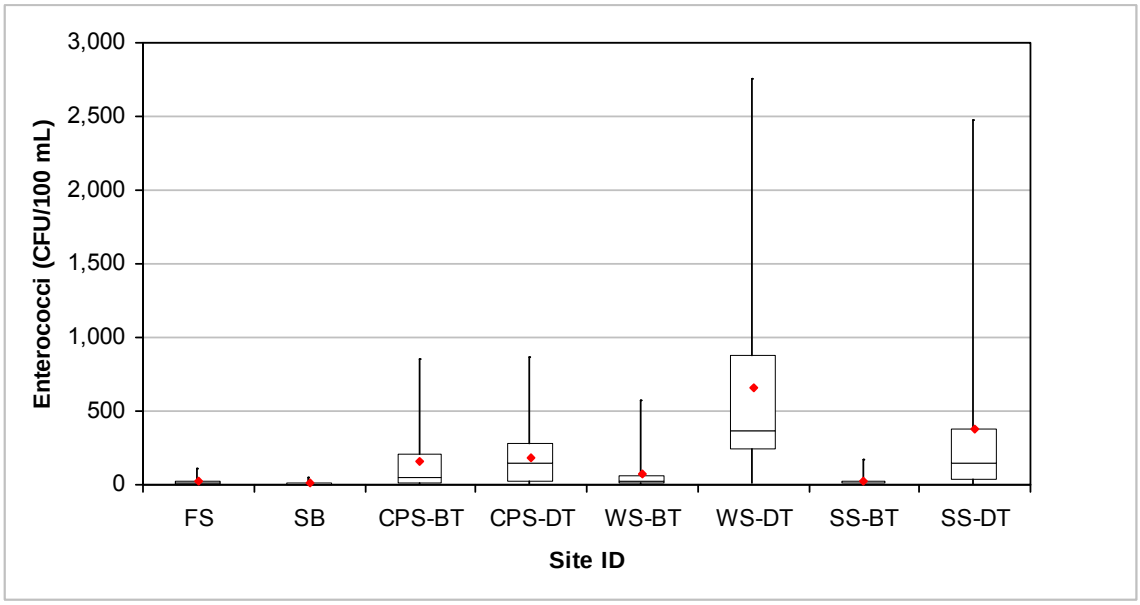
* Each box exhibits the inner quartiles, the whiskers represent the outer quartiles, the median is represented by a solid line and the mean is presented as a red diamond.

Figure 27. Box and whisker plot* depicting baseline Enterococci concentrations at each of six sites sampled in the vicinity of Daytona Beach, Florida.



* Each box exhibits the inner quartiles, the whiskers represent the outer quartiles, the median is represented by a solid line and the mean is presented as a red diamond.

Figure 28. Box and whisker plot* depicting baseline Enterococci concentrations at each of eight sites sampled in the vicinity of Daytona Beach, Florida and Myrtle Beach, South Carolina.



* Each box exhibits the inner quartiles, the whiskers represent the outer quartiles, the median is represented by a solid line and the mean is presented as a red diamond.

Figure 29. Locations of outfalls in the vicinity of sites sampled at Canes Patch Swash in Myrtle Beach, South Carolina.

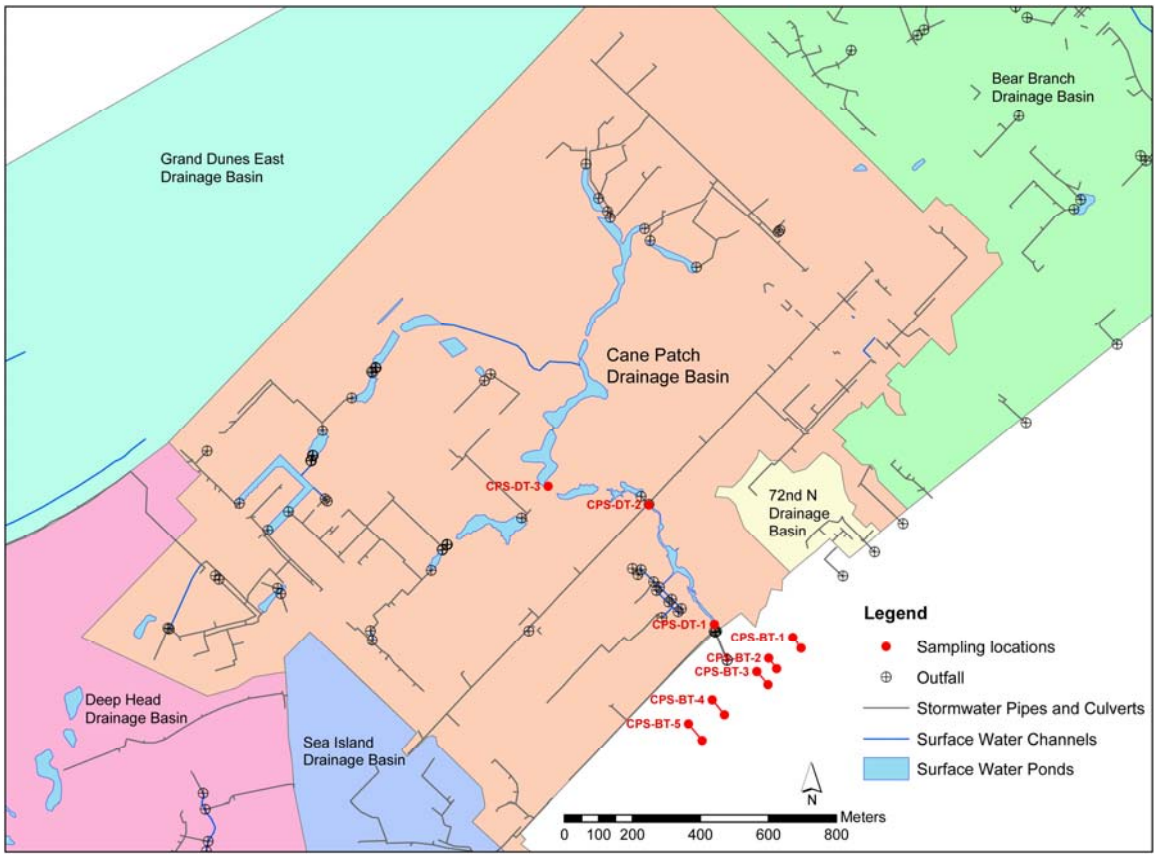
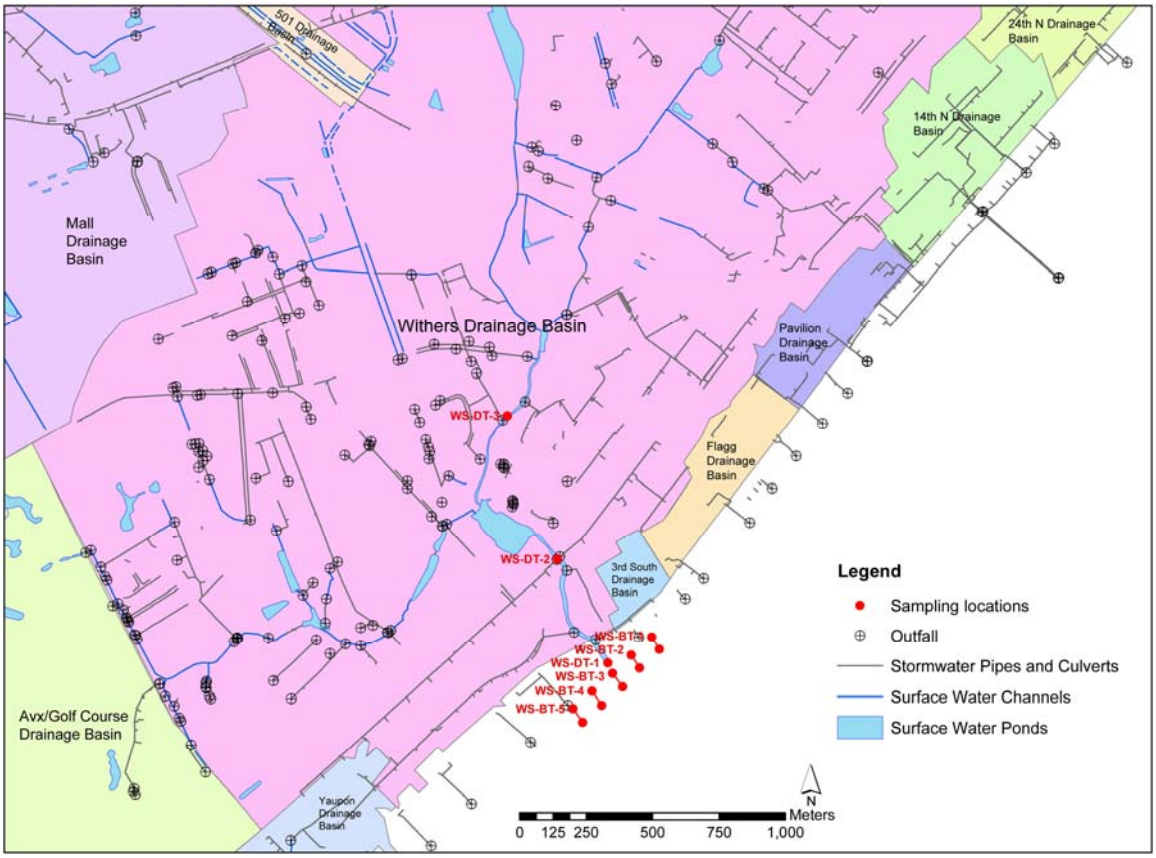


Figure 30. Locations of outfalls in the vicinity of sites sampled at Withers Swash in Myrtle Beach, South Carolina.



TABLES

Table 1. Summary of deviations from the Quality Assurance Project Plan and the Sampling and Analysis Plan.

SOUTH CAROLINA				
Sample Date	**Exception**	**Notes 1**	**Notes 2**	**Notes 3**
12/16/2008	qPCR, Sample Collection	all qPCR filters discarded due to gross overages in holding times	sample collection window greater than two hours (last sample was collected at 4:20 PM)	
12/18/2008	qPCR	two qPCR filters for each site	qPCR holding time slightly exceeded	
12/21/2008	qPCR	two qPCR filters for each site		
12/23/2008	qPCR, Sample Collection	three qPCR filters for each site	DT#3 not filtered for qPCR across all beaches due to time constraints	sample collection window greater than two hours (last sample was collected at 11:30 AM)
12/26/2008	qPCR	three qPCR filters for each site		
12/28/2008	qPCR	three qPCR filters for each site		
12/30/2008	qPCR	three qPCR filters for each site		
1/2/2009	qPCR	three qPCR filters for each site		
1/4/2009	qPCR, Sample Collection	three qPCR filters for each site	CPS-BT#1 and CPS-BT#2 samples not collected due to beach renourishment construction denying access	
1/6/2009	qPCR	three qPCR filters for each site		
1/8/2009	qPCR	three qPCR filters for each site		
1/11/2009	qPCR	three qPCR filters for each site		
1/13/2009	qPCR, Rain Event	three qPCR filters for each site	field blank samples associated with rain event not collected because weather data were not available until after the sampling event (i.e., rain event not confirmed until after sample collection)	
1/15/2009	qPCR	three qPCR filters for each site		
1/18/2009	qPCR, Ancillary Data	three qPCR filters for each site	pH data not recorded for CPS due to meter malfunction	
1/29/2009	qPCR	one qPCR filter for each site	qPCR holding time exceeded	

Table 1 (cont'd). Summary of deviations from the Quality Assurance Project Plan and the Sampling and Analysis Plan.

FLORIDA					
Sample Date	**Exception**	**Notes 1**	**Notes 2**	**Notes 3**	**Notes 4**
12/16/2008	qPCR, Ancillary Data	used 2 20 mL aliquots of sterile phospate buffer to rinse all qPCR filters	FS field blank for qPCR exceeded holding time by two hours; SB duplicate and field blank exceeded holding time by 48 minutes	unsure whether conductivity measurement was temperature corrected (i.e., conductivity or specific conductance)	
12/21/2008	qPCR, Ancillary Data	FS-1 filter 5: only 80 mL sample filtered and only one 10 mL phosphate buffer rinse was filtered for qPCR	unsure whether conductivity measurement was temperature corrected (i.e. conductivity or specific conductance)		
12/23/2008	Sample Collection, DOC, Ancillary Data	no DOC field blanks collected at FS or SB due to lack of clean (baked) glass bottles	no initial rinse completed for FS-1, FS-2 or FS-3 DOC samples	unsure whether conductivity measurement recorded was temperature corrected (i.e., conductivity or specific conductance)	
12/29/2008	Sample Collection, qPCR, Ancillary Data	samples collected on 12/29 instead of 12/28 as originally planned due to travel issues returning from Christmas holiday	sample collection window greater than two hours; sample collection occurred between 9:56 AM and 12:07 PM	FS-1 and FS-2 filter 5 slightly over holding time	unsure whether conductivity measurement recorded was temperature corrected (i.e., conductivity or specific conductance)
12/30/2008	Sample Collection, qPCR, DOC, Ancillary Data	FS-2, FS-3 and Duplicate qPCR filter 5 slightly over holding time	no FS DOC field blank collected due to lack of clean (baked) glass bottles	DOC filtration completed on 12/31 due to lack of clean (baked) glassware	unsure whether conductivity measurement recorded was temperature corrected (i.e., conductivity or specific Conductance)
1/2/2009	qPCR	used 2 20-ml aliquots of sterile phospate buffer for each qPCR filter blank			
1/11/2009	Ancillary Data	no photographs collected at FS			
1/13/2009	Sample Collection	field blanks not collected due to lack of sterile phosphate buffer (were instead collected on 1/15)	DOC field blanks collected outside of 2 hour sampling window (11:45 AM)		
1/15/2009	qPCR	all qPCR filters were accidentally allowed to thaw for over 24 hours due to lack of adequate dry ice			
1/18/2009	Sample Collection	SB-3 sample collected outside of 2 hour sampling window (11:07 AM)			
1/30/2009	qPCR, DOC	FS duplicate qPCR sample exceeded holding time by 5 minutes	DOC filtration completed on 2/01 due to lack of personnel availability		

Table 2. GPS coordinates for sites sampled in Myrtle Beach, South Carolina and Daytona Beach, Florida.

State	Sampling Location	Site ID	Latitude (N)	Longitude (W)
South Carolina	Canes Patch Beach	CPS-BT1	33° 44.269'	78° 49.235'
South Carolina	Canes Patch Beach	CPS-BT2	33° 44.237'	78° 49.282'
South Carolina	Canes Patch Beach	CPS-BT3	33° 44.216'	78° 49.305'
South Carolina	Canes Patch Beach	CPS-BT4	33° 44.173'	78° 49.391'
South Carolina	Canes Patch Beach	CPS-BT5	33° 44.136'	78° 49.437'
South Carolina	Canes Patch Swash	CPS-DT1	33° 44.293'	78° 49.384'
South Carolina	Canes Patch Swash	CPS-DT2	33° 44.486'	78° 49.504'
South Carolina	Canes Patch Swash	CPS-DT3	33° 44.519'	78° 49.695'
South Carolina	Withers Swash Beach	WS-BT1	33° 40.873'	78° 53.343'
South Carolina	Withers Swash Beach	WS-BT2	33° 40.837'	78° 53.394'
South Carolina	Withers Swash Beach	WS-BT3	33° 40.801'	78° 53.441'
South Carolina	Withers Swash Beach	WS-BT4	33° 40.766'	78° 53.492'
South Carolina	Withers Swash Beach	WS-BT5	33° 40.731'	78° 53.539'
South Carolina	Withers Swash	WS-DT1	33° 40.822'	78° 53.452'
South Carolina	Withers Swash	WS-DT2	33° 41.036'	78° 53.569'
South Carolina	Withers Swash	WS-DT3	33° 41.330'	78° 53.684'
South Carolina	Surfside Beach	SS-BT1	33° 36.850'	78° 57.765'
South Carolina	Surfside Beach	SS-BT2	33° 36.814'	78° 57.808'
South Carolina	Surfside Beach	SS-BT3	33° 36.770'	78° 57.849'
South Carolina	Surfside Beach	SS-BT4	33° 36.682'	78° 57.888'
South Carolina	Surfside Beach	SS-BT5	33° 36.682'	78° 57.927'
South Carolina	Surfside Swash	SS-DT1	33° 36.843'	78° 57.921'
South Carolina	Surfside Swash	SS-DT2	33° 36.974'	78° 58.244'
South Carolina	Surfside Swash	SS-DT3	33° 36.998'	78° 58.535'
Florida	Florida Shores Beach	FS-1	29º 11.011’	80º 59.056’
Florida	Florida Shores Beach	FS-2	29º 10.907’	80º 59.003’
Florida	Florida Shores Beach	FS-3	29º 10.817’	80º 58.965’
Florida	Silver Beach	SB-1	29º 12.854’	80º 59.986’
Florida	Silver Beach	SB-2	29º 12.754’	80º 59.935’
Florida	Silver Beach	SB-3	29º 12.654’	80º 59.890’

Table 3. Ancillary measurements recorded during each sampling visit.

Measurement	Description	Units/Format	MQOs
Date and Time	Date and Time of day	mm/dd/yy; hh:mm	5 minutes
Air temperature	Measurement taken from nearby weather station* each sampling day	Quantitative: °C	+/-1°
Water temperature	Measured by YSI thermometer at sampling location on center transect (1m deep, 0.3m below surface) on every visit	Quantitative: °C	+/-1°
Cloud Cover	Evaluated by approximate areal coverage: **S**unny (<20% cloud cover), **M**ostly **S**unny (20-50% cover), **C**loudy (50-70% cover) **M**ostly **C**loudy (70-99% cover), **O**vercast (100% cover)	Categorical: S, MS, C, MC, O	Field Person or Team Consensus
Rainfall	Measurements taken from nearby weather station each sampling day for rainfall since last sample collection; current conditions such as rain, lightning, hail, etc. should also be noted	Quantitative: rain in inches Descriptive: current conditions	+/- 0.25 Inches
Wind speed (local)	Measurements taken from nearby weather station* each sampling day	Quantitative: miles per hour	5 mph
Wind direction	Compass direction to nearest semi-quadrant leeward measured on wind gauge	Categorical: N, NE, E, SE, S, SW, W, or NW	Weather station
Current Direction	Described in relation to shoreline facing out	Descriptive: e.g. onshore, right, etc.	Field Person or Team Consensus
Wave height (avg)	Meter stick measurement at central sampling point	Quantitative: meters	0.2 m
Boats	Approximate number of **S**ailboats, **R**owboats, and **P**owerboats/Jet skis in the water, within 500 m of sampling area	Categorical: S, R, P; None, 1-5, 5-10, 10–20, 20-30, etc.	Field Person or Team Consensus
Animals/Birds	Animals and birds potentially affecting the water (within approximately 20 m of sampling area in water or laterally within 20 m of outer transects on beach), also includes number of fowl or other birds in the air near the sampling area	Descriptive: types of animals Quantitative: numbers of each type on beach and in water	Field Person or Team Consensus

Table 3. Ancillary measurements recorded during each sampling visit.

Measurement	Description	Units/Format	MQOs
Debris	Approximate amount of **W**oody debris, **P**lant matter, and **T**rash/Litter within bathing area; evaluated on areal coverage basis in 10x40m plot around center transect (20m parallel to shore on either side of transect, 5m onshore, and 5m out from shoreline)	Categorical; W, P, T; 0=Absent (0%), 1=Sparse (<10%), 2=Moderate (10-40%), 3=Heavy (40-75%), and 4=Very Heavy (>75%)	Field Person or Team Consensus
pH	Measured by YSI pH meter at sampling location on center transect (1m deep, 0.3m below surface) on every visit	Quantitative: pH units	0.2 units
Turbidity	Measured by turbidity meter from water sample collected in 500 mL DI-rinsed Nalgene bottle at sampling location on center transect (1m deep, 0.3m below surface) on every visit	Quantitative: Nephlometric Turbidity Units (NTUs)	Range dependent; see Standard Methods 2130B
Salinity	Measured by YSI salinity meter at sampling location on center transect (1m deep, 0.3m below surface) on every visit	Quantitative: parts per thousand	1 part per thousand
Conductivity	Measured by YSI conductivity meter at sampling location on center transect (1m deep, 0.3 m below surface) on every visit	Quantitative: microSiemens or milliSiemens, as appropriate	Range dependent
Presence of SSOs , CSOs, leakage of sanitary sewers, and location of storm sewers	Information regarding possible local SSOs, leakage of sanitary sewers that could have affected the beach, presence of municipal storm sewers, quality of the sanitary sewer system, recent malfunctions, accidental bypasses, etc. determined through interviews with local managers	Descriptive: location of SSO and other sources of sanitary sewer contamination	Field or team contact person
Geographical Position	Coordinates taken using handheld GPS unit in 3 places for each transect	Quantitative: Lat/long, ddd°mm'ss.s"	0.1 seconds
Beach Facilities	Facilities at the beach or accessible to beach goers such as: public restrooms, camping, picnic areas, food stands, city parks, etc.	Descriptive: description and location of facilities relative to the beach (i.e., on beach, walking distance, adjacent to beach, but not on beach, etc)	Field Person or Team Consensus

Table 4. Concentration of Enterococci and *Pseudomonas* in samples collected at five beach and three ditch transects in the vicinity of Canes Patch Swash, Myrtle Beach, South Carolina.

Sample ID	Sample Date	Enterocci (CFU/100mL)	*Pseudomonas* (CFU/100mL)	Sample ID	Sample Date	Enterocci (CFU/100mL)	*Pseudomonas* (CFU/100mL)
CPS-BT1	16-Dec-08	16	16	CPS-DT1	28-Dec-08	228	2
CPS-BT2	16-Dec-08	8	120	CPS-DT2	28-Dec-08	96	20
CPS-BT3	16-Dec-08	8	<4	CPS-DT3	28-Dec-08	8	18
CPS-BT4	16-Dec-08	8	72	CPS-DT2-D	28-Dec-08	68	54
CPS-BT5	16-Dec-08	16	56	CPS-DT2-FLDB	28-Dec-08	<4	<2
CPS-DT1	16-Dec-08	96	100	CPS-BT1	30-Dec-08	24	<2
CPS-DT2	16-Dec-08	156	600	CPS-BT2	30-Dec-08	4	<2
CPS-DT3	16-Dec-08	24	24	CPS-BT3	30-Dec-08	12	<2
CPS-BT1-D	16-Dec-08	8	28	CPS-BT4	30-Dec-08	8	<2
CPS-DT2-FLDB	16-Dec-08	<4	<4	CPS-BT5	30-Dec-08	8	<2
CPS-BT1	18-Dec-08	32	20	CPS-DT1	30-Dec-08	168	34
CPS-BT2	18-Dec-08	20	8	CPS-DT2	30-Dec-08	132	18
CPS-BT3	18-Dec-08	12	10	CPS-DT3	30-Dec-08	40	12
CPS-BT4	18-Dec-08	16	10	CPS-BT1	2-Jan-09	28	4
CPS-BT5	18-Dec-08	8	16	CPS-BT2	2-Jan-09	20	2
CPS-DT1	18-Dec-08	310	590	CPS-BT3	2-Jan-09	12	<2
CPS-DT2	18-Dec-08	136	>600	CPS-BT4	2-Jan-09	16	<2
CPS-DT3	18-Dec-08	20	14	CPS-BT5	2-Jan-09	12	<2
CPS-BT1	21-Dec-08	200	8	CPS-DT1	2-Jan-09	28	12
CPS-BT2	21-Dec-08	204	2	CPS-DT2	2-Jan-09	52	18
CPS-BT3	21-Dec-08	192	8	CPS-DT3	2-Jan-09	8	8
CPS-BT4	21-Dec-08	256	16	CPS-BT1-D	2-Jan-09	4	4
CPS-BT5	21-Dec-08	260	44	CPS-BT1-FLDB	2-Jan-09	<4	<2
CPS-DT1	21-Dec-08	312	56	CPS-BT3	4-Jan-09	64	6
CPS-DT2	21-Dec-08	228	14	CPS-BT4	4-Jan-09	96	22
CPS-DT3	21-Dec-08	24	20	CPS-BT5	4-Jan-09	96	16
CPS-BT5-D	21-Dec-08	256	24	CPS-DT1	4-Jan-09	272	38
CPS-BT1	23-Dec-08	16	20	CPS-DT2	4-Jan-09	220	18
CPS-BT2	23-Dec-08	8	2	CPS-DT3	4-Jan-09	<4	8
CPS-BT3	23-Dec-08	4	<2	CPS-BT4-D	4-Jan-09	44	8
CPS-BT4	23-Dec-08	8	<2	CPS-BT1	6-Jan-09	536	16
CPS-BT5	23-Dec-08	16	8	CPS-BT2	6-Jan-09	384	12
CPS-DT1	23-Dec-08	160	122	CPS-BT3	6-Jan-09	484	26
CPS-DT2	23-Dec-08	76	206	CPS-BT4	6-Jan-09	480	6
CPS-DT3	23-Dec-08	16	44	CPS-BT5	6-Jan-09	524	4
CPS-BT4-FLDB	23-Dec-08	<4	<2	CPS-DT1	6-Jan-09	350	50
CPS-BT1	26-Dec-08	12	4	CPS-DT2	6-Jan-09	320	34
CPS-BT2	26-Dec-08	20	2	CPS-DT3	6-Jan-09	8	24
CPS-BT3	26-Dec-08	12	<2	CPS-BT3-D	6-Jan-09	412	22
CPS-BT4	26-Dec-08	40	<2	CPS-BT1	8-Jan-09	116	18
CPS-BT5	26-Dec-08	80	<2	CPS-BT2	8-Jan-09	228	14
CPS-DT1	26-Dec-08	300	6	CPS-BT3	8-Jan-09	430	<2
CPS-DT2	26-Dec-08	>240	18	CPS-BT4	8-Jan-09	320	<2
CPS-DT3	26-Dec-08	32	18	CPS-BT5	8-Jan-09	370	6
CPS-DT1-D	26-Dec-08	272	28	CPS-DT1	8-Jan-09	290	44
CPS-BT1	28-Dec-08	<4	2	CPS-DT2	8-Jan-09	300	32
CPS-BT2	28-Dec-08	20	<2	CPS-DT3	8-Jan-09	40	14
CPS-BT3	28-Dec-08	4	<2	CPS-BT4-D	8-Jan-09	320	6
CPS-BT4	28-Dec-08	<4	<2	CPS-BT4-FLDB	8-Jan-09	<4	<2
CPS-BT5	28-Dec-08	4	<2	CPS-BT1	11-Jan-09	824	10

* Represents data collected during a rain event (defined as > 0.25 inches of rain in the 12 hour period prior to sampling).

Table 4 (cont'd). Concentration of Enterococci and *Pseudomonas* in samples collected at five beach and three ditch transects in the vicinity of Canes Patch Swash, Myrtle Beach, South Carolina.

Sample ID	Sample Date	Enterocci (CFU/100mL)	*Pseudomonas* (CFU/100mL)
CPS-BT2	11-Jan-09	688	4
CPS-BT3	11-Jan-09	852	20
CPS-BT4	11-Jan-09	768	10
CPS-BT5	11-Jan-09	732	14
CPS-DT1	11-Jan-09	860	36
CPS-DT2	11-Jan-09	690	76
CPS-DT3	11-Jan-09	44	22
CPS-BT5-D	11-Jan-09	876	8
CPS-BT1*	13-Jan-09	184	124
CPS-BT2*	13-Jan-09	244	106
CPS-BT3*	13-Jan-09	224	108
CPS-BT4*	13-Jan-09	184	124
CPS-BT5*	13-Jan-09	230	50
CPS-DT1*	13-Jan-09	470	168
CPS-DT2*	13-Jan-09	1110	62
CPS-DT3*	13-Jan-09	56	78
CPS-DT1-D*	13-Jan-09	420	138
CPS-BT1	15-Jan-09	200	2
CPS-BT2	15-Jan-09	208	2
CPS-BT3	15-Jan-09	164	6
CPS-BT4	15-Jan-09	108	4
CPS-BT5	15-Jan-09	232	2
CPS-DT1	15-Jan-09	248	14
CPS-DT2	15-Jan-09	176	50
CPS-DT3	15-Jan-09	4	8
CPS-DT2-D	15-Jan-09	116	68
CPS-DT2-FLDB	15-Jan-09	<4	<2
CPS-BT1	18-Jan-09	80	4
CPS-BT2	18-Jan-09	104	16
CPS-BT3	18-Jan-09	140	20
CPS-BT4	18-Jan-09	124	4
CPS-BT5	18-Jan-09	120	6
CPS-DT1	18-Jan-09	680	16
CPS-DT2	18-Jan-09	540	22
CPS-DT3	18-Jan-09	<4	14
CPS-DT3-D	18-Jan-09	<4	10
CPS-BT1*	29-Jan-09	88	<2
CPS-BT2*	29-Jan-09	56	<2
CPS-BT3*	29-Jan-09	36	6
CPS-BT4*	29-Jan-09	40	2
CPS-BT5*	29-Jan-09	<4	2
CPS-DT1*	29-Jan-09	350	30
CPS-DT2*	29-Jan-09	1120	<2
CPS-DT3*	29-Jan-09	72	92
CPS-BT1-D*	29-Jan-09	60	4
CPS-BT1-FLDB*	29-Jan-09	36	<2

* Represents data collected during a rain event (defined as > 0.25 inches of rain in the 12 hour period prior to sampling).

Table 5. Concentration of Enterococci and *Pseudomonas* in samples collected at five beach and three ditch transects in the vicinity of Withers Swash, Myrtle Beach, South Carolina.

Sample ID	Sample Date	Enterocci (CFU/100mL)	*Pseudomonas* (CFU/100mL)	Sample ID	Sample Date	Enterocci (CFU/100mL)	*Pseudomonas* (CFU/100mL)
WS-BT1	16-Dec-08	<4	<4	WS-DT1	28-Dec-08	460	4
WS-BT2	16-Dec-08	88	12	WS-DT2	28-Dec-08	310	16
WS-BT3	16-Dec-08	80	20	WS-DT3	28-Dec-08	1020	28
WS-BT4	16-Dec-08	112	32	WS-BT3-D	28-Dec-08	12	2
WS-BT5	16-Dec-08	40	52	WS-BT3-FLDB	28-Dec-08	<4	4
WS-DT1	16-Dec-08	160	36	WS-BT1	30-Dec-08	32	<2
WS-DT2	16-Dec-08	184	40	WS-BT2	30-Dec-08	28	4
WS-DT3	16-Dec-08	2000	80	WS-BT3	30-Dec-08	<4	2
WS-BT5-D	16-Dec-08	84	<4	WS-BT4	30-Dec-08	4	<2
WS-BT4-FLDB	16-Dec-08	<4	<4	WS-BT5	30-Dec-08	32	10
WS-BT1	18-Dec-08	8	<2	WS-DT1	30-Dec-08	460	42
WS-BT2	18-Dec-08	24	<2	WS-DT2	30-Dec-08	1530	66
WS-BT3	18-Dec-08	4	8	WS-DT3	30-Dec-08	800	100
WS-BT4	18-Dec-08	16	10	WS-BT1	2-Jan-09	16	<2
WS-BT5	18-Dec-08	<4	6	WS-BT2	2-Jan-09	24	<2
WS-DT1	18-Dec-08	16	6	WS-BT3	2-Jan-09	28	<2
WS-DT2	18-Dec-08	640	84	WS-BT4	2-Jan-09	4	<2
WS-DT3	18-Dec-08	>600	24	WS-BT5	2-Jan-09	12	<2
WS-BT1	21-Dec-08	28	<2	WS-DT1	2-Jan-09	64	<2
WS-BT2	21-Dec-08	32	<2	WS-DT2	2-Jan-09	96	<2
WS-BT3	21-Dec-08	32	<2	WS-DT3	2-Jan-09	680	20
WS-BT4	21-Dec-08	24	12	WS-BT5-D	2-Jan-09	20	<2
WS-BT5	21-Dec-08	24	2	WS-BT5-FLDB	2-Jan-09	<4	<2
WS-DT1	21-Dec-08	>240	40	WS-BT1	4-Jan-09	16	4
WS-DT2	21-Dec-08	>240	>120	WS-BT2	4-Jan-09	28	2
WS-DT3	21-Dec-08	>240	28	WS-BT3	4-Jan-09	16	4
WS-DT3-D	21-Dec-08	>240	24	WS-BT4	4-Jan-09	12	<2
WS-BT1	23-Dec-08	28	4	WS-BT5	4-Jan-09	32	8
WS-BT2	23-Dec-08	48	8	WS-DT1	4-Jan-09	272	12
WS-BT3	23-Dec-08	20	4	WS-DT2	4-Jan-09	1180	28
WS-BT4	23-Dec-08	40	8	WS-DT3	4-Jan-09	310	32
WS-BT5	23-Dec-08	20	6	WS-DT1-D	4-Jan-09	320	14
WS-DT1	23-Dec-08	108	144	WS-BT1	6-Jan-09	<4	2
WS-DT2	23-Dec-08	>240	>120	WS-BT2	6-Jan-09	8	6
WS-DT3	23-Dec-08	>240	82	WS-BT3	6-Jan-09	8	4
WS-DT3-FLDB	23-Dec-08	<4	<2	WS-BT4	6-Jan-09	12	2
WS-BT1	26-Dec-08	160	2	WS-BT5	6-Jan-09	8	<2
WS-BT2	26-Dec-08	128	<2	WS-DT1	6-Jan-09	340	46
WS-BT3	26-Dec-08	200	<2	WS-DT2	6-Jan-09	340	24
WS-BT4	26-Dec-08	284	6	WS-DT3	6-Jan-09	>600	22
WS-BT5	26-Dec-08	188	<2	WS-DT2-D	6-Jan-09	520	68
WS-DT1	26-Dec-08	>240	40	WS-BT1	8-Jan-09	72	36
WS-DT2	26-Dec-08	532	18	WS-BT2	8-Jan-09	36	2
WS-DT3	26-Dec-08	>240	32	WS-BT3	8-Jan-09	36	6
WS-BT2-D	26-Dec-08	160	<2	WS-BT4	8-Jan-09	32	4
WS-BT1	28-Dec-08	24	<2	WS-BT5	8-Jan-09	60	14
WS-BT2	28-Dec-08	20	<2	WS-DT1	8-Jan-09	500	36
WS-BT3	28-Dec-08	28	8	WS-DT2	8-Jan-09	910	90
WS-BT4	28-Dec-08	84	4	WS-DT3	8-Jan-09	1190	66
WS-BT5	28-Dec-08	32	<2	WS-DT3-D	8-Jan-09	920	50

* Represents data collected during a rain event (defined as > 0.25 inches of rain in the 12 hour period prior to sampling).

Table 5 (cont'd). Concentration of Enterococci and *Pseudomonas* in samples collected at five beach and three ditch transects in the vicinity of Withers Swash, Myrtle Beach, South Carolina.

Sample ID	Sample Date	Enterocci (CFU/100mL)	*Pseudomonas* (CFU/100mL)
WS-DT3-FLDB	8-Jan-09	<4	<2
WS-BT1	11-Jan-09	290	38
WS-BT2	11-Jan-09	450	12
WS-BT3	11-Jan-09	570	18
WS-BT4	11-Jan-09	280	6
WS-BT5	11-Jan-09	310	36
WS-DT1	11-Jan-09	380	14
WS-DT2	11-Jan-09	240	46
WS-DT3	11-Jan-09	1210	50
WS-BT1-D	11-Jan-09	300	48
WS-BT1*	13-Jan-09	>800	34
WS-BT2*	13-Jan-09	>800	16
WS-BT3*	13-Jan-09	>800	116
WS-BT4*	13-Jan-09	>800	34
WS-BT5*	13-Jan-09	>800	18
WS-DT1*	13-Jan-09	1820	28
WS-DT2*	13-Jan-09	2740	94
WS-DT3*	13-Jan-09	>2000	66
WS-BT2-D*	13-Jan-09	>800	46
WS-BT1	15-Jan-09	28	<2
WS-BT2	15-Jan-09	60	4
WS-BT3	15-Jan-09	28	2
WS-BT4	15-Jan-09	28	10
WS-BT5	15-Jan-09	24	<2
WS-DT1	15-Jan-09	24	<2
WS-DT2	15-Jan-09	92	36
WS-DT3	15-Jan-09	2760	6
WS-BT3-D	15-Jan-09	28	<2
WS-BT3-FLDB	15-Jan-09	24	<2
WS-BT1	18-Jan-09	80	4
WS-BT2	18-Jan-09	92	4
WS-BT3	18-Jan-09	56	10
WS-BT4	18-Jan-09	28	8
WS-BT5	18-Jan-09	28	16
WS-DT1	18-Jan-09	1800	56
WS-DT2	18-Jan-09	>2000	38
WS-DT3	18-Jan-09	>2000	40
WS-BT3-D	18-Jan-09	80	14
WS-BT1*	29-Jan-09	<4	4
WS-BT2*	29-Jan-09	8	8
WS-BT3*	29-Jan-09	12	16
WS-BT4*	29-Jan-09	92	40
WS-BT5*	29-Jan-09	40	36
WS-DT1*	29-Jan-09	400	>120
WS-DT2*	29-Jan-09	760	116
WS-DT3*	29-Jan-09	1190	98
WS-BT4-D*	29-Jan-09	92	30
WS-BT4-FLDB*	29-Jan-09	<4	<2

* Represents data collected during a rain event (defined as > 0.25 inches of rain in the 12 hour period prior to sampling).

Table 6. Concentration of Enterococci and *Pseudomonas* in samples collected at five beach and three ditch transects in the vicinity of Surfside Swash, Surfside, South Carolina.

Sample ID	Sample Date	Enterocci (CFU/100mL)	*Pseudomonas* (CFU/100mL)	Sample ID	Sample Date	Enterocci (CFU/100mL)	*Pseudomonas* (CFU/100mL)
SS-BT1	16-Dec-08	4	<4	SS-DT1	28-Dec-08	40	20
SS-BT2	16-Dec-08	10	<4	SS-DT2	28-Dec-08	50	132
SS-BT3	16-Dec-08	4	<4	SS-DT3	28-Dec-08	810	88
SS-BT4	16-Dec-08	10	<4	SS-BT4-D	28-Dec-08	20	<2
SS-BT5	16-Dec-08	12	<4	SS-BT4-FLDB	28-Dec-08	<4	<2
SS-DT1	16-Dec-08	96	48	SS-BT1	30-Dec-08	4	2
SS-DT2	16-Dec-08	400	160	SS-BT2	30-Dec-08	4	<2
SS-DT3	16-Dec-08	1300	<4	SS-BT3	30-Dec-08	4	<2
SS-DT1-D	16-Dec-08	350	176	SS-BT4	30-Dec-08	8	<2
SS-DT1-FLDB	16-Dec-08	<4	<4	SS-BT5	30-Dec-08	28	2
SS-BT1	18-Dec-08	8	6	SS-DT1	30-Dec-08	20	12
SS-BT2	18-Dec-08	8	10	SS-DT2	30-Dec-08	40	50
SS-BT3	18-Dec-08	8	<2	SS-DT3	30-Dec-08	>600	74
SS-BT4	18-Dec-08	<4	6	SS-BT1	2-Jan-09	16	<2
SS-BT5	18-Dec-08	<4	2	SS-BT2	2-Jan-09	8	<2
SS-DT1	18-Dec-08	56	34	SS-BT3	2-Jan-09	16	<2
SS-DT2	18-Dec-08	160	130	SS-BT4	2-Jan-09	8	6
SS-DT3	18-Dec-08	>600	22	SS-BT5	2-Jan-09	8	<2
SS-BT1	21-Dec-08	12	<2	SS-DT1	2-Jan-09	12	12
SS-BT2	21-Dec-08	28	2	SS-DT2	2-Jan-09	36	60
SS-BT3	21-Dec-08	32	<2	SS-DT3	2-Jan-09	710	62
SS-BT4	21-Dec-08	24	<2	SS-DT1-D	2-Jan-09	12	18
SS-BT5	21-Dec-08	16	14	SS-DT1-FLDB	2-Jan-09	<4	<2
SS-DT1	21-Dec-08	28	22	SS-BT1	4-Jan-09	12	<2
SS-DT2	21-Dec-08	304	16	SS-BT2	4-Jan-09	<4	<2
SS-DT3	21-Dec-08	>240	14	SS-BT3	4-Jan-09	<4	<2
SS-DT2-D	21-Dec-08	296	>120	SS-BT4	4-Jan-09	8	<2
SS-BT1	23-Dec-08	20	4	SS-BT5	4-Jan-09	8	<2
SS-BT2	23-Dec-08	8	4	SS-DT1	4-Jan-09	88	34
SS-BT3	23-Dec-08	28	2	SS-DT2	4-Jan-09	36	>120
SS-BT4	23-Dec-08	8	10	SS-DT3	4-Jan-09	1550	84
SS-BT5	23-Dec-08	8	4	SS-DT2-D	4-Jan-09	16	>120
SS-DT1	23-Dec-08	40	36	SS-BT1	6-Jan-09	16	10
SS-DT2	23-Dec-08	>240	>120	SS-BT2	6-Jan-09	12	2
SS-DT3	23-Dec-08	>240	28	SS-BT3	6-Jan-09	28	4
SS-BT2-FLDB	23-Dec-08	<4	<2	SS-BT4	6-Jan-09	8	4
SS-BT1	26-Dec-08	8	<2	SS-BT5	6-Jan-09	28	14
SS-BT2	26-Dec-08	4	2	SS-DT1	6-Jan-09	24	14
SS-BT3	26-Dec-08	<4	2	SS-DT2	6-Jan-09	132	92
SS-BT4	26-Dec-08	8	2	SS-DT3	6-Jan-09	1940	106
SS-BT5	26-Dec-08	12	<2	SS-DT3-D	6-Jan-09	2070	82
SS-DT1	26-Dec-08	4	28	SS-BT1	8-Jan-09	8	2
SS-DT2	26-Dec-08	92	>120	SS-BT2	8-Jan-09	<4	4
SS-DT3	26-Dec-08	>240	144	SS-BT3	8-Jan-09	12	4
SS-BT3-D	26-Dec-08	4	2	SS-BT4	8-Jan-09	<4	<2
SS-BT1	28-Dec-08	12	<2	SS-BT5	8-Jan-09	12	2
SS-BT2	28-Dec-08	20	<2	SS-DT1	8-Jan-09	44	18
SS-BT3	28-Dec-08	12	4	SS-DT2	8-Jan-09	316	124
SS-BT4	28-Dec-08	28	6	SS-DT3	8-Jan-09	>600	60
SS-BT5	28-Dec-08	32	6	SS-BT1-D	8-Jan-09	<4	<2

* Represents data collected during a rain event (defined as > 0.25 inches of rain in the 12 hour period prior to sampling).

Table 6 (cont'd). Concentration of Enterococci and *Pseudomonas* in samples collected at five beach and three ditch transects in the vicinity of Surfside Swash, Surfside, South Carolina.

Sample ID	Sample Date	Enterocci (CFU/100mL)	*Pseudomonas* (CFU/100mL)
SS-BT1-FLDB	8-Jan-09	<4	<2
SS-BT1	11-Jan-09	156	20
SS-BT2	11-Jan-09	160	24
SS-BT3	11-Jan-09	168	4
SS-BT4	11-Jan-09	168	4
SS-BT5	11-Jan-09	136	20
SS-DT1	11-Jan-09	140	10
SS-DT2	11-Jan-09	330	102
SS-DT3	11-Jan-09	290	40
SS-BT2-D	11-Jan-09	220	8
SS-BT1*	13-Jan-09	568	96
SS-BT2*	13-Jan-09	692	6
SS-BT3*	13-Jan-09	680	6
SS-BT4*	13-Jan-09	608	36
SS-BT5*	13-Jan-09	560	10
SS-DT1*	13-Jan-09	104	36
SS-DT2*	13-Jan-09	450	112
SS-DT3*	13-Jan-09	>2000	56
SS-BT3-D*	13-Jan-09	552	6
SS-BT1	15-Jan-09	44	<2
SS-BT2	15-Jan-09	56	2
SS-BT3	15-Jan-09	36	<2
SS-BT4	15-Jan-09	40	4
SS-BT5	15-Jan-09	24	6
SS-DT1	15-Jan-09	96	10
SS-DT2	15-Jan-09	236	60
SS-DT3	15-Jan-09	2470	68
SS-BT4-D	15-Jan-09	40	2
SS-BT4-FLDB	15-Jan-09	<4	<2
SS-BT1	18-Jan-09	8	4
SS-BT2	18-Jan-09	16	2
SS-BT3	18-Jan-09	28	4
SS-BT4	18-Jan-09	28	12
SS-BT5	18-Jan-09	20	12
SS-DT1	18-Jan-09	4	12
SS-DT2	18-Jan-09	88	42
SS-DT3	18-Jan-09	1230	112
SS-BT4-D	18-Jan-09	28	6
SS-BT1*	29-Jan-09	4	<2
SS-BT2*	29-Jan-09	<4	4
SS-BT3*	29-Jan-09	4	4
SS-BT4*	29-Jan-09	4	4
SS-BT5*	29-Jan-09	10	2
SS-DT1*	29-Jan-09	100	46
SS-DT2*	29-Jan-09	690	>120
SS-DT3*	29-Jan-09	1210	>120
SS-BT5-D*	29-Jan-09	<4	4
SS-BT5-FLDB*	29-Jan-09	4	<2

* Represents data collected during a rain event (defined as > 0.25 inches of rain in the 12 hour period prior to sampling).

Table 7. Mean Enterococci and *Pseudomonas* concentrations in baseline and rain event samples collected in the vicinity of Silver Beach and Florida Shores, Florida and Canes Patch Swash, Withers Swash and Surfside Swash, South Carolina.

	Baseline Measurements		Rain Event Measurements	
Site ID	Mean Enterococcus Concentration (CFU/100mL)	Mean Pseudomonas Concentration (CFU/100mL)	Mean Enterococcus Concentration (CFU/100mL)	Mean Pseudomonas Concentration (CFU/100mL)
FS-1	16	0.07	24	0
FS-2	18	0	21	0
FS-3	26	0	22	0
FS*	20	0.02	22	0
SB-1	14	0	13	0
SB-2	10	0.07	3.0	0
SB-3	12	0.07	9.0	0
SB*	12	0.04	8.3	0
CPS-BT-1	160	10	136	62
CPS-BT-2	147	14	150	53
CPS-BT-3	171	6.9	130	57
CPS-BT-4	161	10	112	63
CPS-BT-5	177	12	115	26
CPS-DT-1	307	80	410	99
CPS-DT-2	240	123	1,115	31
CPS-DT-3	19	18	64	85
CPS-BT*	163	11	129	52
CPS-DT*	189	74	530	72
CPS*	173	35	279	60
WS-BT-1	56	6.4	400	19
WS-BT-2	76	3.9	404	12
WS-BT-3	79	6.1	406	66
WS-BT-4	69	7.3	446	37
WS-BT-5	58	11	420	27
WS-DT-1	362	34	1,110	74
WS-DT-2	610	52	1,750	105
WS-DT-3	992	44	1,595	82
WS-BT*	67	6.9	415	32
WS-DT*	654	43	1,485	87
WS*	231	17	816	53
SS-BT-1	23	3.4	286	48
SS-BT-2	24	3.7	346	5.0
SS-BT-3	27	1.7	342	5.0
SS-BT-4	25	3.9	306	20
SS-BT-5	25	5.9	285	6.0
SS-DT-1	49	22	102	41
SS-DT-2	176	95	570	116
SS-DT-3	916	64	1,605	88
SS-BT*	25	3.7	313	17
SS-DT*	380	60	759	82
SS*	86	21	480	41

* = Includes all transects in this category.

Table 8. Concentration of Enterococci and *Pseudomonas* in samples collected at three beach transects in the vicinity of Silver Beach, Daytona Beach, Florida.

Sample ID	Sample Date	Enterocci (CFU/100mL)	*Pseudomonas* (CFU/100mL)	Sample ID	Sample Date	Enterocci (CFU/100mL)	*Pseudomonas* (CFU/100mL)
SB-1	16-Dec-08	4	<1	SB-1	4-Jan-09	19	<1
SB-2	16-Dec-08	16	<1	SB-2	4-Jan-09	15	<1
SB-3	16-Dec-08	4	<1	SB-3	4-Jan-09	20	<1
SB-1-D	16-Dec-08	5	<1	SB-1	6-Jan-09	39	<1
SB-1-FLDB	16-Dec-08	<1	<1	SB-2	6-Jan-09	29	<1
SB-1	18-Dec-08	20	<1	SB-3	6-Jan-09	22	<1
SB-2	18-Dec-08	7	1	SB-1	8-Jan-09	2	<1
SB-3	18-Dec-08	7	1	SB-2	8-Jan-09	5	<1
SB-1	21-Dec-08	21	<1	SB-3	8-Jan-09	6	<1
SB-2	21-Dec-08	6	<1	SB-2-D	8-Jan-09	2	<1
SB-3	21-Dec-08	6	<1	SB-1	11-Jan-09	6	<1
SB-1	23-Dec-08	1	<1	SB-2	11-Jan-09	6	<1
SB-2	23-Dec-08	<1	<1	SB-3	11-Jan-09	5	<1
SB-3	23-Dec-08	<1	<1	SB-1	13-Jan-09	32	<1
SB-2-D	23-Dec-08	<1	<1	SB-2	13-Jan-09	29	<1
SB-1	26-Dec-08	15	<1	SB-3	13-Jan-09	47	<1
SB-2	26-Dec-08	8	<1	SB-1	15-Jan-09	4	<1
SB-3	26-Dec-08	16	<1	SB-2	15-Jan-09	5	<1
SB-1	29-Dec-08	16	<1	SB-3	15-Jan-09	4	<1
SB-2	29-Dec-08	5	<1	SB-2-D	15-Jan-09	5	<1
SB-3	29-Dec-08	12	<1	SB-3-FLDB	15-Jan-09	<1	<1
SB-1	30-Dec-08	5	<1	SB-1	18-Jan-09	<1	<1
SB-2	30-Dec-08	9	<1	SB-2	18-Jan-09	<1	<1
SB-3	30-Dec-08	2	<1	SB-3	18-Jan-09	5	<1
SB-3-D	30-Dec-08	2	<1	SB-1*	30-Jan-09	13	<1
SB-2-FLDB	30-Dec-08	<1	<1	SB-2*	30-Jan-09	3	<1
SB-1	2-Jan-09	18	<1	SB-3*	30-Jan-09	9	<1
SB-2	2-Jan-09	13	<1	SB-2-D*	30-Jan-09	6	<1
SB-3	2-Jan-09	16	<1	SB-2-FLDB*	30-Jan-09	<1	<1

* Represents data collected during a rain event (defined as > 0.25 inches of rain in the 12 hour period prior to sampling).

Table 9. Concentration of Enterococci and *Pseudomonas* in samples collected at three beach transects in the vicinity of Florida Shores, Daytona Beach, Florida.

Sample ID	Sample Date	Enterocci (CFU/100mL)	*Pseudomonas* (CFU/100mL)	Sample ID	Sample Date	Enterocci (CFU/100mL)	*Pseudomonas* (CFU/100mL)
FS-1	16-Dec-08	10	<1	FS-1	04-Jan-09	5	<1
FS-2	16-Dec-08	17	<1	FS-2	04-Jan-09	16	<1
FS-3	16-Dec-08	70	<1	FS-3	04-Jan-09	7	<1
FS-1-D	16-Dec-08	11	<1	FS-1	06-Jan-09	4	<1
FS-1-FLDB	16-Dec-08	<1	<1	FS-2	06-Jan-09	9	<1
FS-1	18-Dec-08	21	1	FS-3	06-Jan-09	3	<1
FS-2	18-Dec-08	22	<1	FS-1	08-Jan-09	28	<1
FS-3	18-Dec-08	14	<1	FS-2	08-Jan-09	30	<1
FS-1	21-Dec-08	3	<1	FS-3	08-Jan-09	92	<1
FS-2	21-Dec-08	4	<1	FS-1-D	08-Jan-09	28	<1
FS-3	21-Dec-08	5	<1	FS-1	11-Jan-09	20	<1
FS-1	23-Dec-08	21	<1	FS-2	11-Jan-09	9	<1
FS-2	23-Dec-08	4	<1	FS-3	11-Jan-09	3	<1
FS-3	23-Dec-08	8	<1	FS-1	13-Jan-09	72	<1
FS-2-D	23-Dec-08	3	<1	FS-2	13-Jan-09	92	<1
FS-1	26-Dec-08	9	<1	FS-3	13-Jan-09	108	<1
FS-2	26-Dec-08	12	<1	FS-1	15-Jan-09	7	<1
FS-3	26-Dec-08	11	<1	FS-2	15-Jan-09	10	<1
FS-1	29-Dec-08	10	<1	FS-3	15-Jan-09	28	<1
FS-2	29-Dec-08	4	<1	FS-2-D	15-Jan-09	10	<1
FS-3	29-Dec-08	3	<1	FS-3-FLDB	15-Jan-09	<1	<1
FS-1	30-Dec-08	16	<1	FS-1	18-Jan-09	4	<1
FS-2	30-Dec-08	22	<1	FS-2	18-Jan-09	2	<1
FS-3	30-Dec-08	13	<1	FS-3	18-Jan-09	8	<1
FS-3-D	30-Dec-08	12	1	FS-1*	30-Jan-09	24	<1
FS-2-FLDB	30-Dec-08	<1	<1	FS-2*	30-Jan-09	21	<1
FS-1	2-Jan-09	15	<1	FS-3*	30-Jan-09	22	<1
FS-2	2-Jan-09	20	<1	FS-2-D*	30-Jan-09	28	<1
FS-3	2-Jan-09	19	<1	FS-2-FLDB*	30-Jan-09	<1	<1

* Represents data collected during a rain event (defined as > 0.25 inches of rain in the 12 hour period prior to sampling).

Table 10. Concentrations of Enterococci and *Pseudomonas* in field blank samples collected in Myrtle Beach, South Carolina and Daytona Beach, Florida.

Sample ID	Enterocci (CFU/100mL)	*Pseudomonas* (CFU/100mL)
CPS-BT4-20081223-FLDB	<4	<2
CPS-DT2-20081228-FLDB	<4	<2
CPS-BT1-20090102-FLDB	<4	<2
CPS-BT4-20090108-FLDB	<4	<2
CPS-DT2-20090115-FLDB	<4	<2
CPS-BT1-20090129-FLDB	36*	<2*
CPS-DT2-20081216-FLDB	<4	<4
WS-BT4-20081216-FLDB	<4	<4
WS-DT3-20081223-FLDB	<4	<2
WS-BT3-20081228-FLDB	<4	4
WS-BT5-20090102-FLDB	<4	<2
WS-DT3-20090108-FLDB	<4	<2
WS-BT3-20090115-FLDB	24	<2
WS-BT4-20090129-FLDB	<4	<2
SS-DT1-20081216-FLDB	<4	<4
SS-BT2-20081223-FLDB	<4	<2
SS-BT4-20081228-FLDB	<4	<2
SS-DT1-20090102-FLDB	<4	<2
SS-BT1-20090108-FLDB	<4	<2
SS-BT4-20090115-FLDB	<4	<2
SS-BT5-20090129-FLDB	4	<2
FS-1-20081216-FLDB	<1	<1
FS-2-20081230-FLDB	<1	<1
FS-3-20090115-FLDB	<1	<1
FS-2-20090130-FLDB	<1	<1
SB-1-20081216-FLDB	<1	<1
SB-2-20081230-FLDB	<1	<1
SB-3-20090115-FLDB	<1	<1
SB-2-20090130-FLDB	<1	<1

* Represents data collected during a rain event (defined as > 0.25 inches of rain in the 12 hour period prior to sampling).

Table 11. Concentrations of Enterococci and *Pseudomonas* in investigative and field duplicate samples collected in Myrtle Beach, South Carolina and Daytona Beach, Florida.

Sample ID	Enterocci - Investigative (CFU/100mL)	Enterocci - Duplicate (CFU/100mL)	*Pseudomonas* - Investigative (CFU/100mL)	*Pseudomonas* - Duplicate (CFU/100mL)
CPS-BT1-20081216	16	8	16	28
CPS-BT5-20081221	260	256	44	24
CPS-DT1-20081226	300	272	6	28
CPS-DT2-20081228	96	68	20	54
CPS-BT1-20090102	28	4	4	4
CPS-BT4-20090104	96	44	22	8
CPS-BT3-20090106	484	412	26	22
CPS-BT4-20090108	320	320	<2	6
CPS-BT5-20090111	732	876	14	8
CPS-DT1-20090113	470	420	168	138
CPS-DT2-20090115	176	116	50	68
CPS-DT3-20090118	<4	<4	14	10
CPS-BT1-20090129	88	60	<2	4
WS-BT5-20081216	40	84	52	<4
WS-DT3-20081221	>240	>240	28	24
WS-BT2-20081226	128	160	<2	<2
WS-BT3-20081228	28	12	8	2
WS-BT5-20090102	12	20	<2	<2
WS-DT1-20090104	272	320	12	14
WS-DT2-20090106	340	520	24	68
WS-DT3-20090108	1190	920	66	50
WS-BT1-20090111	290	300	38	48
WS-BT2-20090113	>800	>800	16	46
WS-BT3-20090115	28	28	2	<2
WS-BT3-20090118	56	80	10	14
WS-BT4-20090129	92	92	40	30
SS-DT1-20081216	96	350	48	176
SS-DT2-20081221	304	296	16	>120
SS-BT3-20081226	<4	4	2	2
SS-BT4-20081228	28	20	6	<2
SS-DT1-20090102	12	12	12	18
SS-DT2-20090104	36	16	>120	>120
SS-DT3-20090106	1940	2070	106	82
SS-BT1-20090108	8	<4	2	<2
SS-BT2-20090111	160	220	24	8
SS-BT3-20090113	680	552	6	6
SS-BT4-20090115	40	40	4	2
SS-BT4-20090118	28	28	12	6
SS-BT5-20090129	10	<4	2	4
FS-1-20081216-P	10	11	<1	<1
FS-2-20081223-P	4	3	<1	<1
FS-3-20081230-P	13	12	<1	<1
FS-1-20090108-P	28	28	<1	<1
FS-2-20090115-P	10	10	<1	<1
FS-2-20090130-P	21	28	<1	<1
SB-1-20081216-P	4	5	<1	<1
SB-2-20081223-P	<1	<1	<1	<1
SB-3-20081230-P	<2	<2	<1	<1
SB-1-20090108-P	<2	<2	<1	<1
SB-2-20090115-P	5	5	<1	<1
SB-2-20090130-P	3	6	<1	<1