

Siesta Key Beach Water Quality Sampling to Determine Sources of Fecal Indicator Bacteria

Final Report

May 20, 2005

Prepared for:



Sarasota County

Water Resources
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FOREWORD

This report was prepared for Sarasota County by Post, Buckley, Schuh & Jernigan (PBS&J) and the University of South Florida under contract number 2004-137.

ACKNOWLEDGEMENTS

Several staff from Sarasota County provided invaluable support and guidance to this project including: Ms. Laura Ammeson, Ms. Theresa Connor, Mr. Bruce Maloney, and Ms. Kathy Meaux.

EXECUTIVE SUMMARY

On April 12, 2004, concentrations of the fecal indicator bacteria groups *Enterococcus* and fecal coliforms were above state-mandated limits, causing recreational water quality to be rated “poor,” and a “no swim” advisory to be issued for Siesta Key Beach. Sarasota County staff immediately began comprehensive water quality monitoring at several locations upstream of the beach area that received the advisory. Samples were collected for total and fecal coliform, fecal streptococcus, and enterococcus bacteria, nutrients (total nitrogen, total phosphorus), turbidity, temperature, salinity, conductance, pH, dissolved oxygen, and water depth. Sample results showed very high concentrations (above Class III recreational water quality standards) of both total and fecal coliform bacteria at numerous locations in the ditch and stormwater pipe drainage system that discharged to Siesta Key beach. However, since the coliform bacteria tests only serves as an indicator of fecal contamination, a definitive source for the high bacteria counts could not be determined.

A study was initiated to determine the cause of the elevated bacterial counts. A sanitary survey of the area identified an underground system of inlets and pipes that delivers stormwater to an underground vault, from which the first flush of stormwater is pumped to a retention pond. Subsurface flow from the retention pond along with excess runoff from the road flow to a ditch that discharges at Siesta Key Beach and empties near the Florida Department of Health (DOH) recreational beach monitoring site. Although the stormwater pipe system should not contain sewage, the observation of high bacterial counts during storm events called this assumption into question. Smoke tests and inspections of the wastewater force main system by Siesta Key Utilities Authority (SKUA) did not reveal any leaks into the stormwater conveyance system.

The study design therefore included sampling of the water and sediments of the various components of the stormwater system and the beach for bacterial levels, as well as PCR-based tests for human-specific viruses and bacteria. Furthermore, indicator bacteria (*Escherichia coli*, which is a species belonging to the fecal coliform group, and enterococci) were subjected to “DNA fingerprinting” by BOX-PCR, and the population similarity of bacteria isolated from various locations was compared. Two sampling events were conducted, one immediately preceded by and coinciding with very heavy rainfall, and the second during a dry period.

High levels of indicator bacteria (fecal coliforms and enterococci) in the stormwater/vault/drainage ditch suggested that these might be environmental reservoirs of indicator bacteria. No human-specific signals were obtained from the PCR tests during either sample event, suggesting that no relationship exists between fecal indicator bacteria in the stormwater system and existing wastewater conveyance systems. Analysis of the *Enterococcus* fingerprints showed that during the rain event, populations in beach water and sediments were similar to populations in the ditch sediments and ditch water, as well as to populations in vault water and water in the stormwater pipe system. Similar results were found for *E. coli* populations.

During the second sample event, carried out during dry antecedent conditions, significantly lower numbers of indicator bacteria were observed. *Enterococcus* populations were even more

similar to each other than they were during the rain event, and fingerprints were shared among all of the locations sampled. *E. coli* populations behaved similarly.

This project represents the “toolbox approach” that has been discussed and advocated in many microbial source tracking (MST) reviews and workshops. Since no definitive, statistically rigorous (i.e., having 95%+ accuracy) test has been identified for MST, investigators must rely upon the weight of evidence approach using a suite of tests to provide convincing evidence of the probable source(s) of fecal/microbial pollution. Conclusions from this stormwater evaluation are as follow:

- There is no evidence of a human source for the indicator bacteria in the stormwater system.
- There is evidence that the stormwater conveyance system is acting as a reservoir, or “breeding ground” for indicator bacteria.
- Rainfall flushes high bacterial loads through the system, and also probably resuspends bacteria living in the sediments of the stormwater pipe, the vault and the ditch, further elevating the load to receiving waters at the beach.
- The microbial pollution delivered to Siesta Key Beach via the stormwater system does not carry the same level of risk that it would if the pollution were from human sewage. However, members of the enterococci, including *Enterococcus faecium* and *Enterococcus faecalis*, are opportunistic pathogens, and elevated levels could conceivably pose a risk for the very young or immunocompromised.
- While waterborne human pathogens are most prevalent in human sewage, other animals do sometimes shed pathogens in their feces that could infect humans. These types of microorganisms are termed “zoonoses” and represent infectious diseases that can be transmitted from animals, both wild and domestic, to humans. Cattle and swine are two of the higher-risk animal groups, as they can shed pathogenic microorganisms (such as *Cryptosporidium*) in their feces. Pets (dogs, cats, birds) and wildlife are also potential sources of fecal coliform bacteria. Although there are no known livestock in the watershed area that drains to the beach, other sources of fecal contamination (e.g., transplanted sod from sod farming areas utilizing livestock manure as fertilizer) may exist and so it cannot be definitively stated that there is no risk to human health from stormwater with high fecal coliform bacterial levels.
- Diversion of the drainage ditch from the beach should decrease or stop the intermittent observations of high bacterial levels at the beach. However, treatment of the diverted runoff should be considered if it will be discharged to other surface waters that would have human contact.

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1.0 Introduction

Siesta Key Beach is located on a barrier island on the west coast of Florida in Sarasota County. It has been consistently listed among the top beaches in the United States and the world. On April 12, 2004, immediately following a significant rainfall event (Figure 1), fecal indicator bacteria levels at Siesta Key Beach were found to be elevated, causing water quality to be rated “poor” for both *Enterococcus* and fecal coliform parameters. As a result, a “no swim” advisory was issued for the recreational beach area. Although this is not a frequent occurrence, Sarasota County sought an explanation for the elevated bacterial numbers.

Sarasota County staff immediately began comprehensive water quality monitoring at several locations upstream of the beach area that received the advisory. Samples were collected for total and fecal coliform, fecal streptococcus, and enterococcus bacteria, nutrients (total nitrogen, total phosphorus), turbidity, temperature, salinity, conductance, pH, dissolved oxygen, and water depth. Sample results showed very high concentrations (above Class III recreational water quality standards) of both total and fecal coliform bacteria at numerous locations in the ditch and stormwater pipe drainage system that discharged to Siesta Key beach. However, since the coliform bacteria tests only serves as an indicator of fecal contamination, a definitive source for the high bacteria counts could not be determined.

The project team consisting of PBS&J of Sarasota, Florida (Ray Kurz, Ph.D., project manager), Valerie J. Harwood, Ph.D. at the University of South Florida, and Biological Consulting Service of North Florida Inc., Gainesville, Florida (Troy Scott, Ph.D. and Jerzy Lukasik, Ph.D were selected to develop and implement a monitoring program to assess the source(s) of bacterial contamination at the beach.

This project represents the “toolbox approach” that has been discussed and advocated in many microbial source tracking (MST) reviews and workshops. Since no definitive, statistically rigorous (i.e., having 95%+ accuracy) test has been identified for MST, investigators must rely upon the weight of evidence approach using a suite of tests to provide convincing evidence of the probable source(s) of fecal/microbial pollution.

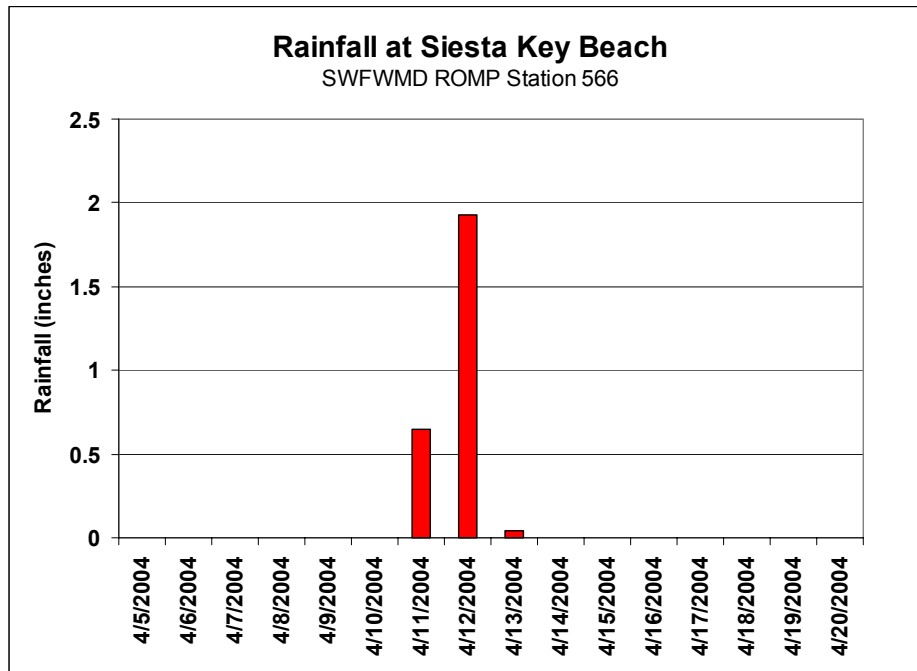


Figure 1. Rainfall, in inches, during the beach advisory at Siesta Key Beach.

Beach Road is a paved thoroughfare that runs parallel to Siesta Key Beach (Figure 2). During roadway improvements in the 1980s, a series of underground pipes were installed under Beach Road which transport stormwater runoff from an approximately 60 acre basin to two outfalls to the Grand Canal and also a concrete vault located further downstream on the west side of the road. A pump system empties the stormwater pipe during the onset of a storm event and pumps the first inch of runoff from the vault into a retention pond located immediately east of the pump station and vault. Subsurface flows from the retention pond drain laterally into an adjacent ditch that flows to the beach and into the Gulf of Mexico. The ditch is heavily shaded by both native (mangrove) and exotic (Brazilian pepper and Australian pine trees) vegetation which have recruited along the banks of the ditch. This vault-pond-ditch system has been considered a possible source of indicator bacteria at Siesta Key Beach.

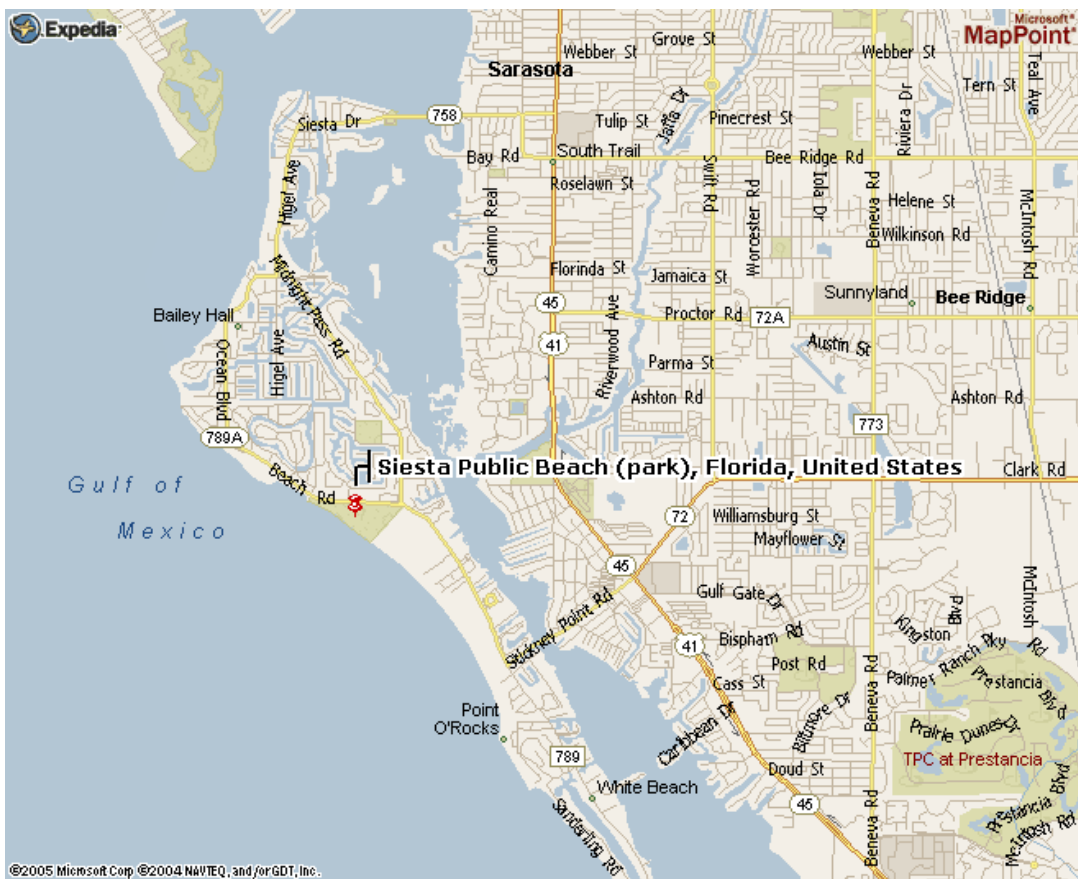
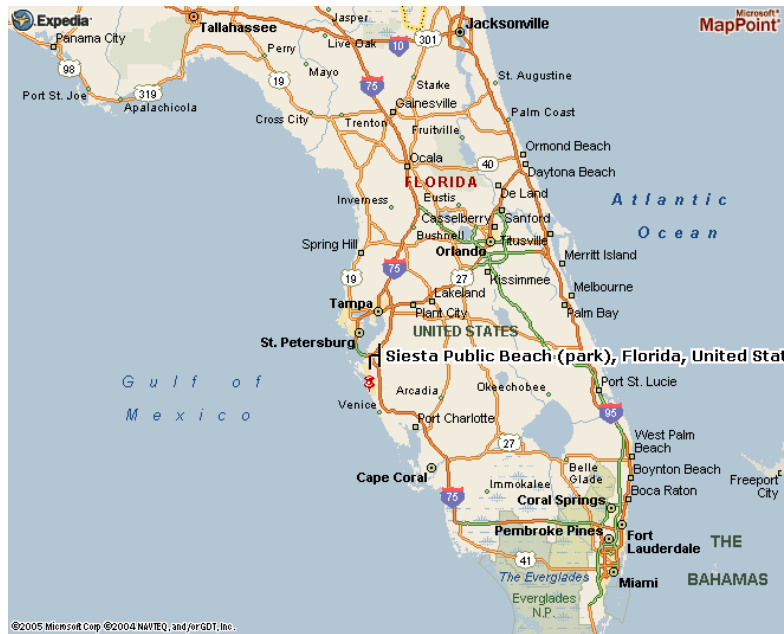


Figure 2. General project location maps of Siesta Key Beach area.

Many Florida coastal counties have participated in the Healthy Beaches water sampling program that began in 1998 and expanded in 2000 to include all coastal counties. County health departments collect samples and analyze them for two groups of indicator bacteria, fecal coliforms and enterococci. If the standards are exceeded (>399 colony forming units (CFU)/100 ml for fecal coliforms and >103 CFU/100ml for enterococci) then retesting takes place or a health advisory is posted. In April of 2004, indicator bacteria levels exceeded these standards at Siesta Key Beach and health warnings were posted.

Indicator bacteria are found in the intestines of humans and other animals and have been used as surrogates for waterborne pathogens since the early twentieth century, but are generally not a health threat themselves. State standards delineate the acceptable levels of these indicators, and any water source containing higher levels would be considered a health risk. This can result in posting “no swim” advisories for recreational beach sites and closing of shellfish harvesting beds, and potentially significant losses in revenue from tourism and commercial fishing.

2.0 Materials and Methods

Two sampling events were conducted during this study; one within 48 hours of heavy rainfall on August 3, 2004, and one during a dry period on August 31, 2004. Rainfall measurements prior to these two events are shown in Figures 3 and 4 from a Southwest Florida Water Management District (SWFWMD) gaging station within Siesta Key Beach park. Water and sediment samples were taken at various points of the vault-pond-ditch system, from two sites at Siesta Key Beach (sediments and flowing water from the ditch to the beach) and from the Gulf of Mexico (Table 1, Figure 5). Fecal coliforms and enterococci (standard indicator bacteria) were enumerated in all samples. Samples were also analyzed by polymerase chain reaction (PCR) for the presence of human-specific microorganisms, which included human polyomaviruses and the enterococcal surface protein (*esp*) gene of *Enterococcus faecium*. *E. coli* and *Enterococcus* isolates cultured from the samples were “fingerprinted” (typed) by BOX-PCR in order to compare the genetic similarity among isolates in each site and to compare the population similarity between sites.

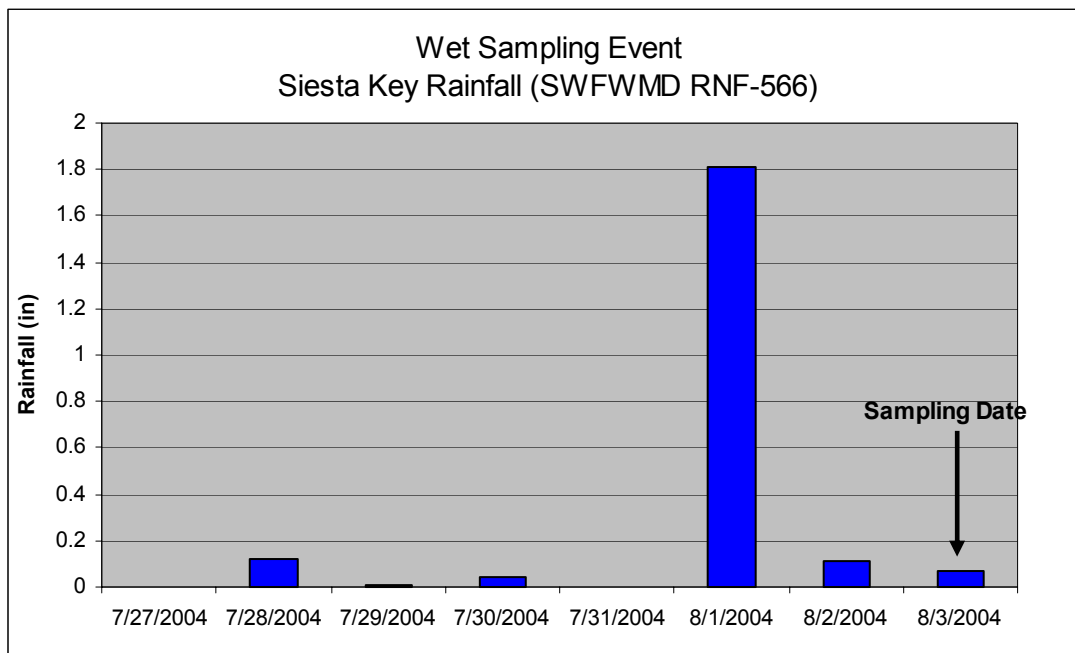


Figure 3. Rainfall conditions during wet sampling event at Siesta Key Beach.

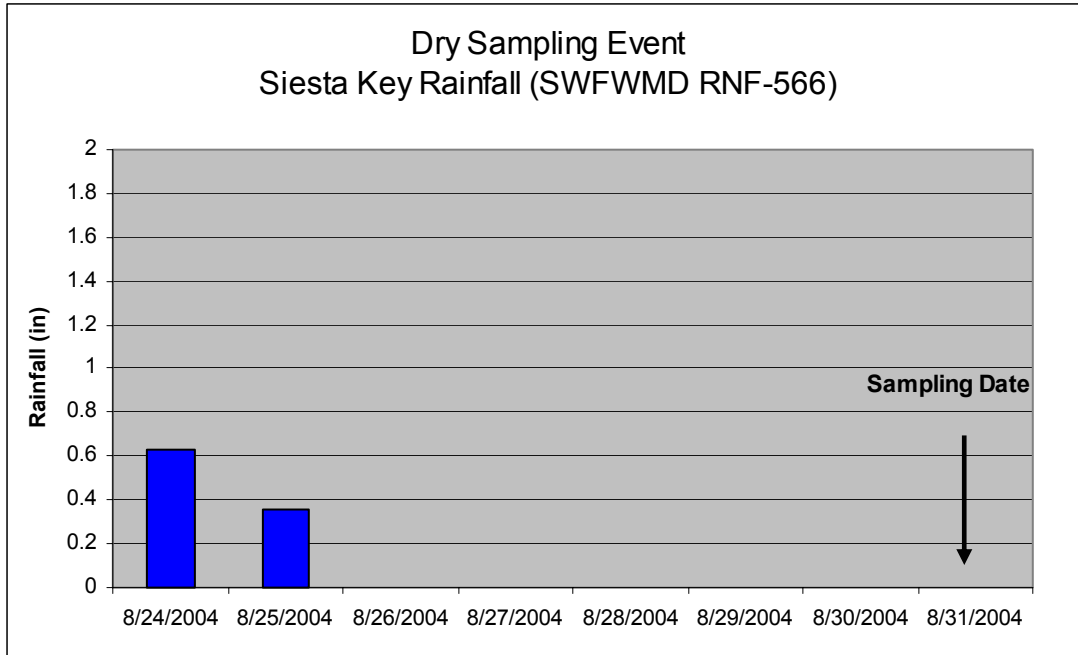


Figure 4. Rainfall conditions during dry sampling event at Siesta Key Beach.

Table 1. Sites sampled 08/03/04 and 08/31/04.

Date	Sites Sampled
08/03/04	Stormwater pipe water; vault water, ditch water, ditch sediment, beach water ¹ , beach sediment (total of 6 sites)
08/31/04	Stormwater pipe water; storm pipe sediment, vault water, pond water, pond sediment, ditch water, ditch sediment, beach water, beach sediment, Gulf water ² , Gulf sediment (total of 11 sites)

¹Beach water and sediment were collected on the beach, within a few yards of the ditch

²Gulf water and sediment were collected in the Gulf of Mexico



Figure 5. Sampling locations within the stormwater system draining to Siesta Key Beach (light blue arrows indicate general direction of stormwater flow).

3.0 Results and Discussion

After the heavy rainfall event on 8/3/04, fecal coliform and *Enterococcus* concentrations in the water column at all sites generally exceeded state water quality standards (Figure 6), but assays for human-specific enterococci and viruses (human polyoma virus) were negative (Appendix C). Bacterial concentrations in sediments were also elevated (Figure 7). Note that comparisons between water column and sediment values should be interpreted with caution, due to differences in units (cfu/100 ml vs. cfu/100 g). Horizontal lines on the graphs indicate Florida's regulatory standards for "poor" water quality in terms of enterococci (104 CFU/100 ml) and fecal coliforms (400 CFU/100 ml). Regulatory thresholds were not added to the sediment graphs because of the difference in units and since sediments are not sampled for regulatory purposes.

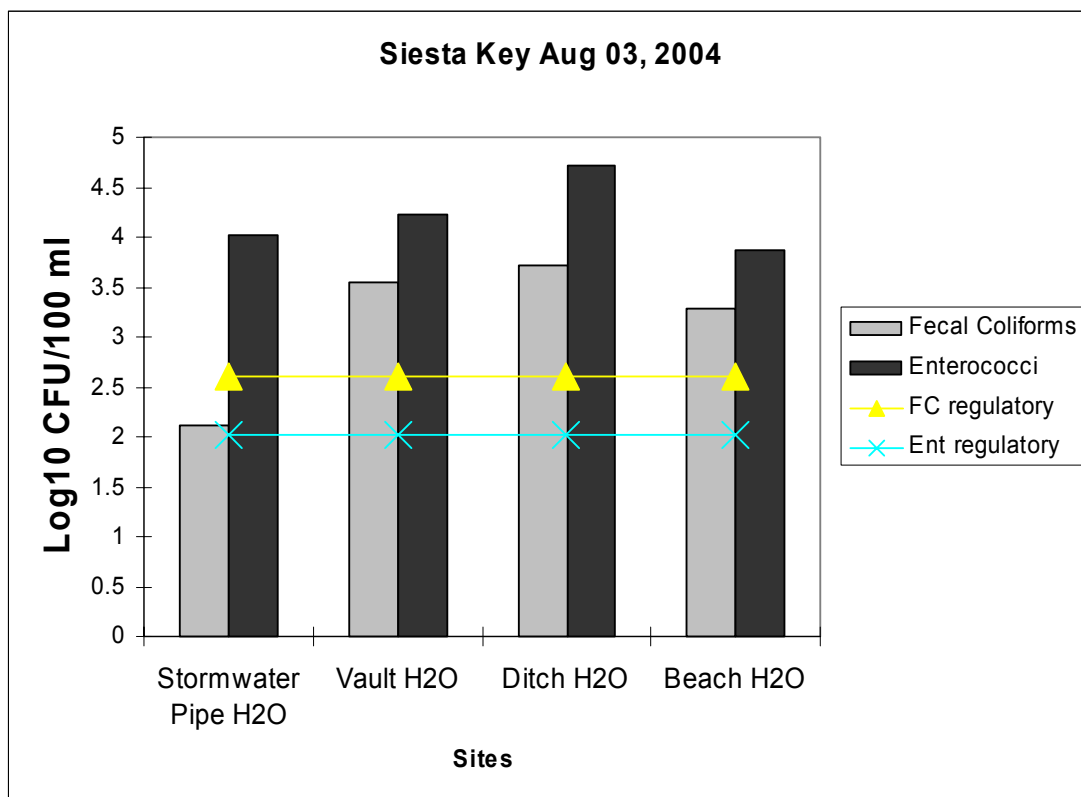


Figure 6. Water column fecal coliform and *Enterococcus* concentrations during wet event on 8/03/04.

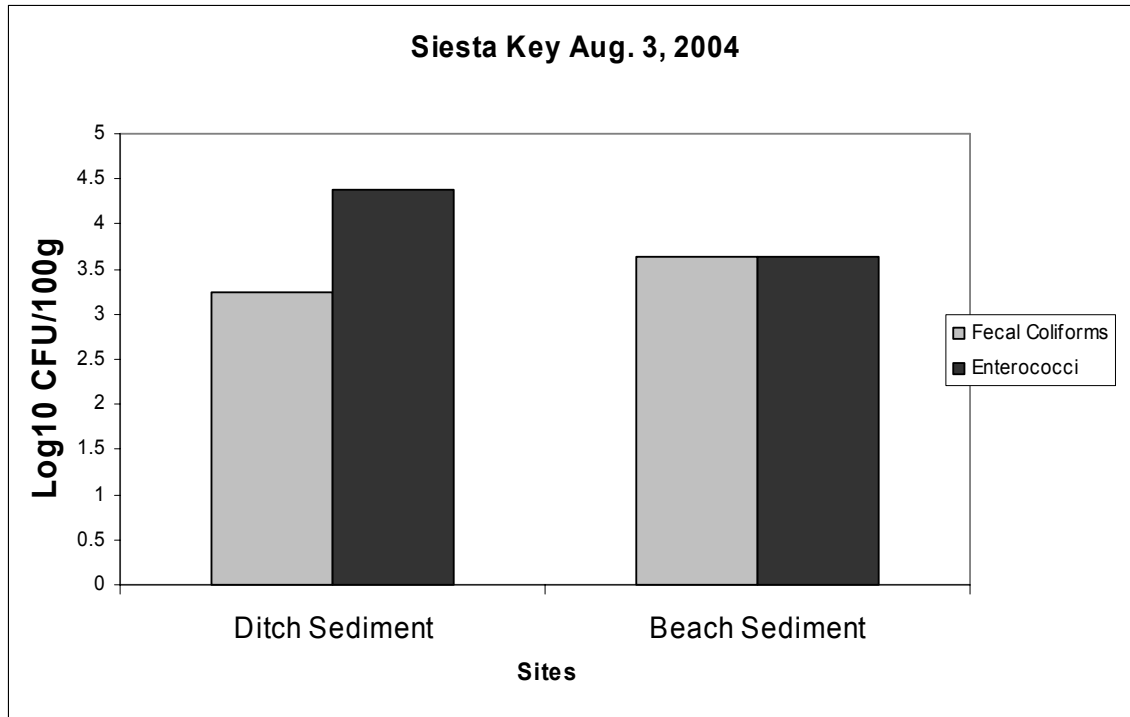


Figure 7. Fecal coliform and Enterococcus concentrations in sediment during wet event on 8/03/04.

The second sampling event, on 8/31/04, occurred after a dry period. Additional sample sites (Table 1, Figures 8 and 9) were added in order to obtain a more complete understanding of the possible sources and sinks of microorganisms in the system. While bacterial counts in the water column were much lower (Figure 8) than on the previous date (Figure 6), concentrations in sediment samples tended to remain elevated (compare Figure 7 to 9). Fecal coliform concentrations were lower than regulatory limits except at the Beach site (taken where ditch water contacted the beach), but *Enterococcus* concentrations continued to exceed regulatory limits in stormwater, vault water, and Beach site water.

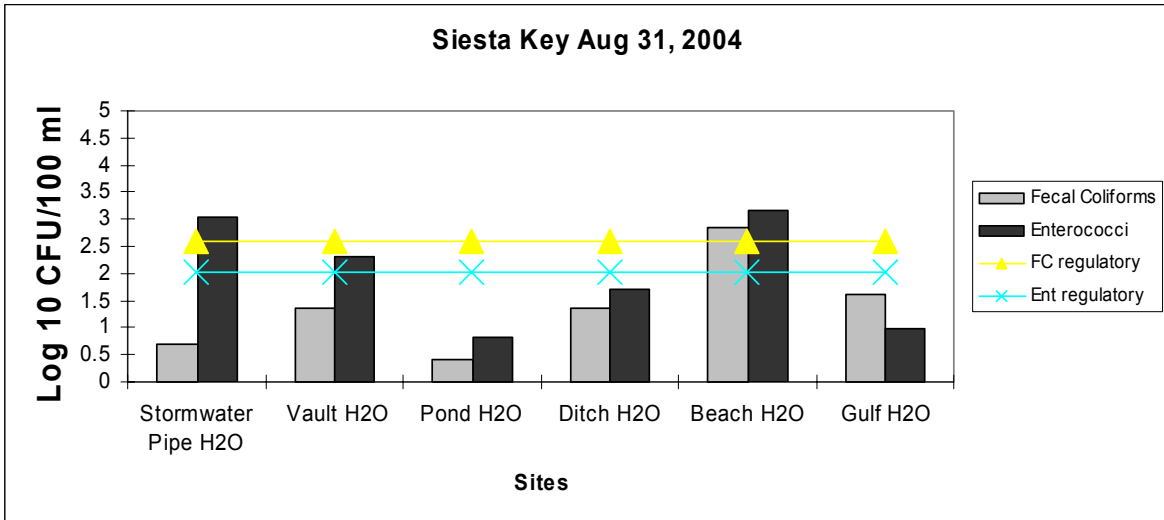


Figure 8. Water column fecal coliform and Enterococcus concentrations during dry event on 8/31/04.

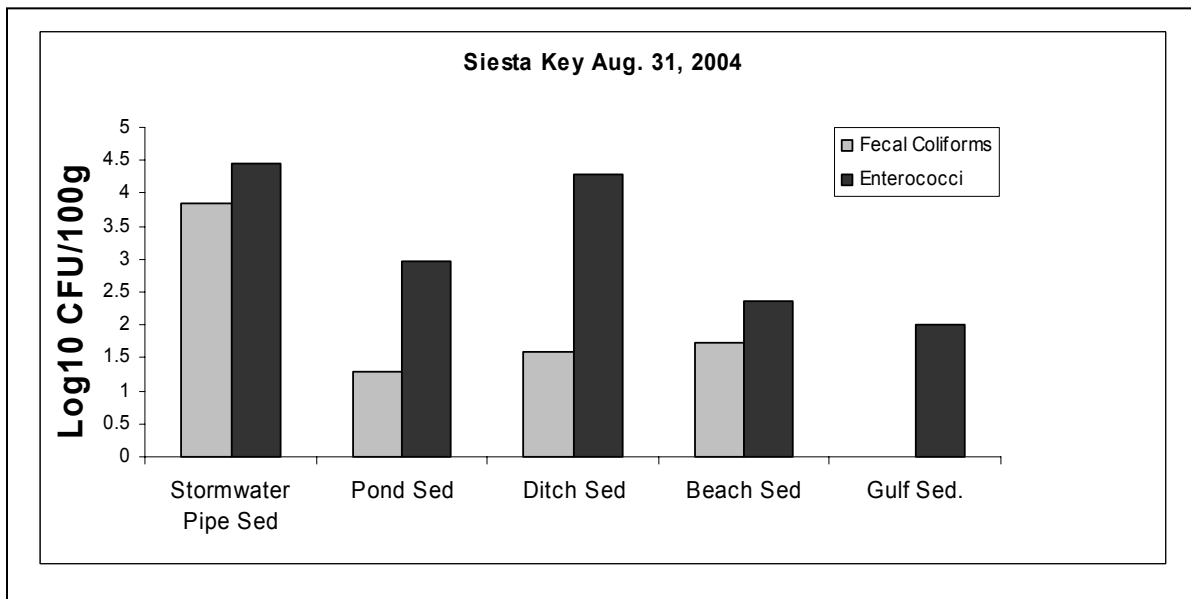


Figure 9. Fecal coliform and Enterococcus concentrations in sediments during dry event on 8/31/04.

Indicator organism concentrations at sites that were sampled on both dates (stormpipe water, vault water, ditch water, and beach water) were compared by a nonparametric version of the paired t-test (Wilcoxon signed-rank sum test). Concentrations were higher during the wet event and the *P*-value was nearly significant for fecal coliforms (0.0571), and was significant for enterococci (*P* = 0.0286). The mean fecal coliform concentration on 8/3/04 (\log_{10} -transformed) was 3.17, while it was 1.57 on 8/31/04. Corresponding means for enterococci were 4.20 on 8/3/04 and 2.56 on 8/31/04.

The DNA “fingerprinting” technique called BOX-PCR was used to type the various *Enterococcus* and *E. coli* species and strains in each sample. Patterns from each site were compared to assess population similarities. The presence of identical strains at the sampling sites would suggest the contribution of bacteria from one site to another and, possibly, replication or regrowth of bacteria within the stormwater drainage system. Although additional analyses are currently being performed on these samples, it is clear that certain strains from the microbial populations are common to the stormwater system, the vault, the ditch, and the beach, suggesting the link between stormwater runoff and beach contamination. Figure 10 shows the BOX-PCR patterns of *Enterococcus* isolates, most of which are from Gulf water sampled on 8/31/04. The patterns are arranged vertically, like a bar code, and each numbered lane contains the pattern from one *Enterococcus* isolate (colony originally picked from mEI agar). Note, for example, that the patterns in lanes 4 and 6 are identical, which means that the genetic composition (DNA) of these bacterial strains are very similar or identical. Figure 11 depicts the results from the stormwater drainage system sediments and vault water, many of which had similar banding patterns.

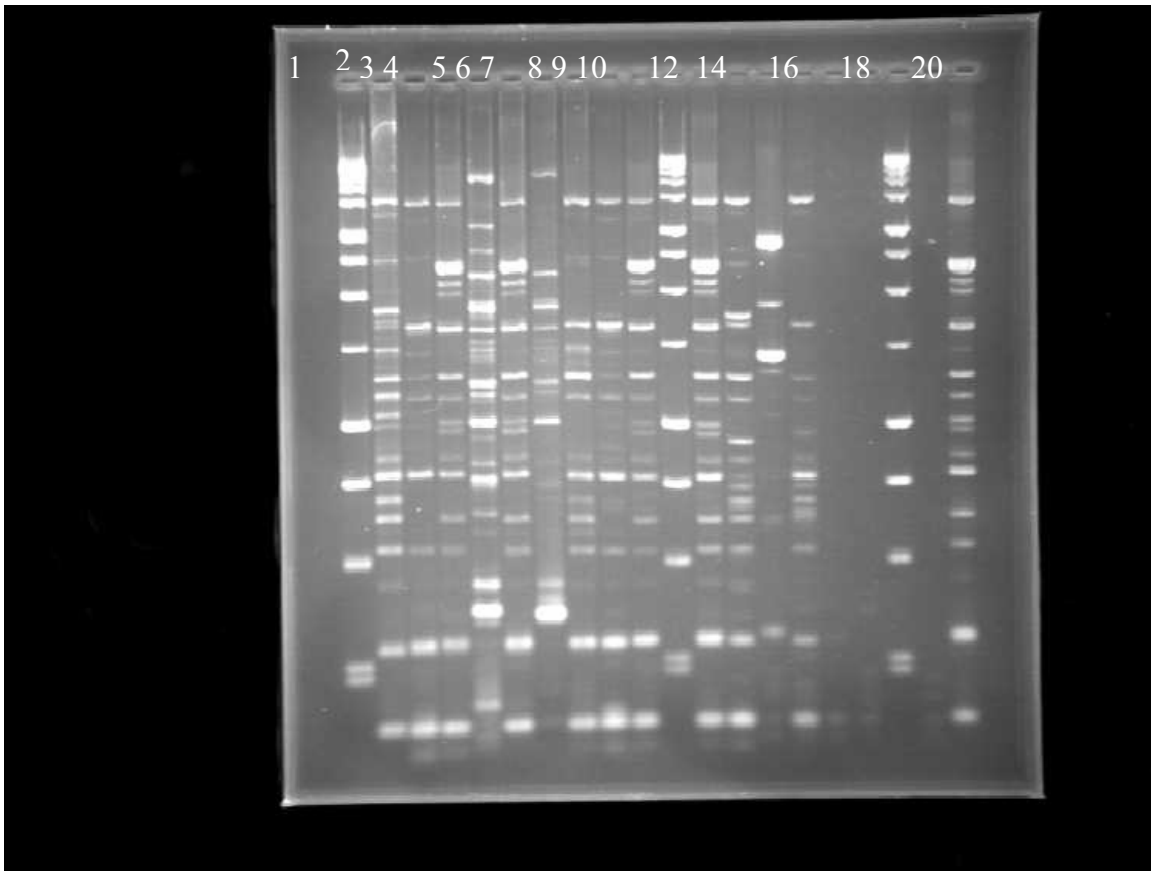


Figure 10. BOX-PCR patterns of Enterococcus isolates from Siesta Key sites sampled 8/31/04. Lanes 1, 11 and 18 standard ladder; lane 2 E. faecalis control strain; lanes 3 – 10 Gulf water isolates; lane 12 Gulf water isolate; lanes 13-14, Gulf sediment isolates; lanes 15 and 20, Beach water isolate.

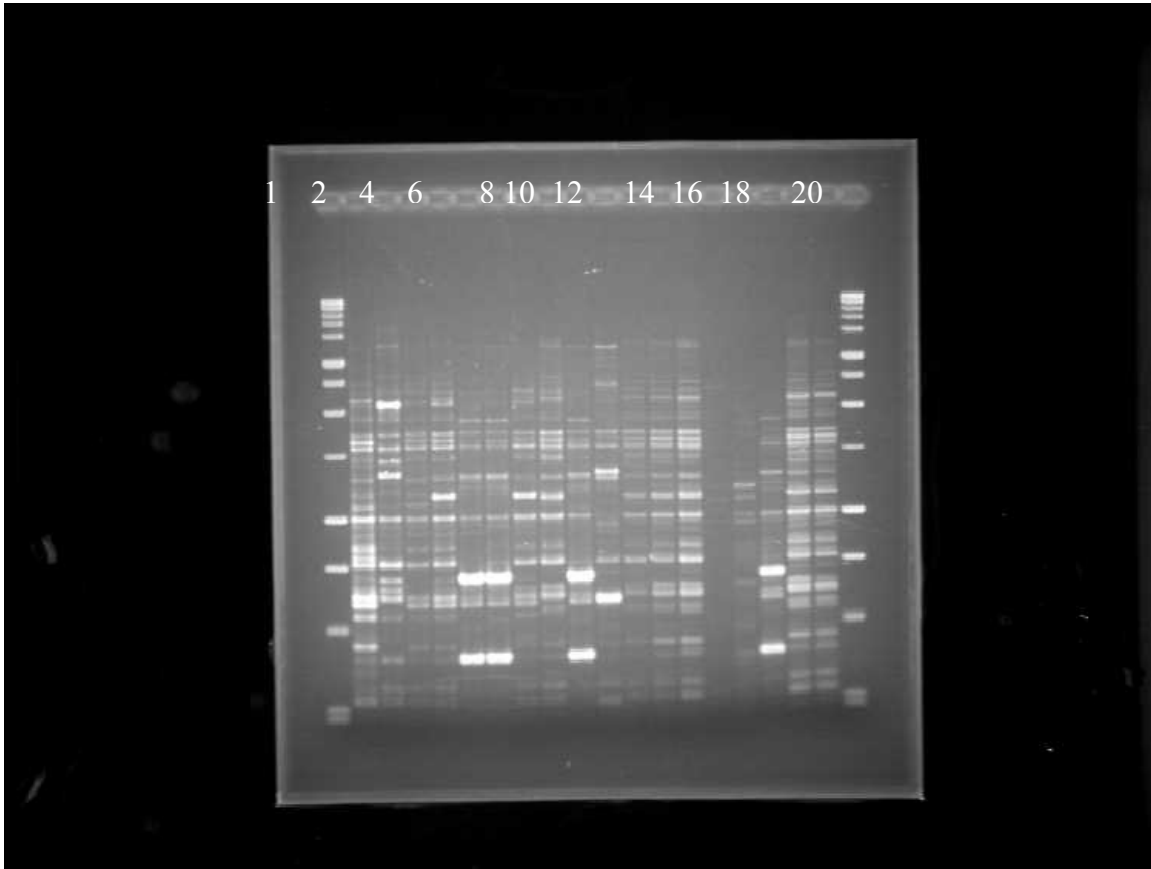


Figure 11. BOX-PCR patterns of E. coli isolates from Siesta Key sites sampled 8/31/04. Lanes 1 and 20, standard ladder; lane 2 E. coli control strain; lanes 3 - 5 stormwater sediment; lanes 6 – 19 vault water.

The lack of a human-related signal in any of the samples collected during this study suggests that contamination to the outfall at the beach is not of human wastewater origin, but is related to stormwater runoff and regrowth or survival of bacteria within the existing stormwater conveyance system. Additional sources of bacterial input at the beach proper is likely from shorebird and wading bird usage of the brackish tidal pool that forms at the outflow point near the Gulf (Figure 12).



Figure 12. Bird usage of the ditch outfall to Siesta Key Beach.

Indicator organism concentrations in all sediment samples from a variety of locations tended to remain elevated in the absence of runoff, while concentrations declined in the water column samples during dry event sampling, suggesting that the sediments are a reservoir for fecal indicator bacteria that are supplied to the stormwater pipe and ditch system during runoff events.

Although microbial pollution delivered to Siesta Key Beach via the stormwater system does not carry the same level of risk that it would if the pollution were from human sewage, members of the enterococci, including *Enterococcus faecium* and *Enterococcus faecalis*, are opportunistic pathogens, and elevated levels could conceivably pose a risk for the very young or immunocompromised. Waterborne human pathogens are most prevalent in human sewage, however, other animals do sometimes shed pathogens in their feces that could infect humans.

These types of microorganisms are termed “zoonoses” and represent infectious diseases that can be transmitted from animals, both wild and domestic, to humans. Cattle and swine are two of the higher-risk animal groups, as they can shed pathogenic microorganisms (such as

Cryptosporidium) in their feces. Pets (dogs, cats, birds) and wildlife are also potential sources of fecal coliform bacteria. Although there is no evidence of livestock contamination in the watershed area that drains to the beach, it cannot be definitively stated that there is no risk to human health from stormwater with high fecal coliform bacterial levels.

Since recreational use of the area is currently regulated by measurements of indicator bacteria, the most direct solution to reducing fecal coliform and *Enterococcus* bacteria loading to the beach would be to reroute the stormwater to a more appropriate receiving water where it will not impact beach water quality. As a result, human contact and risk of infection would thus be greatly reduced (Figure 13). Water quality treatment for bacteria may also be required to reduce the risk of other potential disease-causing organisms that may be present in the runoff.



Figure 13. Human contact at the ditch outfall to the beach.

Following is a summary of our findings from this stormwater evaluation:

- There is no evidence of a human source for the indicator bacteria in the stormwater system.
- There is evidence that the stormwater conveyance system is acting as a reservoir, or “breeding ground” for indicator bacteria.
- Rainfall flushes high bacterial loads through the system, and also probably resuspends bacteria living in the sediments of the stormwater pipe, the vault and the ditch, further elevating the load to receiving waters at the beach.
- Diversion of the drainage ditch from the beach should decrease or stop the intermittent observations of high bacterial levels at the beach. However, treatment of the diverted runoff should be considered if it will be discharged to other surface waters that would have human contact.
- It is anticipated that once the sediment and surface water runoff originating from the stormwater conveyance system is diverted or removed from discharging at the beach, indicator bacteria levels should decline within the beach monitoring area and future advisories will be minimized or eliminated. However, large storm events (hurricanes, El Nino events) or sources outside of this drainage area could still cause advisories depending upon local tidal currents.

4.0 References

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Appendix A

QA/QC Plan for USF Laboratory Analysis

Modifications Made 05/05/05

QUALITY ASSURANCE/QUALITY CONTROL

PROJECT PLAN

for

**WATER QUALITY MONITORING/MICROBIAL ECOLOGY LABORATORY
AT THE UNIVERSITY OF SOUTH FLORIDA:**

**BACTERIAL ENUMERATIONS AND MICROBIAL
SOURCE TRACKING METHODS**

Prepared by

**Valerie J. Harwood, Ph.D.
Stephaney D. Shehane, Ph.D.**

**Department of Biology
BSF 203
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July 12, 2004

Laboratory Quality Assurance/Quality Control Plan
Water Quality Monitoring/Microbial Ecology Laboratory
Valerie J. Harwood, Ph.D.- Principle Investigator

A. Quality Policy Statement and Commitments by Top Management

The laboratory is committed to upholding the highest degree of professionalism and expertise in all aspects of Environmental and Molecular Microbiology. The laboratory focuses on identification of microbial indicators found in water and wastewater, as well as in identification of potential sources of fecal contamination (Microbial Source Tracking) and microbial population dynamics in environmental waters.

B. Identification of approved signatories for the laboratory

Dr. Harwood prepares, oversees and validates final results, supervises analyses, and directs the environmental and molecular laboratories.

All laboratory reports will be signed and approved by Dr. Valerie J. Harwood

C. List of all Test Methods under which testing is being performed

Standard Operating Procedures – All standard operating procedures (EPA methods, Standard Methods) are available to all personnel in the SOP notebook or in reference manuals.

1. SM9222D (Fecal Coliform Bacteria) Membrane Filtration Method in accordance with Standard Methods for the Examination of Water and Wastewater.

Summary of Method:

Fecal coliforms are analyzed by the membrane filtration technique using membrane fecal coliform (mFC) media. The medium is prepared by dissolving 52 g of dehydrated medium per liter of deionized water, followed by heating while stirring with a magnetic stir bar. The suspension is boiled for one minute, followed by the addition of 10 ml 1% rosolic acid in 0.2 N NaOH per liter. Liquefied media is dispensed into plates, which are kept refrigerated for up to 2 weeks.

For analysis of water samples that have been passed through membrane filters, each filter is placed on an mFC media plate. The plates are placed into whirl-pack bags with waterproof enclosures and incubated submerged in a water bath at $44.5 \pm 0.2^\circ \text{C}$ for 24 +/- 2 hours. Blue colonies are counted as fecal coliforms. Pink, cream, gray or other non-blue colored colonies are not considered fecal coliforms.

Quality Control:

Escherichia coli C-3000 (ATCC 15597) is used as a positive control for verification of media and processing integrity. Colonies that grow and exhibit dark blue pigmentation are considered as positive verification of fecal coliform bacteria. Filtering sterile buffered water and a non-target organism, such as *Ent. faecalis*, through a membrane filter and incubating the media along with positive control sample serves as a negative control.

2. SM9230C (*Enterococcus spp.*) or EPA Method 1600 - Membrane Filtration Method in accordance with the Clean Water Act and Standard Methods for the Examination of Water and Wastewater.

Summary of Method:

The medium used in this assay is mEI agar, which is prepared by dissolving 71.2 g dehydrated mE agar (Difco) and 750 mg indoxyl β -D-glucoside per liter deionized water and autoclaving for 15 minutes at 121° C. Autoclaved media is cooled to 45-50° C in a water bath, and to each liter of media is added 10 ml of a 24 mg/ml nalidixic acid solution and 0.2 ml of a 10% 2,3,5-triphenyltetrazolium chloride (TTC) solution; both reagent supplements are dissolved in sterile deionized water. Media is dispensed into plates and allowed to solidify. Plates are stored in the dark at 4°C and kept for a maximum of two weeks.

After water samples have been passed through membrane filters, filters are placed on mEI media plates and incubated at 41° C for 24 +/- 2 hours. Enterococci colonies are small, gray colonies with a blue fringe. Only colonies with this appearance are counted as enterococci.

Quality Control:

Enterococcus faecalis (ATCC 35550) is used as a positive control for verification of media and processing integrity. Colonies that grow and exhibit dark blue to blue-gray pigmentation are considered as positive verification of *Enterococcus spp.* Filtering sterile buffered water and a non-target organism, such as *E. coli*, through a membrane filter and incubating the media along with positive control sample serves as a negative control.

3. Modified EPA Method 1103 - Membrane Filtration Method for *Escherichia coli*.

Summary of Method:

E. coli are analyzed by membrane filtration using mTEC agar plates. The medium is prepared by mixing 45.6 g of dehydrated mTEC agar per liter of deionized water. The

suspension is dissolved by boiling while stirring with a magnetic stir bar, and sterilized by autoclaving for 15 minutes at 121° C. Media is then dispensed into sterile petri dishes and solidified agar plates are stored in the refrigerator for a maximum of two weeks.

After water samples have been passed through membrane filters, each filter is placed on an mTEC media plate and incubated for 2 hours at $35 \pm 0.2^\circ \text{C}$, followed by 22-24 hours at $44.5 \pm 0.2^\circ \text{C}$, submerged in a water bath. After incubation, red or magenta colonies are counted as *E. coli*.

Quality Control:

Escherichia coli C-3000 (ATCC 15597) is used as a positive control for verification of media and processing integrity. Colonies that grow and are red/magenta are considered as a positive result for *E. coli* using mTEC medium. Filtering sterile buffered water and a non-target organism, such as *Ent. faecalis*, through a membrane filter and incubating the media along with positive control sample serves as a negative control.

4. Overall Quality Control for Membrane Filtration Analyses

Membrane Filters – Upon receipt, each lot number of membrane filters is logged and tested for sterility by placing filter on Tryptic Soy Agar (TSA) and incubating at 35°C for 24 hours.

At least once per year, each analyst must successfully perform a blind sample and/or authentic sample that is known or has been performed by another trained analyst with statistically similar results.

D. Laboratory Equipment and Calibration and/or Verification of Test Procedures Used

1. Laboratory equipment

The facility is equipped with a full-scale laboratory capable of performing a wide variety of analyses. The laboratory has a total of ~1500 sq. ft of research space. Equipment includes: an autoclave, high speed refrigerated centrifuges, microcentrifuges, a deionized water system, Reagent grade (Milli-Q) water system, refrigerated recirculating water bath, fecal coliform recirculating water bath, electrophoretic power and associated gel supplies, PCR thermocyclers, fluorometers, incubators, balances, pH meters, -80°C freezers, refrigerators/freezers, mixing platforms, and UV transilluminators. All are routinely certified, monitored, and/or calibrated.

2. Calibration and Maintenance of Laboratory equipment

Modifications Made 05/05/05

2.1 pH meters - All pH meters are calibrated within ± 0.1 units using two point calibration (4.0, 7.0, 10.0) prior to each use. All pH calibration buffers (NIST Traceable) are aliquotted and used only once and stocks are discarded upon expiration. Electrodes are maintained according to manufacturer's instructions.

2.2 Balances - All balances are calibrated monthly using ASTM (NIST traceable) type weights. In addition, professional calibration of all balances occurs at least once annually.

2.3 Incubators - All incubators are maintained at their desired temperature ± 0.5 °C or ± 0.2 °C, depending on application. Incubator temperatures are monitored using bulb thermometers immersed in glycerol, which are calibrated by a NIST traceable thermometer. Temperatures are recorded daily on log sheets.

2.4 Autoclave - Each autoclave cycle is recorded in a log book that indicates the date, contents, sterilization time, temperature, and analyst's initials. Sterilization efficiency is monitored monthly using spore ampules of *Bacillus stearothermophilus* as a control.

2.5 Sterilization procedures - All items are sterilized in the autoclave at 121°C for a minimum of 15 minutes. Biohazardous wastes are sterilized for a minimum of 30 minutes.

2.6 Refrigerators - All refrigerators/freezers are monitored to maintain a temperature of 1-8 °C or -20 to -15°C, respectively, by a bulb thermometer immersed in glycerol.

3. Procedures for Achieving Traceability of Measurements

All measurements by analytical equipment are recorded and dated by each user after use. Log sheets are filed for reference for up to 3 years.

4. Quality assurance of accuracy and precision of data

Quality assurance (Internal standards, duplicate samples) measures are listed with individual SOPs within the QA document.

E. Laboratory setup and procedure

1. Laboratory setup and environment

1.1 Bench space - All laboratory areas have sufficient bench space for reagent and supply storage and operation of equipment. Excess space is available for performing laboratory

work.

1.2 Lighting - sufficient overhead fluorescent lighting is present in each room. Emergency lighting that has its own power supply is also present in each room.

1.3 Waste disposal - Routine materials are placed in trashcans; infectious wastes and potential pathogens are collected in specialized containers and marked to be sent for incineration.

1.4 Safety considerations - General safety procedures are followed: Lab coats and gloves are worn. Chemical waste is stored in designated containers and appropriate safety cabinets are used for storage of chemicals.

1.5 Chemicals - All chemicals and reagents are stored in clearly labeled bottles and labeled with date received and opened and are discarded according to manufacturer's instructions. Precautions and reactivity are indicated on storage containers. Chemical waste is stored in designated labeled containers and sent for appropriate disposal. Safety cabinets are used for storage of chemicals. Materials Safety and Data Sheets (MSDS) are filed and are available for reference by lab personnel.

2. Bacteriological assays

2.1 Grab Sampling – Water samples for bacteriological assays are collected by the grab sample method as in Standard Methods for the Examination of Water and Wastewater (9060A). Polyethylene bottles are pre-sterilized by autoclaving and closed with a screw-cap lid. Sampling technicians are to wear latex gloves and change gloves between each sample collection. All specimens collected are labeled properly in the field with sampling site, date and time of collection and initials of technician collecting. Samples will be kept on ice until delivery at the laboratory for processing. A field log sheet shall accompany all samples with all needed information documented on the form the sample. The time specimens are received in the laboratory is also documented on the field log sheet along with the initials of person receiving specimens.

2.2 Membrane Filtration equipment - All membrane filtration manifolds are constructed from reinforced plastic and are verified for proficiency by authorized laboratory personnel prior to use. Pumps are also inspected and cleaned on a bimonthly basis to ensure proper functioning of the equipment.

2.3 Membrane filters - All filters are composed of cellulose ester fibers. They are white, grid-marked, 47mm in diameter, 0.45µm pore size, and purchased pre-sterilized.

2.4 Petri dishes - Presterilized plastic petri dishes (filled with the appropriate medium) are used for routine bacterial analyses using membrane filtration.

2.5 Sample containers - Sample containers are wide mouth plastic bottles with airtight caps or presterilized polyethylene 50 ml test tubes with airtight caps.

2.6 Laboratory bacterial control strains - Positive controls for the various assays are the following: Fecal coliform bacteria and *E. coli* – *E. coli* ATCC #15597, Enterococci – *Enterococcus faecalis* ATCC #35550

Stocks are obtained from the American Type Culture Collection and maintained by initially re-hydrating the freeze-dried culture and propagating according to ATCC instructions for each organism. Once a high-concentration broth culture of the organism has been grown, 500 µl aliquots of the suspension are mixed with 6 drops of glycerol in 1 ml cryovials and preserved at -80° C.

3. Molecular Biology Quality Control

3.1 Analyses- Molecular biology (PCR, Rep-PCR) is performed in an isolation room separated from live bacterial cultures and free DNA.

Experiments are performed in a UV cabinet and all equipment is exposed to ultraviolet radiation for a minimum of 15 minutes before use. Analyses are performed using separate autoclavable pipettors with aerosol resistant tips and latex gloves.

3.2 Laboratory bacterial control strains - Negative and positive controls are used in all PCR reactions. Negative controls consist of reactions containing no template DNA and only water. Positive controls for the various assays are as follows: ESP- *Enterococcus faecium* C68, rep-PCR- *E. coli* ATCC #9637, *Bacteroides* Human and Ruminant- TOPO SureShot *E. coli* clone containing plasmids for HF183 or RF128 sequences.

G. DNA Extraction

DNA is extracted using the DNeasy Tissue kit (Qiagen, Valencia, CA) according to manufacturer's instructions for Gram-negative and Gram-positive bacteria.

1. Determination of DNA Concentration

DNA concentration is determined using a Beckman DU 640 spectrophotometer according to manufacturer's instructions.

H. Data reporting and Statistical Analysis

All data will be entered in Data Log Sheets (DLS) and transferred to computer spreadsheet files for analysis. DLSs will be kept in a binder in the laboratory. At their weekly meeting, Dr. Harwood and Dr. Shehane will confirm that the data has been correctly transferred from DLS sheets to computer spreadsheets.

ANOVA will be used to compare variation in indicator organism concentrations between sites. The SPSS program will be used for ANOVA and related analyses, including linear regression. Linear regression will be used to correlate indicator organism concentrations to watershed impact level, e.g. *E. coli* numbers and *Enterococcus* numbers within the Cedar River compared to those in Nassau County. Binary logistic regression will be used to determine correlations between indicator values and binary data such as the presence/absence of human markers. Discriminant analysis will be used for multivariate analysis of many indicator parameters against a specific outcome (e.g. presence of human-specific markers).

I. Procedure for Handling Collected Samples

1. Transport of Samples

All samples are received cold or on ice and temperature is verified upon receipt by measuring temperature of ice or water in the shipping container. Once received, samples are immediately labeled, recorded, processed, then stored in the refrigerator until the following day to ensure proper sample analysis.

2. Holding times

All bacteriological samples are stored for a maximum of 12 hours.

3. Sample storage

Water samples are maintained at 4°C and analyzed upon receipt.

4. Record keeping

Laboratory worksheets and notebooks are maintained to record sample information. Sample information is recorded and contains the following information:

- 4.1. Name of sampling site
- 4.2. Sample identification code
- 4.3. Sample type (water, sediment, etc.)
- 4.4. Date and time of collection
- 4.5. Analyses to be performed
- 4.7. Name of technician and organization
- 4.8. Transportation condition (temperature, etc.)

5. Chain of Custody Forms

Chain of custody forms are used when samples are transferred between parties. These forms follow state-applicable guidelines and are filed upon receipt.

J. Corrective action contingencies

1. Unacceptable results

If unacceptable results are obtained, tests with additional positive and negative controls are conducted after calibration of all equipment used in the procedure to determine the source of the problem

2. Departure from documented procedures or standard specifications

If a methodology is deemed inaccurate or unreliable for a particular sample, alternative methodologies will be independently pursued. If results from new procedures are consistent, standard operating procedures may be modified. Dr. Harwood must approve deviations from standard procedures.

K. Procedures for data reduction, verification, validation, and reporting of results

1. Data reduction

All statistical analyses are performed using analytical computer software. Results are compiled into reports and are stored as a hard copy and in a computer database, and backed up by external electronic storage devices.

2. Accuracy of transcriptions

Sample collection sheets and laboratory data sheets are compared and verified before

report preparation and are saved and available for confirmation of results.

3. Data Validation

Dr. Harwood will monitor compliance with internal audits.

4. Reporting

Copies of all data, reports, and monitoring forms, as well as final reports, are supplied to the primary investigator, Dr. Harwood, and filed for further use.

L. Procedures for training new personnel

1. Training of personnel

Dr. Harwood trains all personnel on the proper use of all equipment prior to beginning work.

2. Training on new equipment or procedures

All personnel are trained on new equipment or procedures, as necessary. All personnel are tested on their knowledge base, and are trained and familiarized with standard research and safety practices.

3. Training on ethical and legal responsibilities

All personnel are trained on proper laboratory procedures with regards to ethical and legal rights and responsibilities, according to University of South Florida guidelines.

4. Access to QA/QC procedures

All lab personnel are provided with access to the Laboratory QA/QC plan. All personnel are required to read the document before beginning work. Revisions to the plan are documented with date and are recorded directly on the document.

M. Record keeping and reporting of results

1. Record keeping

Records are maintained in bound notebooks and on the College of Arts and Sciences

Modifications Made 05/05/05

server, as well as on CDs. All records are stored for a minimum of 5 years. Records include raw data, calculations, and quality control data.

2. Reporting of Results

Results are reported as direct quantitative counts or as probable pollution source. Reports include methodology used, positive and negative controls used, overall results, and interpretation of final results.

N. Appendix

Sample Collection and Processing Forms:

1. Physical/Chemical Water Quality Field Data Sheet
2. Microbial Indicator Processing and Raw Data Sheet

Modifications Made 05/05/05

Modifications Made 05/05/05

Chain of Custody Form Water Quality Laboratory – USF Department of Biology

Project Name _____

Date/Time of Sampling _____

Field Technician(s) _____

Date/Time Samples Relinquished _____

Samples Relinquished To (Lab) _____

Samples Received By (Name) _____

Samples Received By (Signature) _____

Site	Sample Matrix	Time	Physical Data (Y or N)	Taken By	Initials

Appendix B

Rep-PCR Analytical Techniques

Rep-PCR for Enterococci

Notice: **IMMEDIATELY** place reactions into thermocycler after addition of template DNA to avoid non-specific amplification!!!

Note- only analyze bands from 3000bp to 250bp following assay (using BioNumerics).

1. Streak from frozen stock onto TSA plate for isolation and grow overnight at 37°C.
2. Pick one colony to inoculate 1.5 ml of BHI in a microcentrifuge tube and grow overnight.
3. Pellet cells by centrifugation for 5 minutes (8000rpm), discard supernatant.
4. Wash cells by vortexing in 500ul of sterile (or buffered) H₂O.
5. Repeat steps 3 & 4 for a total of 2 washes.
6. Resuspend enterococci in 250ul of deionized sterile H₂O. (Perform PCR **IMMEDIATELY** following this step!)
7. Use 1ul of the cell suspension for PCR.

Prepare PCR master mix as follows...

Reagent	Volume	Final Concentration
Sterile DNA-free H ₂ O	11.6 µl	-
Gitschier Buffer	5.0 µl	5x
Dimethyl Sulfoxide 10% (DMSO)	2.5 µl	
BOXA2R Primer*	1.5 µl	0.6 uM
Bovine Serum Albumin 2% (BSA)	0.4 µl	
dNTP Mixture	2.0 µl	1 mM
Taq Polymerase	1.0 µl	
template	1.0 µl	
Total volume per reaction: 25µl		

*Sequence of BOXA2R primer: 5' - ACG TGG TTT GAA GAG ATT TTC G -3' (Malathum et al, 1998)

Biometra T-personal Thermal Cycler Program #7-2 (REP A2R)

- 95°C – 7 min (initial denaturation)
 - 90°C – 30 sec (denaturation)
 - 40°C – 1 min (annealing)
 - 65°C – 8 min (extension)
- } 35 cycles
- 65°C – 16 min (final extension)
- TOTAL running time: ~6.5 hours

Electrophoresis-if using Owl Buffer Puffer chamber

- 1.5% Agarose Gel, 0.5cm thick, prepared with 250 ml of 1x TBE and 3.75 grams of agarose.
- Gel bed is 20x25mm and will have two combs, creating a double run.
- Owl Buffer Puffer chamber is used because it recirculates the buffer for the prevention of ion gradient formation and heat build up.
- Run at 45 volts for 14 hours in 1x TBE buffer to **fill line** (~ 2 liters) on chamber. Do not run past 14 hours or the top row of isolates will run into the bottom row.
- Load 5µl of PCR product with 3µl of loading dye.
- Use 5µl of 1kb ladder (Fisher) where appropriate.

Electrophoresis-if using Bio-Rad chamber

- 1.5% Agarose Gel prepared with 120 ml of 1x TBE and 1.8 grams of agarose if you are using a 15 X 15 cm gel tray.
- Run at 60-65V for 6 hours.

Staining gel and digital imaging

- 25 ul of 1% ethidium bromide into 300 ul 1X TBE in a dark container.
- Place gel in container and gently rock for 30 minutes.
- Place only the gel on the transilluminator (Fotodyne imaging system). Settings should be close to 4 (aperture), 12 (zoom), and 1.5 (focus).
- Set somewhere between 30 to 45 exposures.

5x Gitschier Buffer (Kogan et al, 1987)

Reagent	Molarity	Volume for 200 ml	Volume for 50 ml
(NH ₄) ₂ SO ₄	1M	16.6 ml	4.15 ml
Tris HCl pH 8.8	1M	67.0 ml	16.75 ml
MgCl ₂	1M	6.70 ml	1.675 ml
1:100 EDTA*	0.5M	1.30 ml	0.325 ml
B-mercaptoethanol	14.4M (commercial stock)	2.08 ml	0.52 ml

*1:100 dilution of a 0.5M EDTA

- Make stock solutions of the first four reagents using ultra pure, autoclaved water.
- When adding together specified volumes to make buffer, do so in a fume hood due to the B-mercaptoethanol (always handle with gloves).

- Store in -20°C freezer in microcentrifuge tubes, or leave in a sterile container at 4 °C.

Loading Dye

- Dissolve 50mg of bromophenol blue and 10 grams of sucrose into 20 ml of autoclaved water.
- Aliquot into microcentrifuge tubes and store at 4°C or -20°C.

10X TBE

- To 500 ml of nanopure water add:

Reagent	For 1 Liter	For 2 Liters
Tris base	108 g	216 g
Boric acid	55 g	110 g
0.5 M EDTA (pH 8.0)	40 ml	80 ml

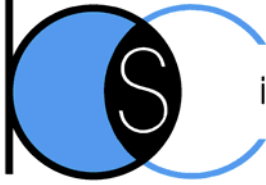
- Bring volume to 1 (or 2) Liter(s)
- Autoclave

References

1. **Kogan, SC., M. Doherty, and J. Gitschier.** 1987. An improved method for prenatal diagnosis of genetic disease by analysis of amplified DNA sequences. *A. N. England J. Med.* **317**: 985-990
2. **Malathum, K., K. Singh, G. M. Weinstock, and B. Murray** 1998. Repetitive Sequence-Based PCR versus Pulsed-Filed Gel Electrophoresis for the Typing of *Enterococcus faecalis* at the Subspecies Level. *J. Clin. Microbiol.* **36**:211-215

Appendix C

Laboratory Results from BCS Laboratory



inc.

Biological Consulting Services
of North Florida, Inc.

September 13, 2004

Valerie J. Harwood, Ph.D.
University of South Florida
Department of Biology, SCA 110
4202 E. Fowler Ave.
Tampa, FL 33620

Dear Dr. Harwood:

Microbial Source Tracking testing on samples collected from Siesta Key and delivered to our laboratory on August 31, 2004 is complete. A complete report is attached. The water and sediment samples were analyzed for the presence of human-specific viruses and virulence factors in *Enterococcus faecium*. Please note that, despite containing high numbers of fecal coliforms and enterococci, no human-specific viruses or molecular markers were detected in any of the samples.

If you have any questions or require any additional information, please do not hesitate to contact me at tscott@gator.net or at the phone numbers listed below.

Best regards,

Troy M. Scott, Ph.D.
Laboratory Director

***4641 NW 6th Street, Suite A, Gainesville, Florida 32609,
Tel. (352) 377-9272, Fax. (352) 377-5630***

Human Enterococcus ID Test

Sampling Site	Client Reference Number	Receipt Date	Process Date	Sample Type	<i>PCR Result (Human Fecal Marker)</i>
Stormwater Pipe A	1-A	8/31/04	8/31/04	Water	Negative
Stormwater Pipe A	1-A sed	8/31/04	8/31/04	Sediment	Negative
Stormwater Pipe B	1-B	8/31/04	8/31/04	Water	Negative
Stormwater Pipe B	1-B sed	8/31/04	8/31/04	Sediment	Negative
Stormwater Pipe C	1-C	8/31/04	8/31/04	Water	Negative
Stormwater Pipe C	1-C sed	8/31/04	8/31/04	Sediment	Negative
Vault	2	8/31/04	8/31/04	Water	Negative
Pond	3	8/31/04	8/31/04	Water	Negative
Pond	4	8/31/04	8/31/04	Sediment	Negative
Ditch	5	8/31/04	8/31/04	Water	Negative
Ditch	6	8/31/04	8/31/04	Sediment	Negative
Beach	7	8/31/04	8/31/04	Water	Negative
Beach	8	8/31/04	8/31/04	Sediment	Negative
Gulf	9	8/31/04	8/31/04	Water	Negative
Gulf	10	8/31/04	8/31/04	Sediment	Negative

Human Polyomavirus Virus ID Test

Sampling Site	Client Reference Number	Receipt Date	Process Date	Sample Type	PCR Result (Human Fecal Marker)
Stormwater Pipe A	1-A	8/31/04	8/31/04	Water	Negative
Stormwater Pipe A	1-A sed	8/31/04	8/31/04	Sediment	Negative
Stormwater Pipe B	1-B	8/31/04	8/31/04	Water	Negative
Stormwater Pipe B	1-B sed	8/31/04	8/31/04	Sediment	Negative
Stormwater Pipe C	1-C	8/31/04	8/31/04	Water	Negative
Stormwater Pipe C	1-C sed	8/31/04	8/31/04	Sediment	Negative
Vault	2	8/31/04	8/31/04	Water	Negative
Pond	3	8/31/04	8/31/04	Water	Negative
Pond	4	8/31/04	8/31/04	Sediment	Negative
Ditch	5	8/31/04	8/31/04	Water	Negative
Ditch	6	8/31/04	8/31/04	Sediment	Negative
Beach	7	8/31/04	8/31/04	Water	Negative
Beach	8	8/31/04	8/31/04	Sediment	Negative
Gulf	9	8/31/04	8/31/04	Water	Negative
Gulf	10	8/31/04	8/31/04	Sediment	Negative

Comments

Water and sediment samples collected from Siesta Key, Florida were received in the laboratory on August 31, 2004 and analyzed for the presence of human specific viruses (polyomaviruses) and a human specific molecular marker in *Enterococcus faecium*. All samples contained high fecal coliform and enterococci counts but tested negative for the presence of either marker. These results indicate that the microbial fecal indicators present in these samples are not of human origin (i.e. sewage, septage). The results should not be construed as being indicative of the absolute absence of human fecal pollution. Additional sampling will confirm results.



Biological Consulting Services
of North Florida, Inc.

August 16, 2004

Valerie J. Harwood, Ph.D.
University of South Florida
Department of Biology, SCA 110
4202 E. Fowler Ave.
Tampa, FL 33620

Dear Dr. Harwood:

Microbial Source Tracking testing on samples collected from Siesta Key and delivered to our laboratory on August 3, 2004 is complete. A complete report is attached. The water and sediment samples were analyzed for the presence of human-specific viruses and virulence factors in *Enterococcus faecium*. Please note that, despite containing high numbers of fecal coliforms and enterococci, no viruses or molecular markers were detected in any of the samples.

If you have any questions or require any additional information, please do not hesitate to contact me at tscott@gator.net or at the phone numbers listed below.

Best regards,

Troy M. Scott, Ph.D.
Laboratory Director

***4641 NW 6th Street, Suite A, Gainesville, Florida 32609,
Tel. (352) 377-9272, Fax. (352) 377-5630***

Human Enterococcus ID Test

Sampling Site	Receipt Date	Process Date	Sample Type	PCR Result (Human Fecal Marker)
Stormwater Pipe A	8/3/04	8/3/04	Water	Negative
Stormwater Pipe B	8/3/04	8/3/04	Water	Negative
Stormwater Pipe C	8/3/04	8/3/04	Water	Negative
Vault	8/3/04	8/3/04	Water	Negative
Ditch	8/3/04	8/3/04	Water	Negative
Ditch	8/3/04	8/3/04	Sediment	Negative
Beach	8/3/04	8/3/04	Water	Negative

Human Polyomavirus Virus ID Test

Sampling Site	Receipt Date	Process Date	Sample Type	PCR Result (Human Fecal Virus)
Stormwater Pipe A	8/3/04	8/3/04	Water	Negative
Stormwater Pipe B	8/3/04	8/3/04	Water	Negative
Stormwater Pipe C	8/3/04	8/3/04	Water	Negative
Vault	8/3/04	8/3/04	Water	Negative
Ditch	8/3/04	8/3/04	Water	Negative
Ditch	8/3/04	8/3/04	Sediment	Negative
Beach	8/3/04	8/3/04	Water	Negative

Comments

Water and sediment samples collected from Siesta Key, Florida, were received in the laboratory on August 3, 2004 and analyzed for the presence of human specific viruses (polyomaviruses) and a human specific molecular marker in *Enterococcus faecium*. All samples contained high fecal coliform and enterococci counts but tested negative for the presence of either marker. These results indicate that the microbial fecal indicators present in these samples are not of human origin (i.e. sewage, septage). The results should not be construed as being indicative of the absolute absence of human fecal pollution. Additional sampling will confirm results.

Appendix D

Field Data Sheets

Sampling #1

Field Data Sheet for Indicator Grab Samples: Water Quality Microbiology/Microbial Ecology Lab., USF Dept. of Biology

Project Name Siesta Key

Sampling Date 8/3/04

Personnel SDS, LB, MD, ~~MS~~

pc
vw
dw
ds
bw
bs

Sampling Site	Sample Type	Time	Temp.	Salinity	pH	GPS	Other (DO, UV, etc.)	Comments (tides, etc.)
1 - Pipe A	H ₂ O	10:00	29.58	0.89	7.22	27°16'09.918"N 82°33'27.942"W	3.11	
2 - Vault	H ₂ O	9:50	27.95	5.87	6.75	27°16'01.692"N 82°32'56.466"W	1.55	
3 - Ditch	H ₂ O	9:35	26.52	12.22	6.55	27°15'57.612"N 82°32'53.916"W	1.38 mg/L	
4 - Ditch	Sediment	"	"	"	"	"	"	"
5 - Beach	H ₂ O	9:20	26.88	9.41	6.60	27°15'50.584"N 82°32'59.888"W	1.25 mg/L	Flowing
6 - Beach	Sediment	"	"	"	"	"	"	"

H₂O
Depth
5 in
12 in
6 in
6 in

Time and Date Received in lab 8/3/04

Received by SDS

Sampling #2

Field Data Sheet for Indicator Grab Samples: Water Quality Microbiology/Microbial Ecology Lab., USF Dept. of Biology

Project Name Siesta Key - USF

Sampling Date 8-31-04

Personnel SDS, MB, MD

GPS
map

Sampling Site	Sample Type/Location	Time	Temp.	Salinity	pH	GPS	DO (DO, mg/L)	Comments (tides, etc.)	Depth
1A	Pipe/sed; Water	10:00AM							
2	Vault/Water	10:30am							
3	Pond/Water	9:37am	30.32	16.20	7.04		5.3 4.70	algae	
4	Pond/sed.	"	"	"	"		"	"	
5	Ditch/Water	9:30am	29.86	15.69	7.09		1.68		
6	Ditch/sed	"	"	"	"		"		
7	Beach/Water	9:20am	28.76	15.28	6.84		2.26		
8	Beach/sed.	"	"	"	"		"		
9	Gulf/Water	9:15am	30.31	33.66	7.19		5.87		
10	Gulf/sed	"	"	"	"		"		

Time and Date Received in lab 8-31-04;

Received by _____



Facsimile Transmittal Sheet

To: Stephaney Shehane From: Wendy Hershfeld
 Fax: (813) 974-~~3077~~ 3263 Pages: 1
 Phone: _____ Date: 8/31/04
 Re: Today's Sampling CC: _____

Urgent For Review Please Comment Please Reply Please Recycle

Comments:

VAULT

T - 28.6 °C
 DO - 0.21 mg/L
 SAL - 13.4 ppt

Manholes

CD1 - Calle del marina (?) T = 31.2 °C
 DO = 0.27 mg/L
 SAL = 1.9 ppt

312 Beach Rd

T = 30.7 °C
 DO = 0.51 mg/L
 SAL = 1.0 ppt

460 Beach Rd

T = 31.9 °C
 DO = 0.21 mg/L
 SAL = 10.3 ppt

* I told you the wrong street name earlier - the road we were on was Beach Rd not Siesta Drive.