

LEGAL COPY

APPLICATION FOR POLLUTION RECOVERY FUND ASSISTANCE

DATE OF APPLICATION: July 29, 2005

A. BASIC ASSISTANCE

1. Applicant: The legal name of the applicant/organization, the organizational unit and the complete mailing address of the applicant.

Name: Cindy K. Morris
Organization: Hillsborough County Health Department
Address: 1105 E. Kennedy Blvd. PO Box 5135 Tampa, FL 33675-5135

2. Project Manager Information: Give name and title of the representative of the applicant who will be the Environmental Protection Commission's principle contact concerning this application

Name Valerie J Harwood
Title Associate Professor, Department of Biology, University of South Florida
Address Dept. Biology SCA 110, 4202 E. Fowler Ave, Tampa FL 33620
Phone Number 813-974-1524
Project Title Analysis of Sources of Fecal Indicator Bacteria Causing Beach Closures in Hillsborough County, Florida
Project Time Start: estimated 11/01/05 End: est 10/31/06
Total Cost of Project \$ 109,251
Total EPC share requested \$ 94,931

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3. Assistance Type: **New or Renewal** (check one)

New - Award of funds for initial request within the project period.

Renewal - Award of funds for a project beyond the current project period.

4. Project Location: The specific location(s) of the project. (Attach Site Map)

Ben T Davis - 7650 Courtney Campbell Causeway, Tampa

Bahia Beach - 611 Destiny Drive, Ruskin

5. Is the Project for:

Restoration of a polluted area

Mitigation of the effects of pollution

Pollution Control Activity to prevent or minimize pollution

Educational

6. Is the Project directed toward restoring an identified "polluted area" (a geographic area destroyed or altered by dredging or filling or contaminated by an emission or discharge), or toward terminating an identified pollution source?

Identify and explain:

The goal of this study is to determine the extent to which human fecal pollution contributes to declining water quality at these heavily used beaches, and to identifying the dominant contributors of bacterial pollution in the area. Not only will biological source of pollution be identified (human sewage or nonhuman), but this information coupled with intensive local sampling will allow Hillsborough County Health Department (HCHD) and the EPC to identify structures such as storm water pipes that may be major contributors of bacterial pollution. This knowledge can lead to termination of a pollution source, thereby protecting public health.

7. Is the harm or potential harm to health, safety or welfare of the public or wildlife actual or potential? Does the project seek to alleviate actual or potential harm and what is the severity of the harm and the causal relationship between the "pollution"

and the harm?

Identify and explain:

The harm is both an actual and potential health threat to the safety & welfare of Hillsborough County residents and visitors to our beaches. The coastal beach water samples collected by the county health departments are analyzed for enterococci and fecal coliform bacteria. Fecal coliform and enterococci are both enteric bacteria that normally inhabit the intestinal tract of humans and animals. The presence of enteric bacteria is an indication of fecal pollution, which may come from stormwater runoff, pets and wildlife, and human sewage. If microbial pathogens are present in recreational waters and are ingested while swimming or enter the skin through a cut or sore, they may cause human disease (i.e., gastroenteritis, dermatitis, wound infections). This research study will assess the presence of high-risk, human sources of fecal indicator bacteria at two beach areas in Hillsborough County, Florida. The beach areas will be Ben T. Davis Beach, at the northern end of Tampa Bay, and Bahia Beach, which is in southern Hillsborough County near the mouth of Tampa Bay. The goal of this study is to determine the extent to which human fecal pollution contributes to declining water quality in these areas, and to apply this information to a risk assessment model. This will allow us to estimate the human health risk associated with recreational use of these waters. This study will aid in protecting public health, as well as economic interests associated with beach use as it defines the relationship between indicator bacteria and high-risk, human sources of bacterial pollution at these beaches.

8. How long has the pollution existed or how long before any harm will be evident?

The beaches in question have been chronically contaminated for many years. In scheduled sampling by HCDOH, samples are taken weekly and advisories are issued when an actual health risk is determined (indicator bacteria levels exceed state standards). This year alone, water quality at Ben T. Davis Beach (South) beach has been rated "Poor" on three sampling dates, while at Ben T. Davis Beach (North) beach, it has been rated "Poor" on six sampling dates. Persons who are exposed to contaminated recreational waters generally become ill within one week of exposure, and symptoms are frequently observed less than 24 hours after exposure. The harm to human health is therefore current and chronic.

9. Identify and describe how the project proposes to alleviate the pollution (addressing technical, practical, and cost effectiveness issues):

This project will aid HCDOH, EPC and other managers in determining the **source** (biological and physical) of bacterial and pollution and assessing the risk posed to human health at beaches by elevated indicator bacteria levels. Fecal contamination from human sources (e.g., sewage) is much more likely to cause disease than contamination storm water of resuspension from sediments. The ultimate goal of this project is to discriminate among these possible sources, which will inform not only public health officials, but will aid in total maximum daily load (TMDL) assessments and other water quality management decisions. Two Hillsborough County beaches that are surrounded by areas of contrasting land use were chosen for this study. Ben T. Davis Beach, located off the Courtney Campbell Causeway at the northern end of Tampa Bay, is surrounded by a highly urbanized watershed. Potential anthropogenic impacts include stormwater runoff and older septic systems in nearby Rock Creek. Bahia Beach, located near rural-suburban Ruskin, Florida, is located in southern Hillsborough County near the mouth of the Manatee River. The human population is much less dense here than in the area around Ben T. Davis Beach, and there are no obvious point or non-point sources of pollution. Nevertheless, water quality, as measured by concentrations of two indicator bacteria groups (*Enterococcus* spp. and fecal coliforms), is frequently unsuitable for recreational use. Monitoring for indicator organisms by Hillsborough County Department of Health (HCDOH) and the Environmental Protection Commission (EPC) at or near the proposed sample sites is already carried out on a weekly basis. The ongoing monitoring will add value to this research, as data that is collected by these agencies can be used. Furthermore, the HCDOH laboratory can process samples for indicator bacteria (fecal coliforms and enterococci), reducing the overall cost of the proposed research.

Alleviation of bacterial pollution at these beaches can only be accomplished by identification of the source of pollution. Quantifying indicator bacteria concentrations cannot accomplish this goal; however, cutting-edge microbial

source tracking methodologies such as those proposed here will allow HCDOH, EPC, FDEP and other watershed managers to make better-informed decisions about land use, infrastructure placement and upgrading, and human use of beaches. Improvements based on this information will lead to better recreational water quality for the citizens of Hillsborough County and its visitors.

10. Is the polluted area one which has previously been subject to commission enforcement and, if so, when and what was the result?

EPC monitors surface water locations in close proximity to these sample sites; the data could be analyzed to determine potential pollution sources in order to reduce health risks to our community. HCDOH is responsible for beach advisories. EPC and HCDOH are interested in working closely on solving issues related to bacterial pollution and beach advisories.

11. If no actual pollution exists and no prior commission enforcement action has occurred, does the project otherwise enhance pollution control activities within the County?

See above.

12. Can this Project be divided into separate and independent parts, and if so,

a) what are they? NO

b) how would the costs be allocated between them? N/A

c) would the applicant be willing to accept only partial funding? NO

13. Are other funding sources committed to the project? Yes

How much and for what? _____

\$14,320 is funded through the Hillsborough County Health Department for personnel to collect samples (Fecal coliform and enterococci) and for laboratory analysis cost.

14. What other funding sources may be available and how much? N/A

15. Why do you believe that this Project is of sufficient importance to justify the expenditure of Pollution Recovery Funds? _____

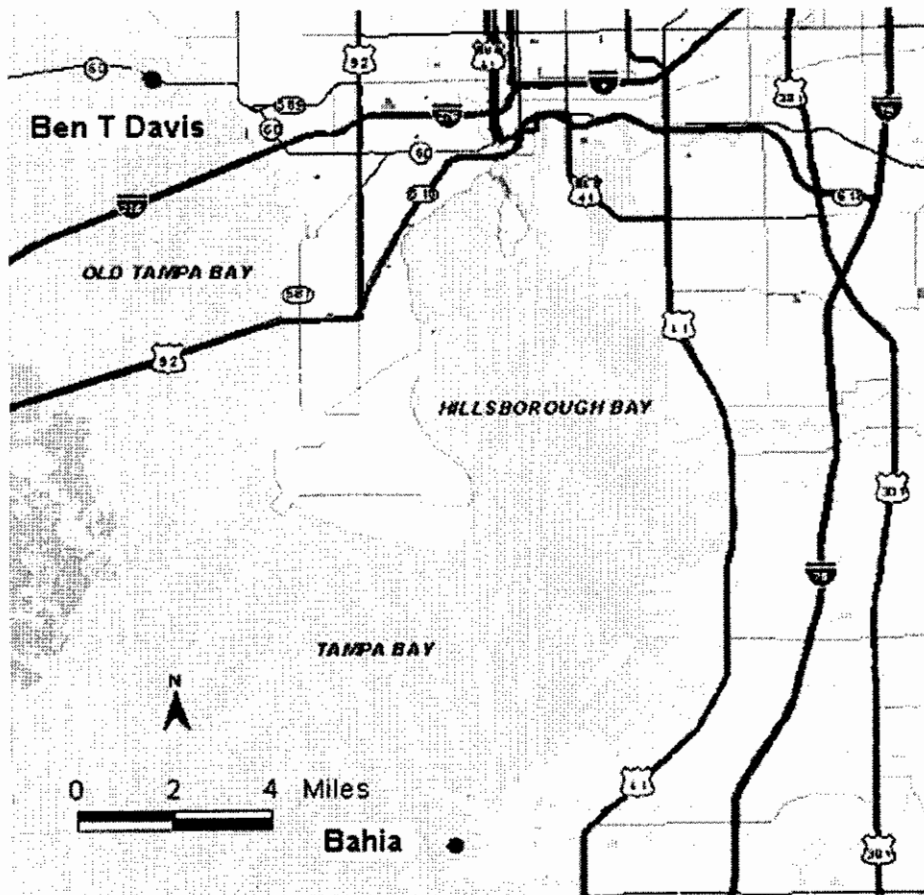
The Hillsborough County residents often voice their concerns regarding beach closures, and the subject is frequently covered by the media (see recent articles about Ben T. Davis Beach in the Tampa Tribune). Residents frequently suspect that water quality problems are associated with failing septic systems, although this is unproven. This research will provide the Health Department and EPC with additional information to address these concerns, as well as providing them with valuable information on which to base land management and water quality management decisions. This study has great implications for the costly process of TMDL assessment and implementation. Determination of bacterial sources is an integral and essential part of the TMDL process, and requires specialized studies such as the one proposed. This study links closely tied interests of public health protection, protection of economic interests, and crucial regulatory decisions that will have implications for Hillsborough County residents and visitors for years to come.

16. Will the project enhance the value of private property, and if so, whose? _____

Yes, if it is determined that human waste via failing septic systems is contributing to bacterial pollution, the County could expand public utilities to surrounding areas possibly through other grant opportunities. If storm water structures are major contributors, they may have to be re-designed to reduce impacts near beaches. Failure to address the problem of bacterial pollution of Hillsborough County beaches could result in property devaluation. Florida has a tourism-based economy that is dependent upon good recreational water quality. As our population grows, so does pressure on the water bodies that receive influent such as treated sewage and runoff. Without attempts to understand how our growing population and its anthropogenic activities affect water quality in Hillsborough County, we could seriously devalue an irreplaceable resource.

Attachment 1 Pollution Recovery Fund Project

**Ben T Davis
Bahia Beach**



Attachment 2 Pollution Recovery Fund Project

Principle Investor and Key Personnel:

Cindy Morris, R.S., Environmental Administrator with the Florida Department of Health, Hillsborough County Health Department, 1105 E. Kennedy Blvd PO Box 5135, Tampa, Florida 33675-5135.

Valerie J. Harwood, Ph.D. Dr. Harwood has carried out numerous water quality studies in areas ranging from Jacksonville, FL to Albuquerque, NM and Orange County, CA. She is recognized as an expert on microbial source tracking, the science of identifying the sources of fecal pollution in environmental waters, and has received funding from federal, state and local agencies including the U.S. Department of Agriculture, the U.S. Environmental Protection Agency, and the Florida Department of Environmental Protection (**see curriculum vitae, next page**). Dr. Harwood will oversee the project, participate in weekly meetings and planning sessions, and will write the Final Report.

Stephaney D. Shehane, Ph.D. Dr. Shehane received her Ph.D. from USF College of Marine Science in 2003 (major professor Dr. Joan Rose). Since that time she has worked as a Postdoctoral Research Associate in Dr. Harwood's laboratory. Her role is Project Manager; she will be responsible for coordinating and carrying out sampling, processing samples, and data analysis, as well as aiding in report writing.



VALERIE J. HARWOOD
CURRICULUM VITAE: 2005

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EDUCATION

- 1992 **Ph.D.** in Biomedical Sciences. Old Dominion University and Eastern Virginia Medical School.
1983 **B.A.** in Biology. State University of New York at Plattsburgh
1980 **B.A.** in French. Iowa State University

ACADEMIC POSITIONS

- 2004- Associate Professor, University of South Florida Department of Biology
1998 - 2004 Assistant Professor, University of South Florida Department of Biology
1995 - 1998 Assistant Professor: University of North Florida Dept. Natural Sciences
1992 - 1995 Postdoctoral: University of Maryland Center of Marine Biotechnology

PROFESSIONAL ORGANIZATIONS

- 1999 - American Water Resource Association
1995 - Council on Undergraduate Research
1989 - Sigma Xi Scientific Honor Society
1989 - American Society for Microbiology

FUNDING: AS PRINCIPLE INVESTIGATOR (since 2001)

- 2005-2006. Tributary Pollution Assessment. Jacksonville Electrical Authority. **\$42,600.**
2004-2005. Identification of the Sources of Fecal Indicator Bacteria in Wakulla County, Florida: A Research Proposal. Florida Dept. Environmental Protection. **\$70,140.**
2004-2005. Microbial Source Tracking: Tools for Refining Total Maximum Daily Load Assessments. Florida Dept. Environmental Protection. **\$295,500 (\$156,000 to vjh).**
2004-2005. Sarasota County, FL. Siesta Key Beach Water Quality Sampling to Determine Sources of Fecal Indicator Bacteria. **\$14,000.**
2004. Long-lasting Effects of a Sewage Spill in McKay Creek & Implications for Human Health. Pinellas County Utilities. **\$30,600.**
2003-2004. Bacteria in Urban Tributaries of the St. Johns River, Jacksonville Florida. Florida Department of Environmental Protection. **\$100,000.**



- 2001-2004. The Use of an Automated Ribotyping Assay for Identification and Source Tracking of Microbial Water Quality Indicators. US Environmental Protection Agency. **\$110,000.**
- 2001-2004. Bacterial Source Tracking Methods to Identify Nonpoint Fecal Pollution in Agricultural Watersheds. US Department of Agriculture. Co-PIs: Bruce Wiggins (James Madison University) and Charles Hagedorn (Virginia Tech). **\$310,000.**
- 2001-2004. Understanding the Sources and Fate of Conventional and Alternative Indicator Organisms in Subtropical Waters. US Environmental Protection Agency. **\$388,000**
- 2001-2003. Determination of the Sources of Fecal Pollution to Orange County, California by Antibiotic Resistance Analysis and Ribotyping. Orange County, Cal. Health Agency. **\$131,000.**
- 2001-2003. Real-Time Detection of Human Pathogens and Identification of the Sources of Indicator Bacteria in the Guana-Tolomato-Matanzas National Estuarine Research Reserve. Cooperative Institute for Coastal and Estuarine Environmental Technology. Co-PI: Daniel V. Lim (USF). **\$198,400.**
- 2001-2002. Upper Hillsborough River Contamination Assessment. Hillsborough County, FL **\$19,680.**
- 2001-2002. Bacterial Source Tracking Using Antibiotic Resistance Analysis in the South Platte River, Denver, CO. City of Denver, CO. **\$18,000.**
2002. Bacterial Source Tracking of Fecal Coliforms from Stormwater by Antibiotic Resistance Analysis in Albuquerque, NM. City of Albuquerque, NM. **\$51,295.**
- FUNDING: AS CO-PRINCIPLE INVESTIGATOR (since 2001)**
(Only funds allocated to Harwood are listed.)
- Development Of Management Tools For Control Of Deposits In Landfill Leachate Drainage Systems. Florida Center for Solid and Hazardous Waste Management. \$70,000 (A.D. Levine, PI).
- 2003-2005. PI: Anita Wright. Improved Methods for Molecular Detection of *Vibrio vulnificus*. National Sea Grant. **\$40,000.**
- 2003-2004. PI: Audrey Levine. Assessment of Biogeochemical Deposits in Landfill Leachate Drainage Systems. Florida Center for Solid and Hazardous Waste Management. **\$30,000.**
- 2002-2003. PI: Andrew C. Cannons. A DNA-based, Fiber Optic Biosensor for the Detection of Bacterial and Protozoan Pathogens. Water Environment Research Foundation. **\$98,000.**
- 2001-2004. PI: Joan B. Rose. Reduction of Pathogens, Indicator Bacteria and Alternative Indicators by Wastewater Treatment and Reclamation Processes. Water Environment Research Foundation. **\$42,100.**



REFEREED PUBLICATIONS (since 2000; out of 22 total)

(Students and postdocs supervised by Harwood are bolded; ^a denotes corresponding author)

2005. **K.L. (Hood) Anderson**, J.E. Whitlock and V.J. Harwood^a. Persistence and differential survival of fecal indicator bacteria in subtropical waters and sediments. *Appl. Environ. Microbiol.* 71: 3041-3048.
2005. V. J. Harwood^a, A. D. Levine, T. M. Scott, **V. Chivukula**, J. Lukasik, S.R. Farrah and J.B. Rose. Validity of the indicator organism paradigm: pathogen reduction and public health protection in reclaimed water. *Appl. Environ. Microbiol.* 71: 3163-3170.
2005. D. F. Moore, V. J. Harwood^a, D. M. Ferguson, J. Lukasik, P. Hannah, M. Getrich and **M. Brownell**. Evaluation of antibiotic resistance analysis and ribotyping for identification of fecal pollution sources in an urban watershed. *J. Appl. Microbiol.* 98: In press.
2005. **S.D. Shehane**, V.J. Harwood, J.E. Whitlock and J.B. Rose. The influence of rainfall on the incidence of microbial fecal indicators and the dominant sources of fecal pollution in a Florida river. *J. Appl. Microbiol.* 98:1127-1136.
2004. V.J. Harwood^a, **N.E. Delahoya**, M.F. Kramer, J.E. Whitlock, J.R. Garey and D.V. Lim. Molecular confirmation of *Enterococcus faecalis* and *E. faecium* from clinical, fecal and environmental sources. *Lett. Appl. Microbiol.* 38:476-482.
2004. V. J. Harwood, **J.P. Gandhi** and A.C. Wright. Methods for isolation and confirmation of *Vibrio vulnificus* from oysters and environmental sources: a review. *Journal of Microbiological Methods.* 59:301-316
2003. V.J. Harwood^a, B. Wiggins, C. Hagedorn, R.D. Ellender, J. Gooch, J. Kern, M. Sampadpour, A.C.H. Chapman, B.J. Robinson and B.C. Thompson. Phenotypic library-based microbial source tracking methods: efficacy in the California collaborative study. *J. Water Health* 01: 153-156.
2003. K. J. Ritter, E. Carruthers, C. A. Carson, R. D. Ellender, V. J. Harwood, K. Kingsley, C. Nakatsu, M. Sadowsky, B. Shear, B. West, J. E. Whitlock, B. A. Wiggins and J. D. Wilbur. Assessment of statistical methods used in microbial source tracking. *J. Water Health* 01:209-224.
2003. S. Choi, W. Chu, J. Brown, S. J. Becker, V. J. Harwood and S. C. Jiang. Application of enterococci antibiotic resistance patterns for contamination source identification at Huntington Beach, California. *Mar. Poll. Bull.* 46:748-755.
2003. T.M. Scott, M.R. McLaughlin, V.J. Harwood, **V. Chivukula**, A. Levine, A. Gennaccaro, J. Lukasik, S.R. Farrah and J.B. Rose. Reduction of pathogens, indicator bacteria and alternative indicators by wastewater treatment and reclamation processes. *Water Sci. Technol.: Water Supply.* 3:247-252.



2002. J.E. Whitlock, D. T. Jones and V. J. Harwood^a. Identification of the sources of fecal coliforms in an urban watershed using antibiotic resistance analysis. *Water Research* 36: 4265-4274.
2002. **J.M. Pisciotta**, D.F. Rath, P.A. Stanek, D.M. Flanery and V.J. Harwood^a. Marine bacteria cause false-positive results in the Colilert-18 rapid identification test for *Escherichia coli* in Florida waters. *Appl. Environ. Microbiol.* 68: 539-544.
2001. V. J. Harwood^a, M. Brownell, **W. Perusek** and J.E. Whitlock. Vancomycin-resistant *Enterococcus* spp. isolated from wastewater and chicken feces in the United States. *Appl. Environ. Microbiol.* 67: 4930-4933.
- 2001 **M. N. Harris**, J.D. Madura, L. Ming and V. J. Harwood^a. Kinetic and mechanistic studies of prolyl oligopeptidase from the hyperthermophile *Pyrococcus furiosus*. *J. Biol. Chem.* 22:19310 – 19317.
- 2001 V. J. Harwood and H.J. Schreier. Prolyl oligopeptidase from *Pyrococcus furiosus*: purification and properties. *Methods Enzymology* 330:445-454
- 2000 V. J. Harwood^a, J. Whitlock and **V. H. Withington**. Classification of the antibiotic resistance patterns of indicator bacteria by discriminant analysis: use in predicting the source of fecal contamination in subtropical Florida waters. *Appl. Environ Microbiol.* 66: 3698-3704.

PEER-REVIEWED, PUBLISHED REPORTS

2004. J.B. Rose, S.R. Farrah, V.J. Harwood, A.D. Levine, J. Lukasik, P. Menendez and T.M. Scott. Reduction of pathogens, indicator bacteria and alternative indicators by wastewater treatment and reclamation processes. Report 00-PUM-2T. Water Environment Research Foundation, Alexandria, VA.



Attachment 3 Pollution Recovery Fund Project

a) Objectives of this Project:

This research study will investigate the sources of fecal indicator bacteria at beaches in Hillsborough County, Florida by conventional microbiology and cutting-edge microbial source tracking techniques.

Objective 1. Determine the source (biological and physical) of indicator bacteria at two beaches: Bahia Beach and Ben T. Davis Beach. Intensive sampling of water and sediments will take place at sites that have received advisories of high indicator bacteria levels (fecal coliforms and/or enterococci).

Objective 2. Use the information obtained on bacterial sources to inform risk assessment models. This will estimate the risk to human health from beach use.

Objective 3. Develop recommendations for remediation of bacterial pollution at the beaches for implementation by HCDOH, EPC and FDEP.



b) Results and/or Benefit Expected:

This project will result in data that will help identify the biological (e.g. human, wildlife, resident, storm water-associated) and physical (e.g. stormwater, sediments) source(s) of bacterial pollution at the beaches. The ultimate goal of this project is to discriminate among these possible sources, which will inform not only public health officials, but will aid in total maximum daily load (TMDL) assessments and other water quality management decisions. Alleviation of bacterial pollution at these beaches can only be accomplished by identification of the source of pollution. Quantifying indicator bacteria concentrations cannot accomplish this goal; however, cutting-edge microbial source tracking methodologies such as those proposed here will allow HCDOH, EPC, FDEP and other watershed managers to make better-informed decisions about land use, infrastructure placement and upgrading, and human use of beaches. Improvements based on this information will lead to better recreational water quality for the citizens of Hillsborough County and its visitors.

c) General Project Information:

Results of this project will be evaluated in collaboration with HCDOH and EPC officials. Strict QA/QC procedures as outlined by the USEPA will be carried out. Data accuracy and precision will



be assessed by QA protocols. This project has many links with current regulatory issues, particularly those of total maximum daily load (TMDL) assessments in Florida. Dr. Harwood is currently finishing two TMDL/land use related studies funded by FDEP. Results from this project will be compared to those, and integrated into TMDL planning for the State of Florida. Hillsborough County is also planning to initiate further TMDL studies that are currently slated to incorporate microbial source tracking studies by Dr. Harwood.

Attachment 4 Pollution Recovery Fund Project

**Scope of Work:
Purpose**

This research study will investigate the sources of fecal indicator bacteria at beaches in Hillsborough County, Florida. Intensive sampling of water and sediments will take place at sites that have received advisories of high indicator bacteria levels (fecal coliforms and/or enterococci). The analysis will include state-of-the-science techniques designed to determine whether bacteria are of human vs. nonhuman (e.g. animal, stormwater) origin. Two beaches that experience frequent advisories will be the focus of this study: Ben T. Davis Beach is located at the northern end of Tampa Bay off the Courtney Campbell causeway, while Bahia Beach is in southern Hillsborough County near the mouth of Tampa Bay. Samples from other beaches



may be analyzed as need dictates. Microbiological analyses will include polymerase chain reaction (PCR)-based tests to determine whether enterococci from human sources are present. Human polyomaviruses, which are nonpathogenic but are found in the feces of most humans, will also be assayed. A sampling plan that will supplement the Environmental Protection Commission's sampling plan for total maximum daily load (TMDL), which will refine their knowledge of pollution "hot spots" in the vicinity of the beaches, will be developed and carried out. The goal of this study is to determine the extent to which human fecal pollution contributes to declining water quality in these areas, and to correlate this information with the human health risk associated with recreational use of these waters. If specific human sources such as failing onsite wastewater treatment (septic) systems or wastewater treatment plant effluent are identified, recommendations will be made for remediating the problems, and future funding from the Environmental Protection Commission Pollution Recovery Fund will be sought.

Background

The goal of bacteriological water quality testing is to protect public health by estimating the risk associated with using the water. Human health risk estimates are generally based on measured levels of bacteria, and the public health response to



elevated levels is to restrict water use of issue advisories against its use. It is time-consuming and expensive to directly quantify disease-causing bacteria and viruses, and virtually impossible to test for all possible pathogens in a water sample. Thus, we quantify fecal indicator bacteria, whose presence should ideally correlate with the presence of human pathogens in the water. Fecal coliform bacteria have been extensively utilized to indicate water quality in recreational waters in Florida, the US and throughout the world. Unfortunately, pathogens, particularly enteric viruses such as Norwalk virus and protozoa such as *Cryptosporidium* and *Giardia*, may well be present in waters where indicator bacteria levels are low. Conversely, high indicator bacteria levels do not always indicate a human health threat, particularly if these organisms are common from a relatively innocuous source such as stormwater that is not impacted by sewage.

One of the major reasons that both fecal coliforms and enterococci are inadequate indicators is that they are present in the gastrointestinal tract of all warm-blooded animals (and some cold-blooded animals). Some animal feces, i.e. those of humans, cattle, and swine, have a higher probability of containing human pathogens than the feces of most other species, therefore many water quality experts would place contamination from these animals in a "high risk" group. Low levels of fecal indicator



bacteria from a high risk animal group would indicate a greater potential health hazard than much higher levels of indicator bacteria from a low risk animal group. Currently, there is no testing method approved by regulatory agencies such as the U.S. Environmental Protection Agency (USEPA) that can be used to determine the source (i.e. cow, human, dog) of fecal indicator bacteria, however such a method would allow much more accurate risk assessment than we can achieve with standard testing methods. It would also allow regulatory agencies to more effectively identify and eliminate the source of bacterial contamination to natural waters, and would contribute greatly to the accuracy of total maximum daily load (TMDL) models.

The enterococci (*Enterococcus* species) are an indicator bacteria group that is recommended by the USEPA as a bacteriological water quality indicator (USEPA, 2000). Some studies have indicated that *Enterococcus* levels are more closely correlated with cases of gastroenteritis in recreational waters than other indicator organisms (Cabelli et al., 1982). The enterococci also survive longer under some environmental conditions, i.e. in saline waters, than fecal coliforms. However, the enterococci share the major disadvantage of the fecal coliform group; they are shed in the feces of all warm-blooded animals and therefore provide no indication of the source of fecal contamination. Like fecal



coliforms, they are sometimes absent in the presence of human pathogens, and are frequently present when pathogens are absent.

Sediments are increasingly considered a possible reservoir of indicator bacteria, serving as a source of both fecal coliforms and enterococci when sediments are disturbed by human or animal activity, or by high flow as is experienced during rainfall events. The prolonged survival of indicator bacteria in sediment coupled with intermittent resuspension can falsely suggest recent contamination. Recent work in Jacksonville Florida by Harwood's laboratory has shown that indicator bacteria concentrations after a sewage spill may remain high in sediments long after the water column is clear (report to Florida Department of Environmental Protection, in preparation). The interaction between bacterial populations in sediments and the water must be further explored to understand the limitations of indicator bacteria as predictors of human health risk.

Microbial Source Tracking. Microbial Source Tracking (MST) is a term that refers to a group of methods that are used to type, or fingerprint, indicator bacteria such as fecal coliforms in order to determine their source, e.g., from human, dog, wild animal, etc. MST techniques that are currently in use measure characteristics such as antibiotic resistance or carbon source utilization to generate the fingerprint (Harwood *et al.*, 2000; Harwood *et al.*, 2003; Wiggins, 1996;), or they may analyze



differences at the level of the DNA fingerprint, as in ribotyping (Dontchev et al., 2003; Parveen et al., 1999) or rep-PCR (Johnson et al., 2004). Many MST techniques rely on the establishment of a large database (library) of "fingerprints" of indicator bacteria from known sources, i.e. humans, cattle, wild animals, etc. Fingerprints of bacteria isolated from water samples can then be statistically compared to the fingerprints in the library, allowing the investigator to determine the source of fecal contamination to the water. Generating fingerprint libraries is time-consuming and expensive, and methods are under development for library-independent determination of bacterial source. PCR is generally utilized to amplify small amounts of DNA into a detectable signal in the methods, therefore they are sensitive to low levels of contamination from a specific source and relatively rapid (Scott et al., 2002). Library-independent methods include detection of human-specific and ruminant specific *Bacteroides* (a fecal anaerobe) (Bernhard and Field, 2000), human-specific *Enterococcus faecium* via the enterococcal surface protein (*esp*) (Scott et al., 2005) and human polyomaviruses (McQuaig et al., 2005)

Study Design

Two Hillsborough County beaches that are surrounded by areas of contrasting land use were chosen for this study. Ben T. Davis



Beach, located off the Courtney Campbell Causeway at the northern end of Tampa Bay, is surrounded by a highly urbanized watershed. Potential anthropogenic impacts include stormwater runoff and older septic systems in nearby Rock Creek. This high-profile, heavily used beach experiences frequent advisories due to bacterial levels (in fact, one was issued July 22, 2005). Bahia Beach, located near rural-suburban Ruskin, is in southern Hillsborough County near the mouth of the Manatee River. The human population is much less dense here than in the area around Ben T. Davis Beach, and there are no obvious point or nonpoint sources of pollution. Nevertheless, water quality, as measured by concentrations of two indicator bacteria groups (*Enterococcus* spp. and fecal coliforms), is frequently unsuitable for recreational use (Table 1). Monitoring for indicator organisms by Hillsborough County Department of Health (HCDOH) and the Environmental Protection Commission (EPC) at or near the proposed sample sites is carried out on a monthly basis.

Table 1. Bacterial counts in Class III (recreational) waters should not exceed these levels per Florida Administrative Code 62-301.530.

Indicator	Geomean (30 day) (CFU/100 ml)	10% must not exceed (CFU/100 ml)	One-time sample (CFU/100 ml)
Fecal coliforms	200	400	800
Enterococci	35	N/A	104



The sampling schedule will have some flexibility so that the team can follow up on observations of high bacteria levels. Follow-up sampling will occur (whenever possible) the day after the initial high-level sample was collected. Same-day follow-up is not possible because the tests require 24 h incubation. Each beach will be sampled a minimum of once per month on a pre-arranged schedule for a total of eight scheduled sample events. Additional sample events (three per beach) are planned following high-bacteria observations. Whenever possible, the HCDOH/USF team will respond to reports of high bacteria levels by sampling the day after the initial samples were taken. The HCDOH laboratory (located in USF's Research Park) will communicate with the USF laboratory immediately following observation of high bacteria levels so timely sampling can be arranged.

Multiple sites will be sampled at each beach in order to (a) determine the variability of bacterial concentrations observed in a confined geographical area and (b) to determine if there is a gradient of pollution that suggests a source. Up to five sample sites per beach will be chosen for intensive sampling efforts in consultation with HCDOH and EPC. Intensive sampling of the sites will include (a) PCR-based detection of human-specific *Enterococcus faecium* (b) PCR-based detection of human polyomaviruses, and (c) measurement of fecal coliform,



Escherichia coli and *Enterococcus* concentrations. The experimental design includes:

- Measurement of fecal coliform and *Enterococcus* concentrations (conducted by HCDOH). Measurement of *E. coli* concentrations (conducted by USF)
- Assessment of the presence of human-specific *Ent. faecium* (USF)
- Assessment of the presence of human polyomaviruses (USF).
- Scheduled sample events will take place monthly, at a previously coordinated and agreed upon sampling date. Only one beach will be sampled during each event, as the geographic separation of the sites would make sample collection and processing very difficult if both were conducted on the same day. Each beach and associated area will be sampled eight times over the course of a year, which will capture both wet and dry seasons in Tampa Bay.
- High-bacteria sampling will take place as opportunities arise. Up to six of these sampling events (three per beach if possible) will be carried out over the course of the study.
- In order to sample "targets of opportunity"; i.e., other beaches that experience high bacteria levels during the



study, or additional sites at the specified beaches, funding for five additional samples is included in the budget.

- Dr. Harwood will write an interim report on the project which will be due after six months, a draft final report (11 months) and a final report, which will be due after twelve months. The conclusions of the final report will be made in collaboration with representatives from HCDOH and EPC, who will correlate the microbiological data with the presence of potential pollution sources such as aging septic systems, central sewer lines, and stormwater runoff.

Participants

Hillsborough County Department of Health (HCDOH) and EPC are currently monitoring beach waters, providing added value to this proposal. HCDOH has agreed to provide a USF student intern to aid in sample collection and processing, to identify areas populated with septic systems and to provide information about their probable installation date, and to help in establishing a GIS database. EPC will extend their monitoring efforts through this study, which will in supplement efforts to develop a TMDL program. Dr. Harwood has carried out numerous water quality studies in areas ranging from Jacksonville, FL to Albuquerque, NM and Orange County, CA. She is recognized as an expert on bacterial source tracking, the science of identifying the sources



of fecal pollution in environmental waters, and is currently funded by many agencies including the U.S. Department of Agriculture, the U.S. Environmental Protection Agency, and the Florida Department of Environmental Protection.

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Deliverables

- Interim report 6 months
- Draft final report 11 months
- Final report 12 months



Attachment 5 Pollution Recovery Fund Project

BUDGET CATEGORIES

	PRF Funds	Federal	Applicant	State	Other
a. Personnel					
1. Harwood (2 weeks summer)	\$4,387				
2. Shehane (50%)	\$27,398				
3. Intern	\$1,440				
4. Rakestraw (10%)			\$4,990		
5. Davis (1.4%)			\$680		
b. Administrative (Bigsby 2.6%)			\$1,750		
c. Materials	\$46,124		\$6,900		
d. Contractual					
e. Construction					
f. Other (USF overhead)	\$15,582				
g. Total Direct Charges (Sum of a. to f.)	\$94,931		\$14,320		

