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The University of Southern Mississippi

EVALUATION OF HUMAN FECAL POLLUTION IN MISSISSIPPI COASTAL AND CREEK WATERS USING LIBRARY INDEPENDENT MARKERS

by

Christopher John Flood

An Abstract of a Dissertation
Submitted to the Graduate School
of The University of Southern Mississippi
in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy

May 2013

ABSTRACT

EVALUATION OF HUMAN FECAL POLLUTION IN MISSISSIPPI COASTAL AND CREEK WATERS USING LIBRARY INDEPENDENT MARKERS

by Christopher John Flood

May 2014

The objective of this study was to determine whether statistically valid correlations could be elucidated between standard indicator bacteria (enterococci and fecal coliforms) from coastal creek and marine samples and the presence of four library independent molecular markers that are human or sewage specific. Eight hundred and nineteen samples were collected between August 2007 and July 2010 to determine enterococcal and fecal coliform counts and the presence of genetic markers for sewage indicator organisms *Methanobrevibacter smithii*, human specific *Bacteroides* sp., *Bacteroides thetaiotaomicron*, and *Fecalibacterium* sp. During the course of this study environmental parameters were measured and statistically analyzed to determine if there was any correlation for the presence of any one of these organisms and the environmental variables.

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The University of Southern Mississippi

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Christopher John Flood

A Dissertation
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Approved:

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Dean of the Graduate School

May 2013

DEDICATION

For Mom.

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TABLE OF CONTENTS

ABSTRACT………………………………………………………………………....ii

ACKNOWLEDGMENTS……………………………………………………......iv

LIST OF TABLES…………………………………………………………………..vi

LIST OF ILLUSTRATIONS ……………………………………………………....vii

LIST OF ABBREVIATIONS………………………………………………………..viii

I. INTRODUCTION……………………………………………………………….1

II. LITERATURE REVIEW…………………………………………………………..6

III. MATERIALS AND METHODS ……………………………………………24

IV. RESULTS……………………………………………………………………...36

V. DISCUSSION ……………………………………………………………….63

Summary
Conclusions

APPENDIXES………………………………………………………………......80

REFERENCES……………………………………………………………………121

LIST OF TABLES

Table

1. Advantages and Disadvantages of Microbial Source Tracking Methodologies….4

2. Summary of the Major Human Library-Independent Microbial Source Tracking Methods Employed in this Study……………………………………………….5

3. Marine and Freshwater Coastal Sampling Sites and their Geographic Locations…………………………………………………………………....24

4. Summarized Experimental Methodologies for all Environmental Samples……..29

5. Average Enterococcal Count (EN), Number of Sampling Dates, and Percent Positive Reactions for each Human Marker (MS, BA) at each of the 12 Study Sites…...39

6. Cross-tabulation (A) Gel Analysis of MS ± vs. BA ±…………………………...41

7. Cross-tabulations of the MultiNA (Capillary) vs. Gel Electrophoresis…………42

8. PCR Results for Bacterial Assays from 5/2009-11/2009………………………..46

9. Krusal-Wallis Results of Water Type Environments in Order of their Mean Ranks for dates 5/12/2009-11/19/2009…………………………………………..48

10. Mann-Whitney Test Results for the Differences in Water Type Environments for Sampling dates 5/12/2009-11/19/2009……………………………………49

11. Spearman's Test Results for the Effects of Salinity and Marker Presence ……...51

12. Pearson Correlation of Human Specific Markers to each other for Sampling dates 5/12/2009-11/19/2009……………………………………………………...54

13. Samples and the Number of Positives for the Three Human Specific Markers at each one of the Sampling Sites 4/21/2010-7/22/2010………………………...55

14. Pearson correlation of *M. smithii*, human *Bacteroides*, and *Bacteroides thetaiotaomicron* ……………………………………………………………...62

LIST OF ILLUSTRATIONS

Figure

1. Coastal Mississippi – Harrison County……………………………………………25

2. Enterococci (log CFU/100mL) for Sampling Months 08/2007-04/2009.............37

3. Enterococcal Counts (log CFU/100mL) Across all Sample Sites for the dates 08/2007-04/2009………………………………………………………...........37

4. Total Marker Positives (*M. smithii* and human *Bacteroides*) at each Site for the dates 08/2007-04/2009……………………………………………….............38

5. CFU/100mL of Enterococci and Fecal Coliforms for each Sampling Location (5/2009 - 11/2009)………………………………...43

6. Seasonal Variation CFU/100mL of Enterococci and Fecal Coliforms for the Sampling Season (5/2009 – 11/2009)……………………...................................44

7. Marker Distributions for all Sampling Sites (5/2009 – 11/2009) ……………….47

8. The Distribution of Enterococci and Fecal Coliforms (CFU/100mL) for each Sampling Site (4/2010 – 7/2010).……………………………………………..56

9. The Distribution of Enterococci and Fecal Coliforms (CFU/100mL) for each Sampling month.………………………………….......................................57

10. This Graph Represents Total Marker Presence (4/2010 – 7/2010) for the Individual Study Sites………………...58

LIST OF ABBREVIATIONS

Klux	Candle lux (1lux = 1 lumen/m²)
UV	Ultraviolet Radiation
mEI	Membrane- Enterococcus indoxyl-beta-D-glucoside
Ppt	Parts per thousand
LB	Luria Bertani (Miller) broth
ºC	Degrees centigrade
mL	Milliliter
PCR	Polymerase chain reaction
rpm	Rotations per minute
USM	University of Southern Mississippi
USEPA	Environmental Protection Agency
µl	Microliter
VBNC	Viable but not culturable
µM	Micrometer
TSV	Taura Syndrome Virus
Ng	Nanogram
DNA	Deoxyribonucleic Acid
mM	Millimolar
CFU	Colony forming units
EN	Enterococci
FC	Fecal coliforms

FWS	Freshwater stream (creek)

NSB	Nearshore Brackish
NSM	Nearshore marine
mm	Millimeter
HuBac	Human *Bacteroides*
Btim	*Bacteroides thetaiotaomicron*
Fecali	Fecalibacterium
HPyV	Human Polyoma Virus
MDEQ	Mississippi Department of Environmental Quality
L	Liter
FIB	Fecal Indicator Bacteria
MS	*M. smithii*
µM	Micromolar
LIM	Library Independent Methods
T-RFLP	Terminal Restriction Length Polymorphism
TAE	Tris-Acetate-EDTA
qPCR	Quantitative PCR
16SrRNA	16S ribosomal RNA
TE	Tris-EDTA
EDTA	Ethylenediaminetetraacetic acid
V	Volts
Bp	Base Pair
s	Seconds

g	Grams
7ACC	Cemetery
7ACT	Trautman Avenue
7A	Trautman Avenue
9	Pratt Avenue
CC1	Coffee Creek
CC2	Coffee Creek
10	U.S. Naval V.A.
10A	Teagarden
AOC	Anniston Oak
CON	Condo
11	Cowan/Lorraine Road
12A	Rodenberg Avenue
CTHC	16th Ave
CH	Courthouse boat launch

CHAPTER I

INTRODUCTION

Coastal recreational waters require proper stewardship to ensure the health and safety of beachgoers. Increased anthropogenic activities contribute to the pollution of the Mississippi Gulf Coast. Among these stressors, fecal pollution and the probability of coming into contact with water-borne disease causing pathogens are of great concern to the general public. Sources of fecal pollution can include leaky septic systems, agricultural runoff, domestic and wild animal waste, storm water runoff, and faulty sewer system infrastructures (Field et al., 2003; Bernhard and Field, 2000).

The association of a health risk in coastal waters is determined by the enumeration of fecal coliforms and/or enterococci levels. While these methods have been useful and have protected the public from disease, the fact remains that indicator bacterial levels cannot be associated with a specific animal. There are other problems associated with these standard methods including (1) the persistence of indicator organisms in waters and sediments; (2) the fact that fecal indicators remain alive in the presence of plant material; (3) the survival of indicator bacteria in beach sand; and (4) the possibility that indicator organisms can exist in areas with no human habitation. Recent investigations have determined that the problems are genuine and inherent in the use of the fecal coliform and enterococci as indicators; however, no suitable alternative to these standard methods has arisen which could serve as a verifiable replacement. Consequently, microbial source tracking has evolved as a way to delineate the possible sources of fecal input in surface waters and to complement viable microbial counts of known indicator species.

There are two forms of microbial source tracking: library-dependent and library-independent methods (Table 1). Library dependent methods isolate potential indicator organisms (coliforms, enterococci, human enteric viruses, bacteriophage, etc.) from a specific animal source, perform standard biochemical testing to identify the isolate, and carry out genetic fingerprinting on each organism. Several thousand fingerprints of different animal isolates constitute a known source library; unknown isolates are compared to the known library and possibly identified as being from a specific animal. Researchers have demonstrated that there is a significant amount of genetic heterogeneity between environmental and human fecal populations of *Eschericia coli*, the use of different statistical procedures can produce conflicting results, and the library based method would require an immense number of sample isolates for it to be viable (Lasalde et al., 2005).

Library-independent methods (LIM) have been developed with the goal of identifying animal-specific sources of fecal pollution using a single gene for identification. LIM methods are rapid, specific, simple, economical, and a variety of methods have been developed and tested for use with environmental samples and in a variety of national locations. Our lab developed and tested a wide variety of alternative *Archeae* molecular markers for the host-specific identification of animal fecal pollution in Mississippi coastal waters. The first use of methanogens as molecular markers was developed in our laboratory and included one sewage specific molecular marker, two domestic ruminant-specific markers, one chicken marker, and one swine marker of fecal pollution (Ufnar et al., 2006). These methods, as well as others listed in Table 2, are currently being examined for application by regulatory agencies as a supplement to the

existing standard methods. Future testing may involve testing only for these alternative molecular markers or a combination of these methods and traditional microbial analysis.

Benefits and broad applications of LIM analysis include an improved understanding of the types of fecal pollution that enter the waters of the northern Gulf of Mexico, enhanced identification of the sources of fecal contamination, and ultimately, better calculation of the risk of increased exposure of the public to human pathogenic microorganisms from contact with Gulf waters. Knowledge of contamination sources is crucial for mitigation and remediation of fecal contamination in coastal waters; thus, the technologies applied and developed by this work have broad application in polluted coastal waters throughout the United States.

This research compared four, human LI methods and their efficacy in the determination of fecal pollution along Mississippi coastal beaches. Comparisons encompassed analyses at specific beach sites at which water samples were collected, transported to the laboratory, filtered to isolate all microbial cells, and extracted to recover total DNA. Primers specific to human *Bacteroides* spp., *Methaonbrevibacter smithii*, *Fecalibacterium*, and *Bacteroides thetaiotamicron* were employed in formulated Polymerase Chain Reactions to amplify known gene sequences representing each of the four human markers. Gel electrophoresis and/or MultiNA capillary electrophoretic analysis of PCR products were conducted to determine if the markers are present at particular sites along the coast. Statistical evaluation was conducted to establish possible correlations involving: the individual markers, the specific coastal sites, and the relationship between markers and indicator species, and certain environmental parameters.

Table 1

Advantages and Disadvantages of Microbial Source Tracking Methodologies

Library-Dependent Microbial Source Tracking Methods		Library-Independent Microbial Source Tracking Methods	
Advantages	Disadvantages	Advantages	Disadvantages
Representative of a specific water body	High cost	Economical	Underdeveloped at present
Large # of libraries in existence	# of isolates needed for a library is unknown	Regional and national applicability	Time needed to identify alternative organisms and develop molecular markers
Software available for isolate comparison	Time intensive	Large number of unknown organisms that may have value as alternative indicators	Cross reactivity of method with other microbial populations must be fully clarified
Numerous research publications available	Confusion about keeping libraries current	Readily applied to environmental samples	Scientific community not fully aware of the potential of this method
Useful in small watershed analysis	Less specificity for national/global scale	Applicable to animal samples	May be geographically specific
		Potential for real-time PCR development	
		Rapid turnaround for sample identification	
		Applicable to high throughput sampling	

Table 2

Summary of the Major Human Library-Independent Microbial Source Tracking Methods Employed in this Study

Organism	Method	Target	Primers
human-*Bacteroides*[a]	PCR	16SrRNA	Bac708R, 5'-CAATCGGAGTTCTTCGTG-3'
			HF183F, 5'-ATCATGAGTTCACATGTCCG-3'
Faecalibacterium[b]	PCR	16SrRNA	HFB-F3, 5'-GCTTTCAAAACTGGTCG-3'
			HFB-R5, 5'-GAAGAGAAAACGTATTTCTAC-3'
Methanobrevibacter smithii[c]	PCR	*nif*H	Mnif-342f, 5'-AACAGAAAACCCAGTGAAGAG-3'
			Mnif-363r, 5'-ACGTAAAGGCACTGAAAAACC-3'
Bacteroides thetaiotaomicron[e]	PCR	16SrRNA	B.thetaF, 5'AACAGGTGGAAGCTGCGGA-3'
			B.thetaR, 5'-AGCCTCCAACCGCATCAA-3'

(a) Bernard and Field 2000;
(b) Zheng et al, 2008;
(c) Ufnar et al. 2006;
(d) McQuaig et al. 2006;
(e) Carson et al. 2005

CHAPTER II

LITERATURE REVIEW

Coastal water quality is a fundamental aspect of a vigorous Gulf of Mexico, sustaining the shellfish industry and providing the basis for an extensive tourism industry. The safety of beaches and quality of life in the Gulf region are highly dependent upon successful stewardship of coastal waters, whose safety with respect to human health is threatened by extensive development and other anthropogenic activities. Water quality is routinely monitored by enumeration of indicator bacteria, which are generally nonpathogenic. These bacteria are associated with a wide variety of fecal inputs from humans and animals, and thus offer no information about the source(s) of pollution that can degrade water quality in coastal areas. This failure impedes the ability of regulatory agencies and managers to protect public health and remediate pollution sources. Microbial (bacterial) source tracking (MST) methods have been developed and tested over the past several decades, showing promise for discriminating between animal and human fecal pollution sources (Field et al., 2003; Meays et al., 2004; Rochelle and De Leon, 2006; Scott et al., 2002; Simpson et al., 2002).

In December 2006, a workshop entitled, "Northern Gulf of Mexico Bacterial Source Tracking Workshop," was held in Biloxi, MS (Ellender et al., 2006). Workshop participants, consisting of researchers from around the country and researchers from federal laboratories and Gulf States representatives, concluded that the most promising source tracking methods were: the human *Bacteroides* (HF8) marker, the *M. smithii* marker and the analysis of optical brighteners. Since that time, the testing of optical brighteners has diminished and three additional human markers, Human Polyomavirus,

Bacteroides thetaiotaomicron and *Faecalibacterium* spp. have been developed. A summary of the human markers is presented below.

Methanobrevibacter. The genus *Methanobrevibacter* is a member of the order *Methanobacteriales* within the domain *Archaea* of the Kingdom *Euryarchaeota* (LeFever and Lewis, 2003). Species within this genus occupy very specific environments. They are found in intestinal tracts of warm blooded animals, anaerobic waste water treatment sludge, termite guts, oral cavities, and decaying plant material (Miller and Wolin, 1983; Lai et al., 2004, Miller and Lin, 2002; Gray et al., 2002; Cabiral et al., 2003; Horz and Conrads 2011; Brusa et al., 1993; and Belay et al., 1998). Research supports that they are the dominant methanogens in animal intestines (Lin & Miller, 1998; Lou et al., 2012).

Despite what is known about their distribution in aquatic and terrestrial environments (Miller 1984), animal intestinal tracts (Miller and Wolin, 1983), oral cavities (Belay et al., 1998), and waste water treatment sludge (Gray et al., 2002; Cabiral et al., 2003), little has been gleaned about methanogen presence and persistence in diverse environments (Ferris et al., 1996; van der Maarel et al., 1999; Lai et al., 2004).

Microbiome studies have concluded that the methanogens *Methanobrevibacter smithii* is only found to inhabit human intestinal and vaginal tract (Miller 1984; Belay et al., 1990). It's unique ability to persist in the complex human microbiome is attributed to the chemical mimicry of its outer surface to carbohydrate formations commonly found in the human (host) digestive track and its ability to regularly express adhesion-like proteins (Samual et al., 2007). *M. smithii* is very competitive of nutrient sources and is able to mitigate the end fermentation products of other host associated bacterial groups (Samual et al., 2007). Methane emissions from respiration studies have indicated that

approximately 33% of the human population in the United States and Great Britain harbor methanogens; *M. smithii* is the most abundant methanogen in the human gut in order of 10^7- 10^{10} per gram (Bond et al., 1971; Lin and Miller, 1998; Ufnar et al., 2006). *M. ruminantium* is considered the dominant methanogen in the rumen of many animals (bovine, ovine, deer, goat, etc.) and is, therefore, a prime candidate for assessing ruminant-specific fecal pollution (Ufnar et al., 2006).

The ability to amplify the *nifH* gene of *M. smithii* from environmental and host samples makes it a good candidate for MST (Ufnar et al., 2006). Researchers have utilized the *nifH* gene to examine the gut microbial communities of host animals including the termite (Braun et al., 1999; Kirshtein et al., 1991; Ueda et al., 1995; and Widmer et al., 1999). The operon containing the *nifH* gene is conserved in methanogens and prokaryotes (Ufnar et al., 2006). The fidelity of the nonfunctional nitrogenase *nifH* gene as being a methanogen specific target has been thoroughly vetted (Ohkuma et al., 1999; Raymond et al., 2004; and Ufnar et al., 2007).

In 2006, Ufnar et al. determined that the *Methanobrevibacter smithii*, assay was rapid, specific, less time consuming and inexpensive when compared to library dependent methods. An assay was developed targeting the *nifH* gene of *M. smithii*. This assay was tested against 27 various methanogens, 19 different bacterial species, 548 environmental bacteria, as well as DNA extracts from humans, sewage, cow, sheep, goat, dog, horse, deer, turkey, goose, and chicken feces to determine if the assay was specific for humans and sewage. *M. smithii* pure culture, human fecal DNA, and sewage were the only samples that tested positive with this assay. In addition, environmental samples collected

during a MS coastal sewage spill confirmed the presence of this gene in contaminated waters, and water samples collected prior to the spill were negative for the gene.

Johnston et al. (2010) approached the detection of the *nifH* gene using a more quantitative real-time qPCR method. In this study the specificity of the primer sets (Mnif 202F 5'- GAA AGC GGA GGT CCT GAA-3' and Mnif 353R 5'- ACT GAA AAA CCT CCG CAA AC 3') were tested against 23 different species of methanogens, 11 of which were members of the genus *Methanobrevibacter.* The *M. smithii* target was detected in all environmental water samples that were spiked with sewage. According to Johnston et al. (2010) the *M. smithii* target sequence was also detected in two water samples spiked with bird guano. More importantly, the detection of the *M. smithii* target sequence in samples spiked with sewage did not correlate with the detection of culturable *E. faecalis* and *E. coli*. Other recent studies have further validated assays for this organism in the monitoring of environmental samples for the possible presence of fecal contamination. Rossario et al. (2009) tested the efficacy of using the *M. smithii* target for monitoring environmental samples in relationship to the detection of a pepper mild mottle virus. The *M. smithii* target was detected at six marine sites during the course of their study.

Bacteroidales. *Bacteroidales* are non-spore forming obligate anaerobes, and comprise a large portion of the human intestinal microbial flora. Species within this genus are known to be resistant to antibiotics, resulting in the highest resistance rates among anaerobic pathogens (Wexler, 2007). The use of this organism, as well as other obligate anaerobes, has been impeded by isolation and cultivation problems which are inherent with all conventional fecal anaerobe assays. Standard biochemical assays are being usurped by improved molecular techniques. To circumvent the inability of

conventional biochemical assays to adequately ascertain the point source of fecal pollution, several labs are utilizing molecular techniques to elucidate the viability of host specific genetic markers in the environment. These molecular based approaches allow the scientific community to reassess antiquated laboratory methods with a new found confidence in each experimental design. At the forefront were the molecular techniques for the isolation of *Bacteroides* sp. as viable fecal indicators, human *Bacteroides* and *Bacteroides thetaiotamicron* have emerged as likely candidates. *Bacteroides* sp. exhibited the characteristics of host specificity that is optimal for identifying the source of fecal contamination (Savichtcheva and Okabe, 2006). *Bacteroides* sp. exist in higher numbers in human than animal host as compared to the abundance of *enterococci* and *E. coli* sp. (Converse et al., 2009). Kreader (1995) suggested that bacteria from the genus *Bacteroides* might be used to distinguish human from nonhuman sources of fecal pollution because (a) *Bacteroides* spp. dominate the human fecal flora, and several species outnumber the coliforms; and (b) early experiments designed to better quantify the persistence of *Bacteroides* spp. in environmental waters supported the value of this organism as a viable indicator. An experiment designed to inoculate Ohio River water samples with whole fecal samples for the PCR detection of *B. distasonis* indicated that temperature variances and predation were both critical in establishing conventional PCR detection limits (Kreader, 1998). Experiments by Okabe et al. (2007) indicated that there was little correlation between the presence of human specific *Bacteroides* and the culturable presence of total and fecal coliforms collected from freshwater river samples. This lack of correlation has been confirmed by other research groups working with the *Bacteroides* 16SrRNA target gene sequence of the human specific *Bacteroides* group.

Human specific *Bacteroides* presence/absence was not directly correlated to any fecal indicator bacteria (FIB) abundance or interactions when assayed from marine samples (Santoro and Boehm, 2007). This study was of particular interest considering that 1/3 of the positive results for *Bacteroides* sp. occurred in an area where the confirmed fecal indicators were not of sewage origin; the sampling station farthest from the impacted tidal outlet in question had the highest occurrence human *Bacteroides* marker (Santoro and Boehm, 2007).

Bacteroides in general are valuable indicators because: 1. the bacterial load of human feces is on the order of 10^{12} per gram, and the predominant bacteria are of the genus *Bacteroides* (Zoetendal et al., 1998), outnumbering surrogate indicators such as *E. coli* and *enterococcus* species by orders of magnitude. 2. A *Bacteroides* genome has been sequenced, providing a basis for understanding the symbiotic role and microbial ecology of this microorganism, and enhancing the potential for development of host-specific molecular diagnostics (Xu et al., 2003; Kreader, 1995). 3. There is an established a protocol for detection of *Bacteroides* spp. that is uniquely associated with human or bovine fecal material (Bernhard and Field, 2000). This method relies on direct detection of strain-specific 16S rRNA gene sequences. They used a double PCR amplification that employs a primary PCR reaction in which DNA from environmental samples provide the template followed by a second amplification in which a small amount of the primary PCR product serves as the template. This allows for the detection of *Bacteroides* target sequences in spite of the very low levels of the obligatory anaerobic bacteria present in the surface waters environment. 4. The poor survival of *Bacteroides* in environmental waters may be a desirable feature since *Bacteroides* proliferating in the

environment longer than a pathogen is highly unlikely. Thus, the resulting test has a low rate of false positives stemming from material other than recent contamination by/with fecal material. 5. Layton et al. (2006) developed bovine and human-specific primers suitable for qPCR that are highly specific for bovine and human *Bacteroides*. In the initial development of a library independent method, Bernhard and Field (2000) used 16S ribosomal RNA markers designed to distinguish human and cow fecal pollution, and to also quantify the effective recovery of these markers from natural waters. Further research identified host specific *Bacteroides-Prevetella* 16S rDNA markers from humans and cows by implementing DNA screening with restriction fragment length polymorphism (T-RFLP). Here, DNA from water samples procured from areas in Tillamook Bay, Oregon, were amplified using *Bacteroides-Prevetella* primers (Bac32F and Bac708R).

Dick et al. (2004) extended this research using a Taq nuclease assay (TNA) that employed a fluorogenic probe and primer set to determine the capture affinity for *Bacteroides* 16S rRNA in primary sewage influent. To validate the quality of these host specific bacterial markers all possible primers sets should be experimentally exhausted. They employed the use of subtractive hybridization in microplate wells to identify host specific *Bacteroides*16S rRNA gene fragments and phylogenetic studies were employed to elucidate the endemism of *Bacteroides* spp. Thus, association of a specific *Bacteroides* spp. and an individual host would be paramount to its effectiveness as fecal contamination marker. Dick et al. (2004) also tested the 16S rRNA gene sequence analysis of *Bacteroides* from the feces of eight hosts: human, bovine, elk, pig, dog, cat, gull, and horse. The results revealed both endemic and cosmopolitan distributions of the

bacterial species. Research on the phylogenetic host relationships of the *Bacteroides-Prevetella* group and their viability in the environment was questioned by Scott et al. (2002), since the persistence of this molecular marker in situ was yet to be fully scrutinized and since little was known concerning the survival and persistence of *Bacteroides* sp. in the environment.

Recently, the isolation of new *Bacteroides* sp. and the revisiting of the genomes of previously known species have yielded a plethora of novel possibilities (Robert et al., 2007). These novel species include *B. plebeius, B. coprocola, B. helcogenes, B. intestinalis, B. finegoldii* and *B. doreii.* In addition, a toxin produced by enterotoxigenic *B. fragilis*, which alters the morphology of human intestinal cells has been sequenced (Chung et al., 1999) and it may be possible to exploit a specific section of this gene when designing genetic probes. Though these species have an extremely high sequence similarity, they may offer unique gene sequences that could better delineate host specificity through stringent primer design and field application.

As noted above, a real time qPCR has been developed for *Bacteroides* sp. There are several advantages to using qPCR as opposed to conventional PCR including the absence of gel analysis, the ability to simultaneously identify and quantify specific genes, a reduction in the time of assay and the cost effectiveness of the assay (Layton et al., 2006). The detection limit of any marker is of inherent importance. Regardless of the particular assay being implemented, a standard detection limit for that marker must be established. It is fair to assume that from the quantitative data generated by a qPCR assay, there may be an efficient way to set the parameters for a standard detection limit for a marker. Recent experiments performed by Seurinick et al. (2005) attempted to

quantify the detection of human specific *Bacteroides*16S rRNA genetic marker in fresh waters. More recent studies have tested the efficacy of using qPCR to monitor environmental waters for the presence of *Bacteroides* spp. (Converse et al., 2009; Shanks et al., 2009). The researchers found that these assays were efficient and reliable at targeting human specific *Bacteroides* spp. in environmental waters. They also discovered that the measured *Bacteroides* sp. found in sewage spiked samples often surpassed that of culturable *Enterococcus* sp. Therefore, qPCR would be an indispensable resource for assigning defined detection limits to the molecular detection of specific genetic markers (Converse et al., 2009).

Bacteroides thetaiotaomicron. In contrast to the *Bacteroides* marker discussed previously (Dick et al., 2004) and to which a variety of procedures has been ascribed to their role in microbial source tracking, *B. thetaiotaomicron* has not been exhaustively tested as a source marker of human pollution. This organism is present at a much higher percentage in humans than in nonhuman species, making it a strong candidate for MST (Carson et al., 2005). *B. thetaiotaomicron* became a candidate for a human specific marker when it was realized that it is a dominant species in the human gut and present in a much higher percentage of fecal samples (Carson et al., 2005). This study indicated that there are advantages in using the *B. thetaiotaomicron* primers instead of the Bernard and Field (2000) human primers since the *B. thetaiotaomicron* assay was sensitive, exhibited lower species overlap, required fewer PCR cycles, and appeared to be a more precise indicator of human fecal contamination.

This bacterium is known to possess a sizeable enzymatic profile that is of tremendous nutrient value to human metabolism (Xu et al., 2003). This organism is often

associated with intra-abdominal sepsis and bacteremia and has been documented to be the second most often encountered disease causing anaerobic gram negative bacillus (Teng et al., 2000). More recently the etiological significance of *B. thetaiotaomicron* has been established by identifying it as the causative agent of a case of meningitis (Feuillet et al., 2005).

Faecalibacterium. Butyrate-producing bacteria play an important role in the maintenance of intestinal health. The taxonomy, structure and dynamics of these anaerobic bacteria have been extensively studied and documented because they comprise a large percentage of the biomass in the human gut and the fact that they could be exploited as potential MST markers. Recently, there has been research aimed at utilizing a *Faecalibacterium* sp*,* formally known as a *Fusobacterium* sp.*,* as a novel MST marker. Using suppression subtractive hybridization (SSH), a new human specific bacterial gene marker, derived from a 16S rRNA gene region of *Faecalibacterium*, has been proposed as a potential MST marker (Zheng et al., 2008). Preliminary results indicate that this *Faecalibacterium* sp. is specific for human sewage, being found in 60.2% of human fecal samples and 100% of sewage samples tested (Zheng et al., 2008).

Human Polyomaviruses. The *Polyomaviridae* are a family of closed, double stranded DNA viruses that have the propensity to infect a wide range of vertebrates. They have an approximate genome of 5,000 base pairs (bp) and these data have been used to construct oncogenic models. Certain polyomaviruses are unique to humans, namely the JC and BK viruses. They appear to be widespread in the human population and are very host specific. Polyomaviruses of humans are acquired early in life and

develop into chronic infections of the kidney persisting indefinitely (Shah, 1996); they are shed in urine and, therefore, are found in sewage (McQuaig et al., 2006).

Bofil-Mas et al. (2000) and Biofil-Mas and Girones (2001) showed that this virus was readily found in sewage, reflecting the potential value of these organisms as a measure of human fecal pollution (Hundesa et al., 2006). A PCR based assay for human polyomavirus was recently described (McQuaig et al., 2006) and has been tested in a validation study in Florida and Mississippi. Recent data suggests that primers specific for the JC and Bk viruses in humans have very little, if any, cross reactivity with bovine and porcine associated viruses (McQuaig et al., 2006). Significant titer volumes have been documented in city sewage waste (Bofil-Mas et al., 2000); the high specificity and sensitivity of these viruses make them appropriate candidates for MST.

A TaqMan based qPCR assay for the detection of polyomaviruses BK and JC in environmental samples was developed (McQuaig et al., 2009). This study concluded that there was a negative statistical correlation between HPyV and bacterial indicators in sewage. This disparity in the rate of decay for HPyV and bacterial indicators in sewage may be more indicative of their relationship in the marine environment.

Use of human and animal markers to detect aquatic pollution. The use of published human and/or animal markers to determine the presence of fecal waste in fresh or salt water bodies, including coastal waters, is a comparatively novel undertaking and has developed using a variety of technological methods (Soule et al., 2006; Shanks et al., 2009; Field and Samadpour, 2007; Korajkic et al., 2010). The human *Bacteroides* marker has been exhaustively vetted in the environment through conventional and real-time qPCR assays (Bower et al., 2005; Layton et al., 2009; Ahmed et al., 2009; Dick et

al., 2004; Kildare et al., 2007 Hong et al., 2008; Flood et al., 2011). However, much of this research has yielded conflicting results regarding marker/standard indicator correlations. In addition, much of the research conducted on marker persistence has taken place in the laboratory, greatly inhibiting accurate extrapolations regarding marker sensibilities to pervasive environmental factors.

Experimental designs eventually evolved to include testing nonhuman fecal sources for amplification of the human- specific and general *Bacteroides-Prevotella* markers (Fogarty and Voytek, 2005; Kildare et al., 2007; Layton et al., 2006). The research conducted by (Fogarty and Voytek, 2005), elucidated cross-reactive amplification patterns in chicken and geese samples. Our research has also indicated that there is cross-reactivity between the human specific *Bacteroides* marker and chicken fecal samples. In addition, we have demonstrated that the marker can be amplified in domestic canine and feline fecal samples.

Persistence and decay of human specific indicators in the natural environment. Relationships affecting the ability of certain organisms to be good predictors of fecal pollution extended beyond their correlation to a host or each other. Once an indicator is exposed to the environment there are numerous biotic and abiotic variables that may affect survivability. Studies to determine which abiotic variables most greatly affect the persistence of detectable *Bacteroides* spp. target genes determined that salinity and temperature had a significant effect on their survivability (Seurinick et al., 2005; Okabe and Shimazu, 2007; Bell et al., 2009). As additional data has been generated by researchers involved in microbial source tracking, it has become evident that a multi-tiered approach and additional time points and physical variables should be considered

when addressing water quality (Santaro and Boehm, 2007). Even when these variables are statistically applied to environmental and microcosm studies, the results are still confounded by geographical and laboratory design variations.

Recently, Balleste and Blanch (2010) reported that *Bacteroides thetaiotaomicron* was less oxygen tolerant than *Bacteroides fragilis*, *B. thetaiotaomicron* was more thermotolerant in the summer months, and that environmental *Bacteroides* sp. exhibited a higher survivability rate. To understand the relationships of human specific fecal indicator bacteria it is paramount to design experiments that focus on elucidating specific correlations between these markers and environmental parameters. The data generated utilizing host specific *Bacteriodales* markers must be scrutinized within the environmental parameters of the sampling area, and they must be compared to previous studies examining their relationship to pathogens and traditional bacterial indicators (Walters et al., 2009). Walters et al. (2006) demonstrated that there is a differential survival rate of bacterial species belonging to the group *Bacteriodales.* Recently, Flood et al. (2011) showed that the presence of *M. smithii* and human *Bacteroides* markers were more prevalent in the coastal creeks that drained directly into the Mississippi Sound, and that these markers did not statistically correlate with the frequency of the markers found in the marine environment. This is indicative of the freshwater environment as a contributor of fecal pollution to the marine environment, but also indicates that the markers experience a differential survival pattern. It has been demonstrated that *Bacteroides* sp. recovered from sewage had a higher rate of decay than fecal coliforms or enterococci (Balleste and Blanch, 2010).

Questions concerning enterococcal persistence and importance in coastal waters. Conventional indicators of fecal pollution should presumably share common attributes with the pathogens they are proxies for. They should exhibit some correlation with the numbers of pathogens shed by the host, be nonpathogenic, easily assayed and enumerated, and share proportional survivability patterns; they should not persist and grow readily in extra intestinal environments (Scott et al., 2002). The United States Environmental Protection Agency (USEPA) has suggested the use of *Escherichia coli* and *Enterococcus spp.* as indicators of water quality for marine waters (USEPA, 2000). As research has progressed in the field of microbial source tracking it has become evident that differential survival rates and the innate ability of these organisms to proliferate and persist in the environment has called into question the efficacy of these organisms as appropriate indicators of fecal pollution. Researchers have attempted to quantify how these organisms react to the many variables encountered when they are introduced to the environment through controlled laboratory microcosm experiments (Anderson et al., 2005; Lee et al., 2006; Hartz et al., 2008). However, it would be more beneficial to strategically sample directly from the environment in a temporally compressed manner to better elucidate enterococcal and human specific marker trends. Marine water sampling strategies should focus primarily on the intertidal wash zone along the beach. These sampling constructs are important for many reasons: (1) the intertidal zone is an area where recreational bathing densities would be the highest; (2) beach sediment should inherently provide enteric bacteria with more nutrients and shelter than the water column; (3) bacterial levels should increase in areas of higher wave energy and higher concentrations of re-suspended particulates. These logical notions are recapitulated

throughout the current MST literature. For instance, research conducted by Alm et al. (2003) investigating the efficacy of examining beach sand for higher enteric bacteria concentrations concluded that, compared to water, enterococcal counts were 4-38 times higher and *E.coli* counts were 3-17 times higher in freshwater sediment samples. The time reported for enterococci survival in sediment varies in the literature. Recent studies by Gast et al. (2011) indicated that enterococci survival may persist in deep sediment (25-70cm). The growth of enterococci in sand seems to be inherently related to the availability of organic matter (Lee et al., 2006). Haller et al. (2009) found that enterococci be cultured from sediment could for up to 90 days after the initial sampling event. In addition to protecting the enterococci from predatory grazers, sediment shields the bacteria from prolonged exposure to UV radiation. Solar radiation is thought to be one of the primary factors in inhibiting bacterial populations, especially in shallow seawater (Sinton et al., 2002). The exact mechanism of photo-inactivation can vary for the particular bacteria in question and the environmental waters that the bacterium is recovered from. There is a knowledge gap as to how sunlight actually causes photo-damage, either by direct UVB destruction of DNA or the increase in reactive oxygen species, to fecal indicator bacteria in the marine setting (Maraccini et al., 2011). A study by Shibata et al. (2004) determined spatial concentrations and prevalence of indicator organisms assayed were tied directly to the particular organism, sampling procedure used, and proximity to the beach. These sentiments reiterate the need for further experiments aimed at determining how these organisms react with their environment. More importantly, these notions serve as a warning to investigators when designing experiments and interpreting results.

Direct pathogen detection. A natural and logical progression in the field of microbial source tracking is to adopt methods to assay for the direct presence of pathogens rather than using traditional indicators. Prohibitive costs and intermittent shedding of pathogenic species hinders the implementation of these direct assays in regular environmental monitoring As technology becomes more readily available and costs are lower, researchers are beginning to field test the efficacy of utilizing these direct pathogen measurements. Though these assays still retain the inherent inability to ascertain viability or infectivity, they do represent a direct method for determining the presence of a particular viral, protozoan, or bacterial pathogen (Stewart et al., 2008). It would be more statistically and biologically relevant to elucidate correlation, if any, between current water quality standards (enterococci) and possible pathogens. Carr et al. (2010) demonstrated that the presence of detectable *Salmonella spp.* did not correlate with enterococci along Mississippi Gulf Coast sampling sites. This discrepancy in correlation between standard fecal indicators and possible pathogenic exposure, whether it is fungal, bacterial, viral, or protozoan in origin, is the current impetus for improved environmental monitoring strategies. Stewart et al. (2008) suggests a tiered approach, ranging from the initial testing of indicators to assays for individual pathogens. A tiered approach should incorporate the known relationships of bacterial indicators, human specific markers (e.g., *M.* smithii, *B. thetaiotaomicron*), and pathogens to each other and environmental variables that may influence their persistence. Epidemiological studies for the geographic area in question would ideally mirror the correlation values for the above biological variables and reported beach associated illness. Direct pathogen detection

would alleviate much of the uncertainty associated with the current MST methods when assessing water quality.

OBJECTIVES

(1) To apply PCR detection of *Bacteroides*, *M. smithii*, *Fecalibacterium* and *B. thetaiotaomicron* markers in coastal waters and compare these results with enterococcal and fecal coliform counts taken at MDEQ sites. Analysis was performed as follows: 7/2007-11/2009 (human *Bacteroides, M. smithii*); 5/2009-11/2009 (human *Bacteroides, M. smithii, Fecalibacterium, Bacteroides thetaiotaomicron*); 4/2010-8/2010 (*Bacteroides, M. smithii, Bacteroides thetaiotaomicron*).

(2) To use appropriate statistical procedures in the analysis of all environmental and biological variables to answer the following questions:

1. Are there any correlations between EN/100mL/FC/100mL counts and sampling areas, i.e., are the counts higher in the freshwater of marine environment?
2. Where is the difference?
3. Are there any correlations between EN and FC counts and sampling areas?
4. Is there any correlation for the presence/absence of the four markers?
5. Is there any relationship for finding a marker positive and a high bacterial count?
6. Are the indicators present more often at any one site?
7. Are the markers and indicator bacteria interacting with salinity, temperature, UV, and turbidity?

CHAPTER III

MATERIALS AND METHODS

Table 3

Marine and Freshwater Coastal Sampling sites and their Geographic Locations

Location	Sites	Coordinates
Cemetery	7ACC	30°20'28.78"N 89° 9'41.30"W
Trautman Avenue	7ACT	30°20'31.06"N 89° 9'36.80"W
Trautman Avenue	7A	30°20.485'N 89°09.621'W
Pratt Avenue	9	30°22.201'N 89°04.783'W
Coffee Creek	CC1	30°22'52.14"N89° 3'22.99"W
Coffee Creek	CC2	30°22'40.51"N89° 31’7.95"W
U.S. Naval V.A.	10	30°22.559'N 89°03.161'W
Teagarden	10A	30°22.643'N 89°02.713'W
Anniston Oak	AOC	30°23'15.40"N 89° 1'8.57"W
Condo	CON	30°23'1.55"N89° 1'30.44"W
Cowan/Lorraine Road	11	30°22.938'N 89°01.578'W
Rodenberg Avenue	12A	30°23.586'N 88°56.291'W
16th Ave	CTHC	30°22'49.65”N 89°02'43.91”W
Courthouse boat launch	CH	30°22'37.59”N 89°02'41.00”W

Coastal sampling locations. Creek and coastal sampling sites are shown in Table 3 and Figure 1. Changes in the coastal sites sampled were evident at Condo (significant Katrina damage) and 7ACT (road construction); the physical natures of the other sites were consistent during the study period. Field observations indicated that these areas had the highest density of recreational beachgoers.

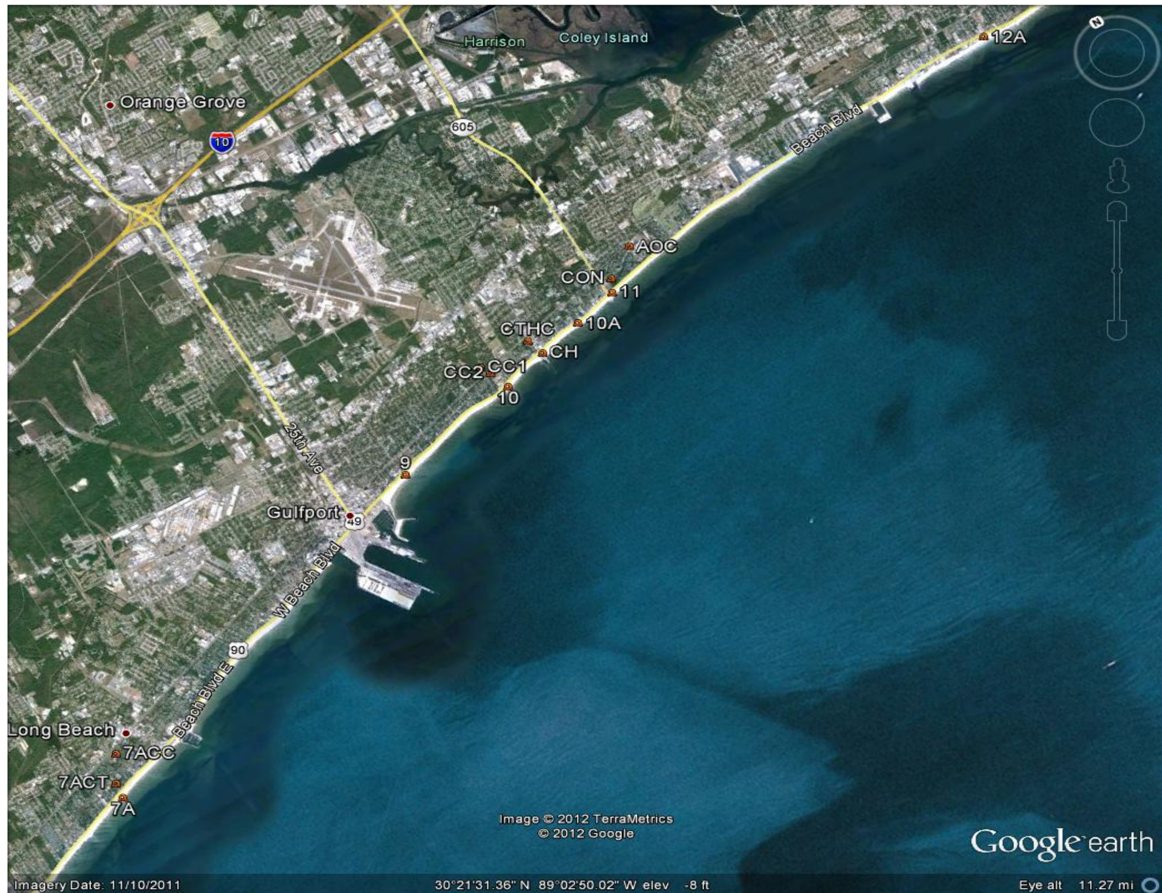

Figure 1. Coastal Mississippi – Harrison County.

Critical to this investigation is the inclusion of samples from freshwater streams that drain to the coastal environment and affect beach water quality. Specific sites within these streams were evaluated for the presence/absence of each marker; fecal coliform and enterococcal counts were also conducted on each stream sample.

Bacterial Indicators. Water samples were processed according to Standard Methods (USEPA, 2006). Briefly, dilutions were prepared for each sample and the CFU/mL calculated. Vacuum filtration was used to filter each sample dilution (0.45 µm, 47mm nitrocellulose membrane) (Pall Corporation, Ann Arbor, Michigan). For each sample dilution two individual filtrations were performed. One filter membrane was placed on a sterile Petri dish (55mm) containing mEI (membrane-Enterococcus indoxyl-beta-D-glucoside) agar (Becton, Dickinson and Company, Sparks, MD), and incubated at 41.5 ºC for 24 hrs. (USEPA, 2006). Countable plates were defined as containing 20-60 viable colonies that are raised and have a blue ring around a white center. The blue ring around the perimeter is consistent with the enterococci's ability to metabolize the indoxyl-beta compound. For fecal coliforms, a filter membrane was placed in a sterile Petri dish 60mm containing an absorbent pad and 2.0ml of mFC broth (EMD Chemicals Inc., Darmstadt, Germany). The mFC plates were incubated for 24hrs at 44.5ºC in a water bath (Norweco, 1997). Countable plates for mFC were defined as having between 20 and 60 colonies that are raised and blue in color.

DNA extractions. DNA extractions were performed using the Mobio Powersoil DNA kit (Mo Bio Laboratories, Carlsbad, CA) and, unless otherwise specified, followed the manufacturer's instructions.

Environmental Parameters. Sample measurements were taken (Appendix B) at each coastal location which included water temperature, turbidity, insolation and salinity. Several of these measurements were omitted on days when there was a device malfunction or when the sample location was inaccessible due to construction. Wind speed and direction, barometric pressure, surface air temperature, tidal cycles, and

average precipitation were gathered from online sources (http://www.nauticalcharts.noaa.gov/csdl/op/nowcoast.htm and http://www.wunderground.com/US/MS/Gulfport.html). Measurements of turbidity and salinity were measured: salinity was measured using a MR100ATC salinity refractometer (Milwauke Instruments, Rocky Mount, NC); turbidity was determined using 30ml of water from each sampling site. Samples were placed in a Hach 2100N IS Laboratory Turbidimeter (Hach Co., Loveland, CO) and the results recorded. Surface water temperature was measured on site using a mercury thermometer. Sunlight exposure may greatly affect the survivability of the near-shore shallow water microbial communities. Relative sunlight exposure was measured in Klux using an Extech EA30 digital light meter (Extech Instruments Corp., Waltham, MA). Measurements of UV A&B were monitored using a solarmeter (Solartech Inc, Harrison Township, MI). There was a significant amount of temporal variation for these measurements and they were considered close approximations for the average UV radiation that influenced each site.

Environmental sample collection for DNA extraction. Water samples were collected from each sampling location for every recorded sampling date. The samples were collected by completely submerging a sterile Nalgene bottle, capping and uncapping the bottle while underwater. The samples were placed on ice while in transit to the lab. Samples processing began within 6 hrs of collection. Each sample was pre-filtered through a 3.0 µm Versapor 3000T membrane (acrylic copolymer embedded on a nylon substrate) using vacuum filtration. Pre-filtering expedited the total processing time. The filtrate was collected in a sterile 1L Erlenmeyer vacuum flask and transferred back to the original sample bottle. The sample was then vacuum filtered through a 0.45 µm, 47mm

nitrocellulose (mixed cellulose ester) membrane. The magnetic filter holders and filtration flasks were all thoroughly sterilized by washing in warm soapy water, rinsing for 5 min, rinsing with 90% ethanol, and UV irradiation for 15 min. The flasks were autoclaved as a final precaution but the magnetic holders were not.

Filter method A1. Final processing of the 0.45 µm filters followed two different protocols for coastal samples. For sampling dates 8/2007–4/2009 the entire filter was placed in a sterile 150mL glass beaker. Autoclaved PBS (1mL), 0.25g of autoclaved beach sand, and an autoclaved magnetic stir bar were placed on top of the filter. The beaker was placed on a stir plate for a minimum of 5 min. This combination approached served to break up the filter before the primary step of DNA extraction. All filter and sand particulate was placed in the DNA extraction tube. The remaining PBS in the beaker was centrifuged at 10,000 X g for 5 min. The pellet was placed in the corresponding DNA extraction tube.

Filter method A2. Processing of filters for DNA extraction post 4/2009 followed a different protocol. The 0.45 µm filters were removed from the magnetic filter holder and placed in sterile plastic Petri dish (55mm). The filter was then cut, following the gridlines, using a sterilized razor blade. All the pieces were placed in the primary tube of the DNA extraction kit (MoBio). This procedure required fewer handling steps, reducing the chance of contamination and expediting processing time.

Filter method A3. The 0.45 membrane filters were folded and placed directly into the beading tube of the MobioPowersoil extraction kit (Mo Bio Laboratories, Carlsbad, CA).

PCR Protocols for the analysis of environmental waters. The experimental protocols for the environmental samples collected from the Mississippi Gulf Coast went through several modifications (see Table 4).

Table 4

Summarized Experimental Methodologies for all Environmental Samples

Sample Seasons	Sampling Dates	# of Samples	Visualization Method	PCR Protocol	Thermocycler Conditions
All Seasons*	8/2007-8/2008	351	Agarose Gel	A & B	1&2
Fall/Winter/Spring*	9/2008-4/2009	126	MultiNA/Gel	A & B	1&2
Summer/Fall	5/2009-11/2009	224	MultiNA	C, D, E & F	3,4 & 5
Summer	4/2010-7/2010	118	Agarose Gel	C, D & F	3 & 5

*Dates within this range represent the same sampling group, separated by visualization methods.

Protocols, sampling dates, number of samples processed, the method of marker visualization, and the PCR method utilized and the thermocycler conditions are summarized above (Table 4). A total of 819 samples were tested during this study. The methods utilized for each sampling trial are discussed in the text below.

Environmental Samples August 2007 - April 2009

(A) For *M. smithii* extracted DNA from environmental samples was amplified in 25 µl reactions containing 12.5 µl of GoTaq Green (Promega® Corporation, Madison, WI.), 0.5µM of both forward and reverse primers, 8.5 µl of nuclease-free water, and 2 µl of varying concentrations of DNA template.

(1) The Master Mix was aliquoted into nuclease-free PCR tubes (Sarstedt AG & Co, Nümbrecht, Germany). The PCR was carried out in an Eppendorf thermocycler (Eppendorf Mastercycler, Hamburg, Germany.). The thermocycler protocol consisted of a lid temperature of 105°C followed by 30 cycles of initial denaturation of 92°C for 30 sec, annealing at 55.1°C for 30 sec, and extension at 72°C for 30 sec, and a final extension of 72°C for 6 min (Ufnar et al., 2006).

(B) *Bacteroides* extracted DNA from environmental samples were amplified in 25 µl reactions containing 12.5µl of GoTaq Green (Promega® Corporation), 0.5 µM of both forward and reverse primers, 9.5 µl of Nuclease-free water, and 1 µl of varying concentrations of DNA template.

(2) The Master Mix was aliquoted into nuclease-free PCR tubes (Sarstedt AG & Co, Nümbrecht, Germany). The PCR was carried out in an Eppendorf thermocycler (Eppendorf Mastercycler). The thermocycler protocol consisted of a lid temperature of 105°C followed by the parameters: 35 cycles of initial denaturation of 94°C for 1 min, annealing at 55°C for 30 sec, and extension at 72°C for 1 min, followed by a final extension of 72°C for 7min (Bernard and Field, 2000).

The PCR product for each potential marker was assayed by standard gel electrophoresis. A 1.5% agarose gel (AquaPor LE, Atlanta, GA.) was cast in 0.5X TAE buffer. A gel run for each sample was performed to determine the presence or absence of the particular marker in question. Each gel contained a 100bp ladder from N.E. Biolabs. Gels were run for 1hr 45min at 72V in a voltage metered electrophoresis box (Fisher Scientific, Model # FB300). Unless otherwise specified, the gels were then stained in 1% ethidium bromide (Invitrogen, Life Sciences, Grand Island, NY.) for 20

min. After the allotted staining time the gels were visualized with the aid of an Alpha Multi Image light cabinet and Alpha Imager software (Cell Biosciences, Santa Clara, CA).

PCR May 2009 – July 2010

(C) *M.smithii* extracted DNA from environmental samples was amplified in 25 µl reactions containing 12.5 µl of EconoTaq Plus (Lucigen® Corporation, Middleton, WI.), 0.5µM of both forward and reverse primers, 8.5 µl of nuclease-free water, and 2 µl of varying concentrations of DNA template.

(D) *Bacteroides* extracted DNA from environmental samples was amplified in 25 µl reactions containing 12.5µl of EconoTaq Plus (Lucigen® Corporation), 0.5 µM of both forward and reverse primers, 9.5 µl of Nuclease-free water, and 1 µl of varying concentrations of DNA template.

(3) The Master Mix was transferred into nuclease-free PCR tubes (Sarstedt AG & Co, Nümbrecht, Germany). The PCR was carried out in an Eppendorf thermocycler (Eppendorf North America, Hauppauge, NY). The thermocycler protocol consists of a lid temperature of 105°C followed by an initial denaturation step of 94.0°C for 3 min 30 sec, followed by 94° C for 45 sec; 45 sec at 65-55°C (step down 1 /2 cycles from 65 to 62°C & 1 /cycle from 62 to° 55 C); 72° C for 30 sec. This was followed by 30 cycles at 94° C for 45 sec; 55°C for 45 sec and 72°C for 30 sec. The final extension was at 72°C for 5 min. The thermocycler was held at 4.0°C until the product was removed.

(E) *Faecalibacterium* extracted DNA from environmental samples was amplified in 25 µl reactions containing 12.5 µl of EconoTaq Plus (Lucigen® Corporation), 0.5µM

of both forward and reverse primers, 8.5 µl of nuclease-free water, and 2 µl of varying concentrations of DNA template.

(4) The Master Mix was transferred into nuclease-free PCR tubes (Sarstedt AG & Co, Nümbrecht, Germany). The PCR was carried out in an Eppendorf thermocycler. The thermocycler protocol consisted of a lid temperature of 105°C followed by 35 cycles of the parameters: initial denaturation at 94°C for 2 min; denaturation at 94°C for 1 min; annealing at 55°C for 1 min; elongation at 72°C for 30s; followed by a final elongation of 72°C for 7 min (Zheng et al., 2008).

(F) For *Bacteroides thetaiotaomicron* extracted DNA from environmental samples were amplified in 25 µl reactions containing 12.5 µl of EconoTaq Plus (Lucigen® Corporation), 0.5µM of both forward and reverse primers, 8.5 µl of nuclease-free water, and 2 µl of varying concentrations of DNA template.

(5) The Master Mix was aliquoted into nuclease-free PCR tubes (Sarstedt AG & Co, Nümbrecht, Germany). The PCR was carried out in an Eppendorf thermocycler (Eppendorf Mastercycler). The thermocycler protocol consisted of a lid temperature of 105°C followed by the parameters: 35 cycles of initial denaturation of 94°C for 1 min, annealing at 56°C for 1 min, and extension at 72°C for 1 min, followed by a final extension of 72°C for 7 min (Teng et al., 2000).

Gel Electrophoresis. The PCR product for each potential marker was assayed by standard gel electrophoresis. A 1.5% agarose gel (AquaPor LE,Atlanta, GA.) was cast in 0.5X TAE buffer. There was a gel run for each experiment to determine the presence or absence of the particular marker in question. Each gel row was run with a 100bp ladder from N.E. Biolabs (Ipswich, MA). The gels were run for 1hr 45min at 72V in a voltage

metered electrophoresis box (Fisher Scientific, Model # FB300). The gels were then stained in 1% ethidium bromide for 20 min. After the allotted staining time the gels will be visualized with the aid of an Alpha Multi Image light cabinet and Alpha Imager software (Cell Biosciences, Santa Clara, CA).

Microchip (MultiNA) Electrophoresis. A protocol for high throughput analysis of EconoTaq derived PCR amplicons from *M. smithii*, human *Bacteroides*, *B. thetaiotaomicron* and *Faecalibacterium* were designed. Analysis was performed using the microchip electrophoretic system MCE-202 MultiNA (Shimadzu Corporation, Kyoto, Japan). The electrophoresis assay was designed according to the following the parameters. Unless otherwise stated, all reagents were purchased from Shimadzu.

Analyses of PCR amplicons ≤500bp were tested as follows: a 1/50 dilution of a 25 bp ladder (Invitrogen Co., catalog No. 10597-011) was made by dispensing 1µl of ladder into 49µl of TE buffer. The solution was gently agitated for ten seconds. A working stock solution of Syber Gold Dye was prepared by dispensing 1 µl of dye into 99 µl of TE buffer (10mM, 1mM EDTA, pH 8.0).The solution was then agitated for 10 sec. The volume of separation buffer was calculated according to the number of samples to be assayed. The diluted Syber Gold was added to the separation buffer until it reached a volume ratio of 1/100. This solution was not agitated but gently swirled for 1.5 minutes. The total volume of marker solution was calculated as (the amount of marker solution needed) = 2 x the number of analyses (samples + ladders + positive and negative controls) + 40µl.

Analyses of PCR amplicons ≥ 500bp were determined using the following protocol: A 1/100 dilution of ΦX174 DNA/Hawaii marker (Promega Co. Madison, WI.)

were made by dispensing 1µl of the marker into 99µl of TE buffer. The solution was agitated for 10 sec. 1µl of SYBER Gold dye was dispensed into 99µl of TE buffer and the solution was agitated for 10 sec. The diluted dye solution was added to the separation buffer until it reached a volume ratio of 1/100. The solution was not agitated but gently mixed for 1.5 minutes. The total volume of marker solution was calculated as (the amount of marker solution needed) = 2 x the number of analyses (samples(X) + ladders (4) + positive and negative controls (2)) + 40µl.

To ensure the integrity of the experiment all of the reagents were allowed to equilibrate at room temperature before they are placed into the MultiNA. Extra attention was paid to how all of the reagents were agitated due to the systems sensitivity to residual micro-cavitations in viscous media. New protocols were developed for chip cleaning (See Appendix A for details), including the design and construction of an inexpensive small scale, stand alone, capillary pump. Other considerations included the inherent salt concentration of the PCR reactions and the ng/µl concentrations of the PCR products. These factors greatly influenced the operational integrity of this assay platform. There were other variables to consider when using this technology and they are discussed in the results.

Comparison of PCR detection of amplicons from human Bacteroides, M. smithii, Faecalibacterium, and B. thetaiotaomicron in coastal waters to enteroccocal counts taken at coastal sampling sites. Several statistical models were applied to compare the relationships of the presence or absence of each of these markers with standard enteroccocal counts and environmental variables taken at the same location. Extensive Kruskal-Wallis, Mann-Whitney, Spearman’s rho, post hoc Tukey HSD, Pearson

correlation, Oneway Anova, Linear regression, and Chi-Square statistical analyses were performed (SPSS V.17.1 software, IBM, Armonk, NY) on all of the collected data to try to elucidate significant correlations. The data was analyzed using a frequentist approach. This approach included linear regressions, analysis of variance, and multivariate statistics.

CHAPTER IV

RESULTS

Indicator bacterial counts and PCR detection of Bacteroides and M.smithii markers in coastal samples (8/2007-4/2009). For all samples from each site, the average enterococcal counts were calculated and the frequency (%) of a positive PCR result for *M. smithii* (%MS) and human *Bacteriodales* (%BA) was tabulated (Table 5, Part A). The highest average EN counts were recorded in creek samples as shown by the measurements for sites 7ACT, 7ACC, CC1, CC2, AOC and Condo. Coastal sample (sites 7A, 9, 10, 10A, 11 and 12A) averages were lower with site 10 having the highest count in this group of samples and station 10A the lowest count. With rare exception, the %BA at each site was higher than the %MS; however, certain sites contained virtually equal percentages of each marker (sites 7A, CC1, 10A, AOC and 11). Not all creeks associated coastal sites showed the trend shown for EN. For example, the percentages of each marker in the creek (7ACC and 7ACT) leading to the coastal 7A site (15/32; 15/ 32; %MS/%BA, respectively) were in the same general range as the coastal site (24/27; %MS/%BA, respectively). The same basic outcome was found at the Turkey creek stations (AOC and Condo) exiting to the coast at site 11. In contrast, the CC1 and CC2 creek stations yielded high percentages of each marker but the concentrations of the markers dropped in the coastal water site (10) by a factor of 3. Station 9 is approximately 1 mile from station 10. Since the prevailing winds are from the southeast, it is possible that the EN and human markers drift with the water from site 10 to site 9, but there is no direct evidence for this conclusion. Site 12A is approximately 10 miles east of site 9 and there is no known source for the human markers at this site. Enterococci counts

(CFU/100mL) were graphed for all sampling months (Figure 2). There was a spike in bacterial counts during the month of August.

Figure 2. Enterococci (log CFU/100mL) for sampling months 08/2007-04/2009.

Enterococcal counts varied between sites. The freshwater creek sites had the highest EN counts (Figure 3).

Figure 3. Enterococcal counts (log CFU/100mL) across all sample sites for the dates 08/2007-04/2009.

Total markers positives were graphed for all sampling sites for the dates 08/2007-04/2009. The Coffee creek system contributed the most marker positives (Figure 4).

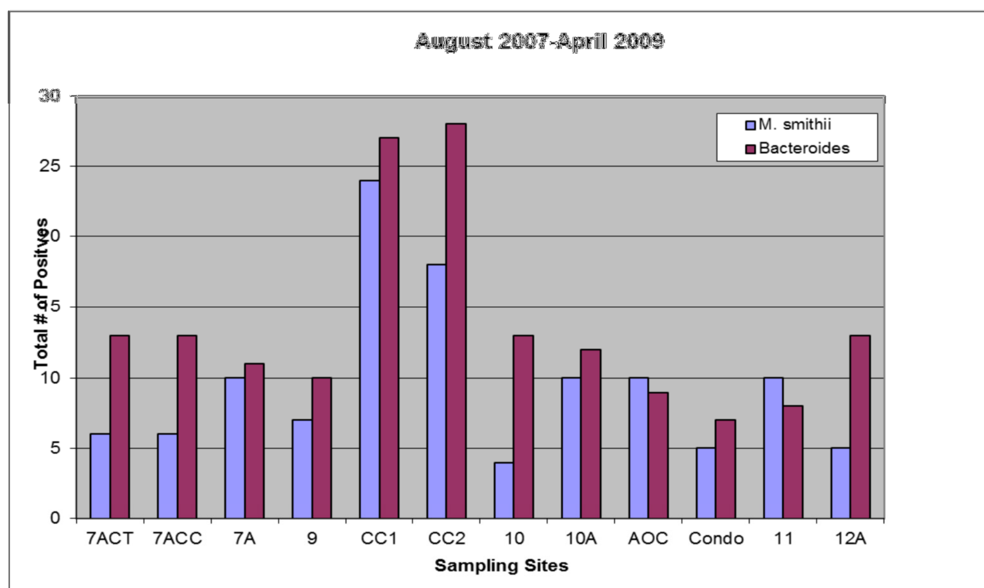

Figure 4. Total marker positives (*M. smithii* and human *Bacteroides*) at each site for the dates 08/2007-04/2009.

Statistical analyses of environmental coastal samples 8/2007-4/2009. To determine whether there was a significant relationship between EN counts at the different sampling sites, a one way ANOVA was performed (using the data set shown in Table 5, Part A and B) and demonstrated that all sites were significantly different from each other $\{F(11,314)=18.34, p < 0.001\}$. Multiple comparisons of all sites tested showed significant differences between creek and coastal sites, with the exception of sites 11 and 12A. These sites were significantly different from CC1, CC2, AOC and Condo.

Table 5

Average Enterococcal Counts (EN), Number of Sampling Dates, and Percent Positive Reactions for each Human Marker (MS, BA) at each of the 12 Study Sites

5.A		7ACT	7ACC	7A	9	CC1
5A .1	EN (N=30)	1781±1832	2024±1535	189±466	385±863	4248±2845
G	%MS	15	15	24	17	61
G	%BA	32	32	27	24	66
5A .2	EN (N=11)	1556±1236	2770±2577	254±390	61±97	3397±2530
G	%MS	9	18	27	9	27
G	%BA	0	27	0	0	55
M	%MS	45	64	64	55	64
M	%BA	9	27	0	0	36

Table 5 (continued)

5.B	CC2	10	10A	AOC	Condo	11	12A
5B.1	3612±2997	257±552	221±335	3640±2918	2916±2317	644±1679	718±142
G	53	10	27	29	14	22	15
G	82	33	29	26	29	22	38
5B.2	2839±32000	93±131	25±37	3503±2158	2136±1516	659±1364	17±45
G	36	27	18	27	9	9	27
G	36	18	18	9	9	9	45
M	64	45	36	64	9	45	45
M	36	18	9	9	9	0	27

Tables 5A and 5B represent the average enterococcal count (EN), number of sampling dates, and the percent positive reactions for each human marker (MS, BA) at each of the 12 study sites. (A). Values for each site from August 2007 to August 2008 (Average enterococcal per 100 ml; Percentage of each human marker detected by gel electrophoresis {G}); (B). Values for each coastal site from September 2008 to April 2009; (Average enterococcal count per 100 ml; Percentage of each human marker detected by gel electrophoresis {G} and by MultiNA analysis (M).

An examination of the relationship between EN and the presence or absence of BA and MS at each sampling site showed significant differences between EN counts and presence or absence of the markers (BA: [t(323)=2.41, p=0.016]; MS: [t(324)=2.79, p=0.006]. A MS± to BA± cross-tabulation (Table 6) demonstrated that when MS was not present in a sample, BA was not present 73% of the time. When MS was present in a sample, BA was present 69% of the time. The BA± to MS± cross-tabulation showed that when BA was not present in a sample, MS was not present 90% of the time; when BA was present in a sample, MS was also present 40% of the time. These data seem to imply that each measure is testing for the same parameter, i.e., fecal pollution of water. Taking into account all samples, both BA and MS were negative 54% of the time, positive 20% of the time, and disagreed 26% of the time.

A comparison of the percentages of each marker as determined by gel electrophoresis and MultiNA analysis is shown in Table 7, parts A and B. These data show that at 11 of the 12 locations, the percentage of the MS marker was higher than the BA marker when tested by the MultiNA method. Gel electrophoresis showed that the MS percentage was higher at 4 sampling sites, BA higher at 3 sites and the remaining sites were equivalent.

MultiNA and gel electrophoresis cross-tabulations demonstrated that when the MultiNA did not indicate the presence of the MS marker in a sample, the gel method did not show the marker 94% of the time. On only one occasion did the MultiNA present a negative result when the gel method was positive. When the MultiNA analysis indicated the presence of the MS marker, the gel method showed 61% dissimilarity with the

capillary method. Both methods agreed on the presence of the MS marker 39% of the time.

Cross-tabulation of the BA marker analyzed by MultiNA and gel electrophoresis showed that when the MultiNA result did not display this marker, the gel method did not show the marker 92% of the time. When the BA marker was found by the MultiNA analysis, the gel electrophoresis method found the marker 80% of the time. Taking into account all measurements, the MultiNA data agreed with the gel electrophoresis data 68% of the time for MS and 90% of the time for BA.

Table 6

Cross-tabulation of Gel Analysis of MS ± vs. BA ±

		BA ±		
		No	Yes	Total
MS ±	Count	202	75	277
	No, % within MS ±	72.9	27.1	100
	No, % within BA ±	89.8	60.0	74.7
	% of Total	54.4	20.2	74.7
	Count	23	50	73
	Yes, % within MS ±	31.5	68.5	100
	Yes, % within BA ±	10.2	40.0	19.7
	% of Total	6.2	13.5	19.7
Totals	Count	225	125	371
	% within MS ±	60.6	33.7	100
	% within BA ±	100	100	100
	% of Total	60.6	33.7	100

Cross-tabulation (A) analysis of MS ± vs. BA ±. The % within MS is a row percentage (202/277) whereas the % within BA is a column percentage (202/225). That is, 72.9% of the "no MS" are also "no BA" but 89.8 % of the "no MS" are also "no BA"; the numbers total 100%.

Table 7 (A & B)

Cross-tabulations of the MultiNA (capillary) vs. Gel Electrophoresis

Table 7, A.

		Gel MS No	Gel MS Yes	Total
MultiNA MS	Count	60	1	64
	No, % within M MS	94	2	100
	No, % within G MS	59	4	49
	% of Total	46	1	49
	Count	41	26	67
	Yes, % within M MS	61	39	100
	Yes, % within G MS	40	96	51
	% of Total	31	20	51
Totals	Count	102	27	132
	Yes, % within M MS	77	21	100
	Yes, % within G MS	100	100	100
	% of Total	77	21	100

Table 7, B.

		Gel BA No	Gel BA Yes	Total
MultiNA BA	Count	103	9	112
	No, % within M BA	92	8	100
	No, % within G BA	96	36	85
	% of Total	78	7	85
	Count	4	16	20
	Yes, % within M BA	20	80	100
	Yes, % within G BA	4	64	15
	% of Total	3	12	15
Totals	Count	107	25	132
	Yes, % within M BA	81	19	100
	Yes, % within G BA	100	100	100
	% of Total	81	19	100

Note. Cross-tabulations of the MultiNA vs. Gel electrophoresis for MS and BA are presented as percentages of positives and negatives expressed as % yes and % no within gel and MultiNA assays for both markers.

Indicator bacterial counts and PCR detection of Bacteroides (HuBac), M. smithii (MS), B.thetaiotaomicron (Btim), and Fecalibacterium (Fecali) markers in coastal samples (5/12/2009-11/19/2009). Samples were collected and assayed between 5/12/2009-11/19/2009, representing 16 individual sampling trips. The distribution of En/100mL and FC/100mL across all sampling sites were graphed (Figure 5). Enterococci

were found in much higher levels in the freshwater creek environments, followed by their effluent waters.

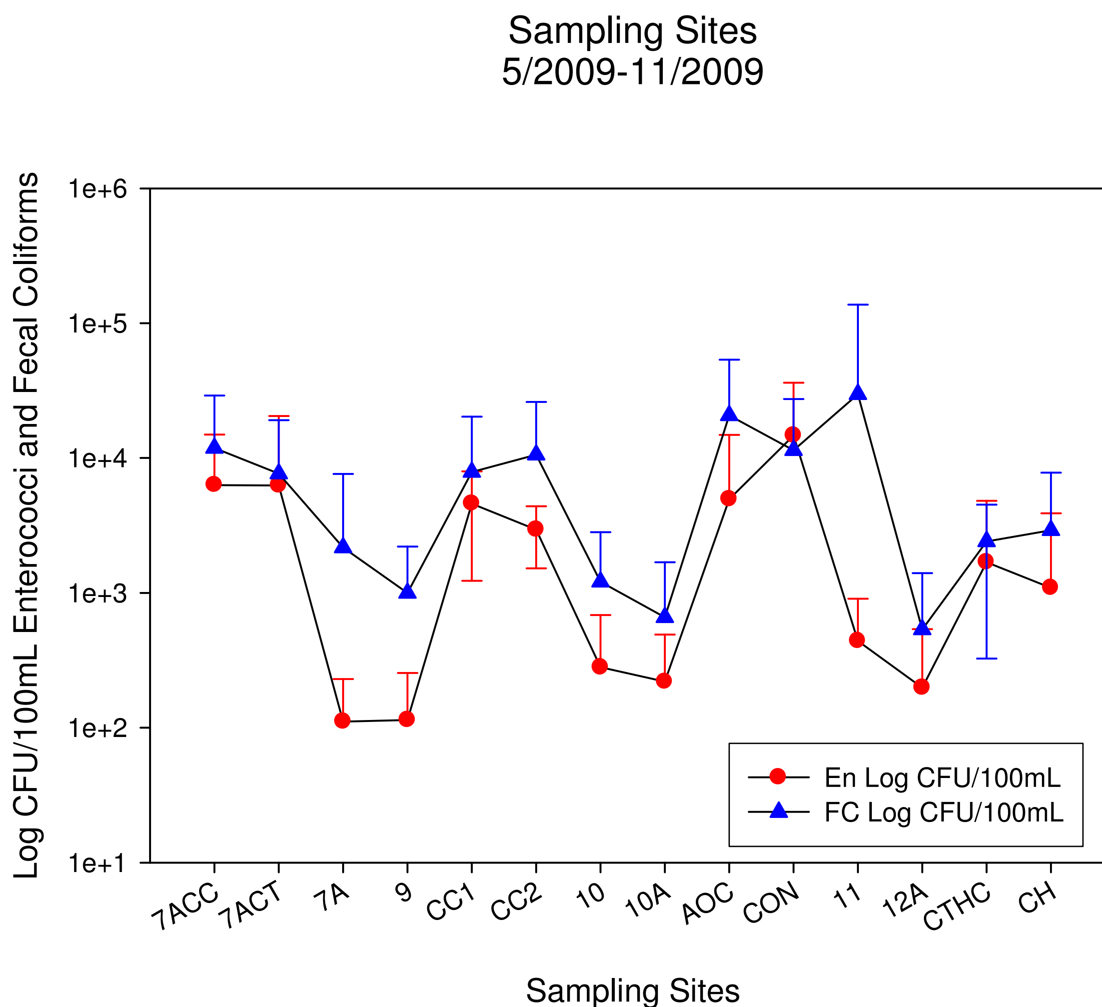

Figure 5. CFU/100mL of enterococci and fecal coliforms for each sampling location (5/2009-11/2009).

Both of the indicator bacteria followed the same trend across sampling locations. The highest concentrations were found in the freshwater creek environment, followed by their respective effluents. The FC/100mL was noticeably higher at site 11, the terminal point of AOC and Condo.

The seasonal variations for each of the bacterial indicators enterococci and fecal coliforms were graphed (Figure 6). Both indicators had their highest spikes in the month of July. Fecal coliforms and enterococci exhibited a precipitous drop in the winter month of December and in late July.

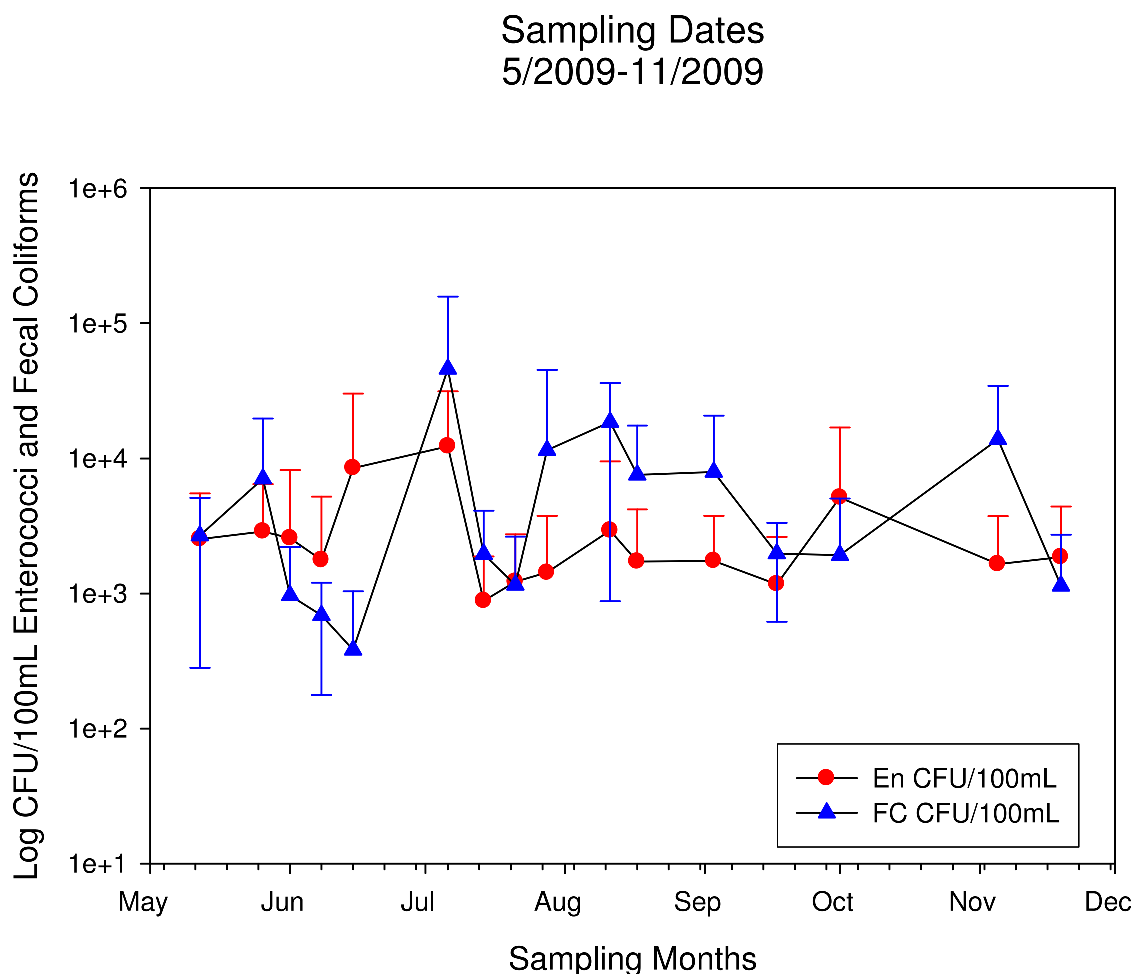

Figure 6. Seasonal variation (CFU/100mL) of enterococci and fecal coliforms for the sampling season (5/2009-11/2009).

Forty percent (311 of 768) of the PCR assays tested positive for the presence of at least one of the human markers (Table 8). Freshwater (FWS), marine (NSM), and near-shore brackish (creek effluent) water sampling sites represented 65%, 13%, and 22%, respectively, of those samples that showed the presence of a marker. Of the four coastal

creeks sampled, CC1 and CC2 contributed the highest number, 18% and 22%, respectively, of the positive markers assayed. Consequently, 29% of the total positives found in the NSB were found at site 10, the terminal point of CC1 and CC2. Sample sites AOC and CONDO represented 9.36% and 11.3% of the total FWS positives assayed respectively. In the NSB environment the associated effluent, site 11, from this creek system represented 26% of the positives assayed. The sample sites 7ACC and 7ACT both contributed 14% of the positives assayed from the FWS environment. The terminal effluent of this creek system, site 7A, contributed 18% of the positives found in the NSB waters. Markers observed at coastal sites impacted by a creek had a 3.2 to 1 chance of being positive when compared to coastal sites not impacted by a creek. The *B. thetaiotaomicron* (*Btim*) marker was positive (100 times) 32% of the time, followed by human *Bacteroides* (HuBac) at 25%, *Faecalibacterium* (Fecali) at 22% and *M. smithii* at 21%. The distribution of the markers was graphed for all sampling sites (Figure 7).

Table 8

PCR Results for Bacterial Assays from 5/2009-11/2009

	MS +/(%)	HuBac +/(%)	Btim +/(%)	Faecali +/(%)	(+) #/%
Total	65(21)	78(25)	100(32)	67(22)	311 (40)
7ACC	6	8	10	5	29 (9)
7ACT	6	6	11	5	29 (9)
7A	3	2	2	4	12 (4)
9	2	2	2	4	10 (3)
CC1	5	10	15	6	36 (12)
CC2	9	14	14	8	44 (14)
10	5	10	5	4	24 (8)
10A	5	3	3	3	14 (5)
AOC	4	5	10	4	23 (7)
CON	5	3	11	4	23 (7)
11	2	5	4	6	18 (6)
12A	3	3	5	4	15 (5)
CTHC	7	3	5	5	19 (6)
CH	3	4	3	5	15 (5)

Note. Percentage of putatively human specific bacterial positives (*M. smithii,* human *Bacteroides, Bacteroides thetaiotaomicron* and *Fecalibacterium*) for each site for sampling dates 5/12/2009-11/19/2009.

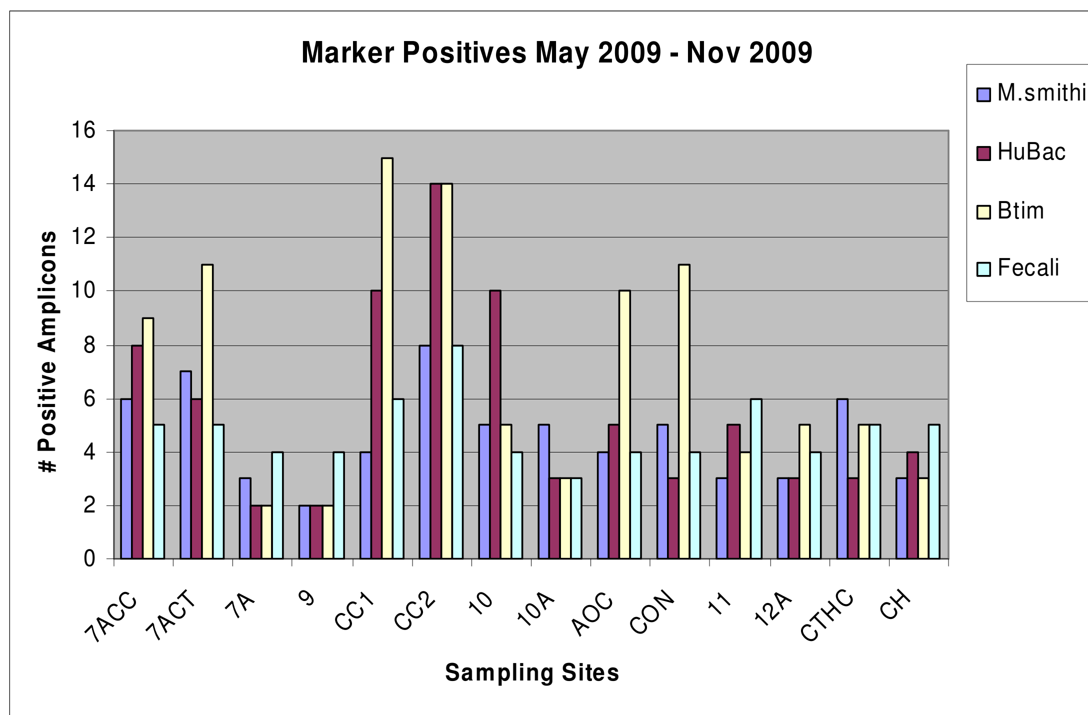

Figure 7. Marker distributions for all sampling sites (5/2009 – 11/2009).

Statistical analyses of environmental coastal samples 5/12/2009-11/19/2009. For these analyses the sampling sites were delineated into subgroups based on their respective water type. The sampling sites were divided into freshwater creeks (FWS; 7ACC, 7ACT, CC1, CC2, AOC, CON, and CTHC), their near-shore brackish effluents (NSB; 7A, 10, 11, and CH), and marine sites that were not directly impacted by associated creek water effluents (NSM; 9, 10A, 12). A one-way ANOVA was run to determine if the microbial variables of En/100mL and FC/100mL differed significantly between the three water types. The data were not normally distributed, having standard deviations much larger than their means. In response to the lack of normality, a Kruskal-Wallis test was performed on the three water types according to their rank sum. This test was the most appropriate due to all sites being delineated into three grouped variables. For EN/100mL the ChiSquare = 126.29 with df = 2 and $P < 0.001$. For FC/100mL the ChiSquare =

64.34 with df = 2 and $P < 0.001$. For EN/100mL the three water types were ranked FWS>NSB>NSM with means 159.38, 72.45, and 51.59 respectively. For water types FWS, NSB, and NSM, N = 111, 64, and 47, respectively. This ranking indicates that the EN/100mL and FC/100mL were significantly different for each of the three water types. For FC/100mL the three water types were ranked FWS>NSB or NSM with means 145.22, 87.05 and 65.15, respectively (Table 9).

Table 9

Krusal-Wallis Results of Water Type Environments in Order of their Mean Ranks for Dates 5/2009-11/2009

Bacterial Count	Water Type	Mean rank
En/100mL	FWS	159.38
	NSM	51.59
	NSB	72.45
FC/100mL	FWS	145.22
	NSM	65.15
	NSB	87.05

To determine any significant difference between the two microbial variables EN/100mL and FC/100mL, a Mann-Whitney test was run grouping the water type variables by twos (Table 10). For water types FWS and NSM there was a significant difference between EN/100mL and FC/100mL with a $P < 0.001$ for both. Water types FWS and NSB showed a significant difference for both EN/100mL and FC/100mL with a

P < 0.001. For the water types NSM and NSB there was an additional Kruskal-Wallis test run which indicated that there was a significant difference for EN/100mL with a ChiSquare = 6.85, df = 1 and P < 0.01 but no significant difference for FC/100mL with a ChiSquare = 3.64, df = 1 and P > 0.055.

Table 10

Mann-Whitney Test Results for the Differences in Water Type Environments for Sampling Dates 5/12/2009-11/19/2009

	Water Type	Mean Rank
En/100mL	NSM	51.59
	NSB	72.45
FC/100mL	NSM	65.15
	NSB	87.05

Additionally to determine if there was any correlation between EN/100mL and FC/100mL, i.e., if one increased did the other increase, a Spearman's rho was run for count data and sampling sites based on water type. For water type FWS there was a low-moderate correlation with a coefficient = 0.261, N = 111, and P < 0.007. The water type NSM had a moderate correlation with a coefficient = 0.419, N = 47 and P < 0.004. The water type NSB had a slightly higher moderate correlation coefficient = 0.561, N = 64, and P < 0.001. To determine if there was any correlation between bacterial counts of EN/100mL and FC/100mL and salinity (ppt) a Spearmans's rho was performed. For EN/100mL the correlation coefficient = -0.596, N = 182 and P < 0.001. For FC/100mL the correlation coefficient = -0.416, N = 182 and P < 0.001. Both tests were significant at

the $P < 0.01$ level with each bacterial group showing an inverse relationship to salinity but with EN/100mL being more greatly impacted. As the sampling sites increased in salinity, the abundance of these bacterial counts decreased. This inverse relation is not only applicable to salinity but can be considered a proxy for the inherent dilution effects of the study sites in question. To test whether the abundance of each of the four indicator groups could be predicted by salinity (ppt) a Spearman's rho was performed. *Btim* had a correlation coefficient = -0.477, $N = 182$ and $P < 0.001$. *HuBac* had a correlation coefficient = -0.221, $N = 182$ and $P < 0.004$. *M. smithii* had a correlation coefficient = -0.153, $N = 182$ and $P < 0.05$. *Fecalibacterium* had a coefficient correlation = -0.011, $N = 182$ and a $P = 0.881$ (Table 11). The indicators *Btim*, *HuBac*, *M. smithii*, and *Fecalibacterium* accounted for the interactions of presence/absence and salinity by 23%, 4%, 2%, 0%, respectively. *Btim* was the most significant of these tests with 23% of its prevalence being explained with its inverse relationship to salinity. However, this magnitude of effect indicates that another variable is accounting for the other 77% of its presence in the water system. Salinity remained relatively constant across the different sampling sites. Salinity values were almost never above zero for freshwater sites and ranges between 26 – 33 ppt for NSB and NSM sampling sites.

Table 11

Spearman's Test Results for the Effects of Salinity and Marker Presence

**sig. @ 0.001	Salinity (ppt)	Salinity(ppt)
M. smithii		
	Correlation coefficient	-0.153*
	Sig. (2-tailed)	0.040
	N	182
Bacteroides sp	Correlation coefficient	-0.221**
	Sig. (2-tailed)	0.003
	N	182
B.thetaiotaomicron	Correlation coefficient	-0.447**
	Sig. (2-tailed)	0.000
	N	182
Fecalibacterium	Correlation coefficient	-0.011
	Sig. (2-tailed)	0.881
	N	182

To determine if there was any interaction between the presence/absence of each of the four indicator groups and water temperature (°C) a Spearman's correlation was performed. For *Btim* the correlation coefficient = 0.042, N = 177 and P > 0.5. For *HuBac* the correlation coefficient = -0.45, N = 177 and P > 0.5. For *M. smithii* the correlation coefficient = -0.104, N = 177 and P > 0.1. For *Fecalibacterium* the correlation coefficient = 0.181, N = 177 and P = 0.016. As temperature increased the

presence of *Fecalibacterium* seemed to increase, but not very significantly. These relationships were essentially uninformative for this data set. To determine if there was any effect of temperature on the bacterial counts of EN/100mL and FC/100mL a Spearman's rho was performed. For EN/100mL the correlation coefficient = -0.157, N =177 and P = 0.036. For FC/100mL the correlation coefficient = 0.007, N = 177 and P = 0.928. EN/100mL was weakly and inversely correlated to temperature. A multiple regression was used to test whether or not EN/100mL or FC/100mL could be predicted by the abundance of the four individual markers assayed. For *Btim* (EN/100mL as the dependent variable) the regression yielded a {$F(4,217) = 4.28$, $P = 0.002$}, and a $R^2 = 0.07$. This indicated that only 7% of CFU's from EN/100mL can be explained by the presence/absence of *Btim*. This relationship is not highly significant and, based on its magnitude of effect, is indicative of the *Btim* variable failing as a good indicator of enterococci. Conversely, the multiple regression for FC/100mL as the dependent variable yielded a P = 0.121, df = 4, F = 1.84, and $R^2 = 0.033$. The Anova test yielded no significance indicating that FC/100mL is not being predicted by any of the variables. However, the closest variable to being a predictor was still *Btim* with a P = 0.032. A Spearman's rho was used to ascertain the effect of turbidity on EN/100mL or FC/100mL bacterial counts and all four molecular markers. For EN/100mL there was a low-moderate negative correlation coefficient = -0.305 with N = 154 and P < 0.001. For FC/100mL there was a low negative correlation coefficient = -0.284 with N = 154 and P < 0.001. *M. smithii* had a correlation coefficient = -0.085 with N = 154 and P = 0.293. The presence of *M. smithii* did not have a significant correlation to turbidity. *HuBac* had a correlation coefficient = -0.193 with N = 154 and P = 0.016. The test was significant

but the inverse correlation was low. For *Btim* the correlation coefficient was -0.264 with a N = 154 and P < 0.001. This test yielded a low-moderate correlation to turbidity but had the most significant P- value for the data set. For *Fecalibacterium* the correlation coefficient = -0.103 with N = 154 and P = 0.203. The test was not significant and there was a very low correlation. The same Spearman's rho was run to determine the effects of solar intensity on these six variables. For EN/100mL the correlation coefficient was -0.361 with N = 177 and P < 0.001. This test had a moderate correlation to UV exposure. FC/100mL had a correlation coefficient = -0.385 with N = 177 and P < 0.001. This test had a slightly higher inverse correlation to UV exposure. *M. smithii* had a correlation coefficient = -0.128 with N = 177 and P = 0.089. *HuBac* had a correlation coefficient = -0.013 with N = 177 and P = 0.860. *Btim* had a correlation coefficient = -0.226 with N = 177 and P < 0.003. Btim had the highest inverse correlation and the most significant P-value. *Fecalibacterium* had a correlation coefficient = -0.036 with N = 177 and P = 0.633. To determine whether or not any of the four bacterial indicators were predominately recovered from a particular water type (FWS, NSB, or NSM) a multiple regression analysis was run for each indicator in all water types. For *M. smithii* the Chi-Square = 6.28, with df = 2 and P < 0.05. For the water types (FWS, NSB, and NSM) *M. smithii* was accounted for 36.6%, 22% and 21% respectively. For *HuBac* the Chi-Square = 11.02, with df = 2 and P < 0.005. For the water types (FWS, NSB and NSM) *HuBac* was accounted for 43.8%, 32.8%, and 16.7%, respectively. For *Btim* the Chi-Square = 48.85, with df = 2 and P < 0.001. For the water types (FWS, NSB and NSM) *Btim* accounted for 68%, 22% and 20.8% of the positives, respectively. This accounts for its overall abundance in freshwater creeks. For *Fecalibacterium* the Chi-Square = 1.64, with

df = 2 and P = 0.440. This test yielded no significance and the percent recovery from water types FWS, NSB, and NSM were almost evenly distributed at 33.0%, 29.7%, and 23%, respectively. All of the markers were found more often in the freshwater creek environments, followed by the near-shore brackish (commingling) environment, and marker presence was least abundant in the marine environment. A Pearson correlation was used to ascertain if any of the four human specific markers were correlated with one another (Table 12).

Table 12

Pearson Correlations of Human Specific Markers to each other for Sampling Dates 5/12/2009-11/19/2009

	Correlations	*Btim*	*HuBac*	*Fecali*	*M.smithii*
Btim	Pearson	1	**0.230****	0.061	**0.138***
	Sig.(2tailed)		**0.001**	0.367	**0.039**
	N	224	**224**	224	**224**
HuBac	Pearson	0.230**	1	0.075	0.090
	Sig.(2tailed)	0.001		0.263	0.179
	N	224	224	224	224
Fecali	Pearson	0.061	0.075	1	0.098
	Sig.(2tailed)	0.367	0.263		0.144
	N	224	224	224	224
M.smithii	Pearson	0.138*	0.090	0.098	1
	Sig.(2tailed)	0.039	0.179	0.144	
	N	224	224	224	224

The above Pearson correlation is representative of how well the putatively human specific markers correlated with each other. Btim and HuBac had the most significant correlation but it was still low - moderate.

Indicator bacterial counts and PCR detection of Bacteroides, M. smithii, and B. thetaiotaomicron markers in coastal samples. 4/21/2010-07/22/2010 represented a total

of 9 individual sampling trips. Seventy three percent (86 of 118) of the samples tested positive for at least one of the human markers assayed (Table 10). FWS, NSB, and NSM sample sites represented 17%, 5% and 2% of the total positives respectively. Both CC1 and CC2 contributed 25% of the total positives in the FWS. The effluent of this creek system, site 10, contributed 59% of the total positives found in the NSB. Sample sites AOC and CONDO contributed 10% of the total positives found in the FWS. Their associated effluent (site 11) contributed 24% of the total positives from the NSB. The sample sites 7ACC and 7ACT both contributed 12% of the total positives found in the FWS. The *Btim* marker was present 38 times (32%) followed by HuBac at 25%, and *M. smithii* at 16% (Table 12). It should be noted that during the course of sampling, site 10A had to be omitted several times due to its inaccessibility due to road construction.

Table 13

Samples and the Number of Positives for the Three Human Specific Markers at Each One of the Sampling sites (4/21/2010-07/22/2010)

Table 13	MS +/(%)	BA+/(%)	Btim+/(%)	(+) #/%
Total	**19(16)**	**29(25)**	**38(32)**	**86(73)**
7ACC	2	3	3	8(7)
7ACT	1	1	4	6(5)
7A	1	1	1	3(3)
9	1	3	1	5(4)
CC1	6	4	5	15(13)
CC2	5	5	5	15(13)
10	1	5	4	10(8)
10A	0	0	0	0(0)

Table 13 (Continued).

AOC	0	2	4	6(5)
CON	0	2	4	6(5)
11	1	3	2	6(5)
12A	1	0	1	2(2)

Note. The above table shows discrete patterns emerging from CC1, CC2, AOC, and CON

Enterococci and fecal coliform count data (CFU/100mL) were graphed for all 14 sampling sites (Figure 8). Both enterococci and fecal coliforms were present in higher numbers in the freshwater creek environments.

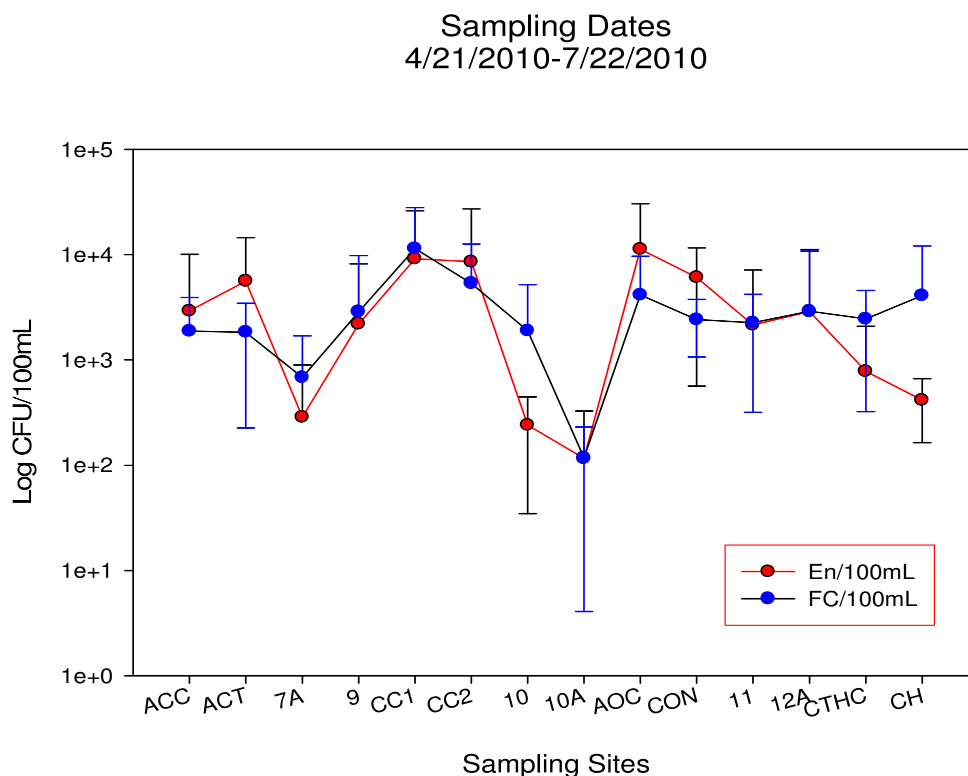

Figure 8. The distribution of enterococci and fecal coliforms (CFU/100mL) for each sampling site (4/2010 – 7/2010).

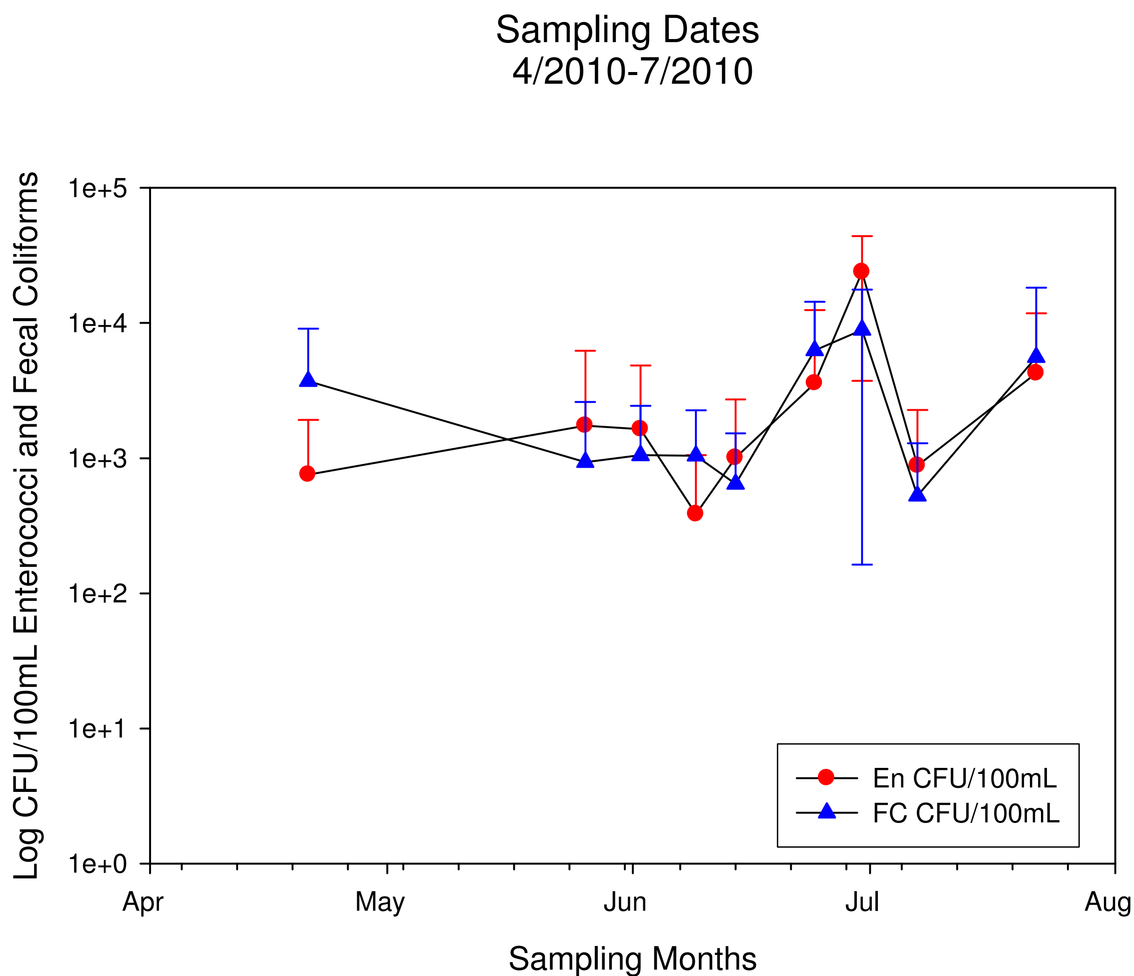

Figure 9. The distribution of enterococci and fecal coliforms (CFU/100mL) for each sampling month.

The overall distribution of each human specific marker *HuBac, M. smithii* and *B. thetaiotaomicron*, was graphed for each site (Figure 10). The freshwater creek systems had the highest number of positives. *B. thetaiotaomicron* was found more abundantly

than any other marker. The Coffee creek system (CC1, CC2, 10) had the highest number of positives and almost proportionate scaling of each individual marker.

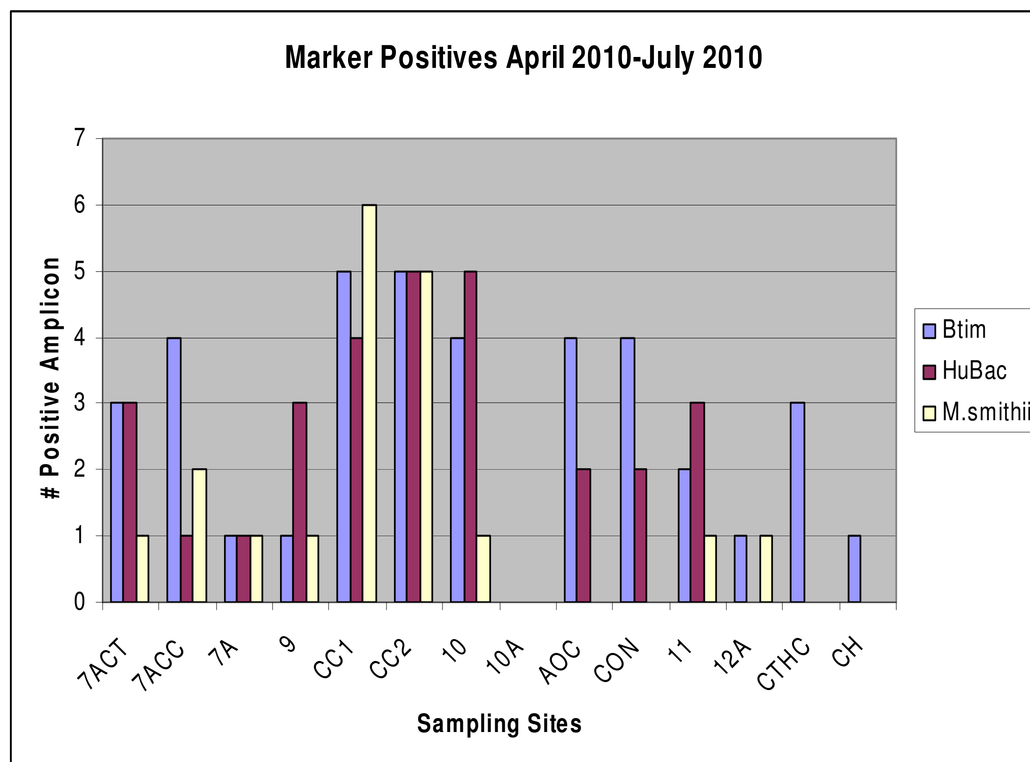

Figure 10. This graph represents total marker presence (4/2010 – 7/2010) for the individual study sites.

Statistical analyses of environmental coastal samples 4/21/2010-07/22/2010. These data points were delineated in the same manner as above, correlating to their respective water types. A one-way Anova was run for the two bacterial variables En/100mL and FC/100mL for the three water types of FWS, NSB, and NSM. The lack of normality found within these data sets mandated the use of additional non-parametric testing procedures. A Kruskal-Wallis test was run to determine if the En/100mL and FC/100mL bacterial counts differed between the three water types. N = 57, 32, and 24 for the three water types FWS, NSB, and NSM respectively. For En/100mL the Chi-Square = 39.893, df = 2, and $P < 0.001$. For FC/100mL the Chi-Square = 16.906, df = 2,

and P < 0.001. N = 53, 31, and 24 for the three water types FWS, NSB, and NSM respectively. The three water groups were ranked in order of their recovery rate for each bacterial variable En/100mL and FC/100mL. For En/100mL sampling sites were ranked in order of FWS>NSB>NSM. For FC/100mL the sampling sites were ranked in order of FWS, NSB>NSM. To determine if there was any correlation between the water types and the two bacterial variables a Mann-Whitney test was run grouping the water type variables by two. For water types FWS and NSM there was a significant difference between EN/100mL and FC/100mL with a P < 0.001 for both. For water types FWS and NSB there was a significant difference between the En/100mL bacterial counts, P < 0.001, but there was no significant difference for the FC/100mL bacterial counts with a P = 0.086. For water types NSM and NSB there was a significant difference between both the En/100mL and FC/100mL bacterial counts with a P < 0.005 and P < 0.007 respectively. To determine if there was any correlation between En/100mL and FC/100mL bacterial counts, i.e., if one increase does the other increase, among water type (sampling area) a Spearman's rho was run. For water type FWS there was a moderate correlation with a coefficient = 0.410, N = 53, and P < 0.003. The water type NSM had a moderate correlation with a coefficient = 0.431, N = 24 and P < 0.04. The water type NSB had a slightly higher moderate correlation coefficient of 0.571, N = 31, and P < 0.002. This indicated that the highest correlation is occurring at the commingling of fresh and salt water. To determine if there was any correlation between bacterial counts of EN/100mL and FC/100mL and salinity (ppt) a Spearman's rho was performed. For EN/100mL the correlation coefficient = -0.558, N = 96 and P = 0.00. For FC/100mL the correlation coefficient = -0.391, N = 91 and P < 0.01. Both tests were significant at P

< 0.01 level with each bacterial group showing an inverse relationship to salinity but with EN/100mL being more greatly impacted. To test whether the abundance of each of the three human specific markers could be predicted by salinity (ppt) a Spearman's rho was performed. *Btim* had a correlation coefficient = -0.254, N = 98 and P = 0.012. *HuBac* had a correlation coefficient = -0.137, N = 98 and P = 0.178. *M. smithii* had a correlation coefficient = -0.218, N = 98 and P = 0.031. The indicators *Btim*, *HuBac*, and *M. smithii* accounted for the interactions of presence/absence and salinity by 6.45%, 1.9%, and 4.75% respectively. *Btim* had the most significant inverse relationship but it was very small. To determine if there was any interaction between the presence/absence of each of the four indicator groups and water temperature (°C) a Spearman's correlation was performed. For *Btim* the correlation coefficient = -0.206, N = 112 and P < 0.04. For *HuBac* the correlation coefficient = -0.109, N = 112 and P = 0.235. For *M. smithii* the correlation coefficient = -0.123, N = 112 and P < 0.2. These tests were essentially uninformative and only elucidated small inverse correlations to temperature. To determine if there was any effect of temperature on the bacterial counts of EN/100mL and FC/100mL a Spearman's rho was performed. For EN/100mL the correlation coefficient = -0.258, N =109 and P < 0.008. For FC/100mL the correlation coefficient = -0.236, N = 104 and P < 0.02. Both EN/100mL and FC/100mL were only moderately and inversely correlated to temperature, with EN/100ml being slightly more correlated. A Spearman's rho was used to ascertain the effect of turbidity on EN/100mL or FC/100mL bacterial counts and all three molecular markers. For EN/100mL there was a negative correlation coefficient = -0.416 with N = 91 and P < 0.001. For FC/100mL there was a negative correlation coefficient = -0.688 with N = 91 and P < 0.001. *M.*

smithii had a correlation coefficient = -0.166 with N = 91 and P = 0.116. The presence of *M. smithii* did not have a significant correlation to turbidity. HuBac had a correlation coefficient = -0.045 with N = 91 and P = 0.671. For *Btim* the correlation coefficient was -0.160 with an N = 91 and P = 0.130. A multiple regression was used to test whether or not EN/100mL or FC/100mL could be predicted by the abundance of the four individual markers assayed. For *Btim* (EN/100mL as the dependent variable) the regression yielded a P = 0.119 with df = 2, F = 2.17, and a $R^2 = 0.038$. This indicated that 0.14% of CFU's from EN/100mL could be explained by the presence/absence of *Btim*. This relationship was not significant and indicated that the *Btim* variable failed as a good indicator of enterococci. Conversely, the multiple regression for FC/100mL as the dependent variable yielded a P = 0.006, df = 3, F = 4.402, and $R^2 = 0.113$ The Anova test was significant and indicated that 1.27% of CFU's from FC/100mL was predicted by *Btim*. The variable *Btim*, despite its low magnitude of effect, still had the highest correlation to FC/100mL. To determine whether or not any of the four bacterial indicators are predominately recovered from a particular water type (FWS, NSB, or NSM) a multiple regression analysis was run for each indicator in all water types. For *M. smithii* the Chi-Square = 4.74, df = 2, N = 118, and P < 0.05. For the water types (FWS, NSB, and NSM) the recovery rate was 23.3%, 9.1%, and 8.0% respectively. For *HuBac* the Chi-Square = 2.71, df = 2, N = 118, and P = 0.257. For the water types (FWS, NSB, and NSM) the recovery rates were 28.3%, 27.3%, and 12.0%, respectively. For *Btim* the Chi-Square = 12.3, df = 2, N= 118, and a P < 0.003. This test was significant for predicting the presence of *Btim* based on water type. For the individual water types (FWS, NSB, and NSM) the recovery rates were 45.0%, 24.2%, and 8.0%, respectively. Again, all of

the markers were recovered most often from the fresh water creek environment, followed by the near-shore brackish (commingling) environment, and was least abundant in the marine environment. It is of interest to note that *Btim* was recovered at a much higher percentage in the freshwater environment and that all of the recovery rates are indicative of the creek systems being a dominate source of human fecal pollution. A Pearson correlation was run to determine if any of the human specific markers correlated with each other (Table 14).

Table 14

Pearson Correlations of M. smithii, human *Bacteroides*, and *Bacteroides thetaiotaomicron*

	Correlations	*Btim*	*HuBac*	*M.smithii*
Btim	Pearson	1	**0.251****	**0.201***
	Sig.(2-tailed)		**0.006**	**0.029**
	N	118	**118**	**118**
HuBac	Pearson	0.251**	1	**0.178**
	Sig.(2-tailed)	0.006		**0.053**
	N	118	118	**118**
M.smithii	Pearson	0.201*	0.178	1
	Sig.(2-tailed)	0.029	0.053	
	N	118	118	118

** Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).

Btim and HuBac had the most significant correlation with a low – moderate magnitude of effect. Btim and M.smithii exhibited an interaction within a less stringent confidence interval, but the interaction is still confined to the parameters of biologically uninformative magnitude of effect.

CHAPTER V

DISCUSSION

For the present, enterococcal measurements are the standard measure of human risk from contact with enteric pathogens in coastal waters; however, recent studies have indicated that there are many factors that mitigate the value of these analyses. For example, enterococci are known to exist in a variety of animals and on plants, and to reproduce in the coastal environment (Signoretto et al., 2005). Furthermore, sediments and beach sand have been shown to harbor enterococci and allow them to persist in the environment (Scott et al., 2002; Hartz et al., 2008). In partial response to the problems experienced by regulators that utilize the enterococcal standard, researchers developed human and animal markers to identify sources of coastal pollution and allow remediation efforts to occur. The question is: In natural samples, are enterococci a reliable indicator of human fecal pollution, and do human markers correlate well with the levels of enterococci observed in coastal samples? For this geographical area, the answer is no.

This research project represented three distinct data sets collected over a period of three years. The delineations between data represent slightly different experimental designs and questions asked. The augmentations follow logical progressions from subsequently collected data. The sampling locations did not change during the course of these investigations except for the addition of sites CTH and CH in the last two years of the study. Because of the damage caused by hurricane Katrina (2005) there were times early in the study when some of the sites were inaccessible.

Environmental Coastal Samples 8/2007 – 4/2009. During this study, *Enterococcus* counts at 12 coastal sampling sites were not positively correlated.

Unquestionably, creek waters contain substantial enterococcal levels and frequently showed the presence of the human markers; however, these measurements did not statistically translate into associated beach water counts of enterococci or the presence of the human markers. During the same period from August 2008 to April 2009, there were 131 enterococcal exceedances (Mississippi uses a single sample count of $\geq$104/100mL to designate a polluted beach) associated with the six coastal sites tested. Forty eight exceedances occurred at station 10, followed by 26 at site 10A, 22 at site 9, 17 at site 11, 13 at site 12A and 5 at site 7A. These data imply that a statistical correlation should occur at site 10 which is influenced by sampling sites CC1 and CC2, but it did not exist. Therefore it must indicate that other factors are at play to create this disparity. Differences do exist between the creek and the beach environments including such variables as fresh vs. salt water, the levels of ultraviolet light exposure, the dilution effect as creek water enters the estuary, and tidal transport at beach sites, as well as differences in turbidity and sediment disturbance. All or a portion of these factors could account for the lack of correlations observed (Ufnar et al., 2007).

Similarly, there was a significant difference between EN and FC counts and the presence or absence of the BA or MS marker in either the creek or coastal samples. This is not unexpected since one measurement is a quantifiable bacterial count (continuous-interval) and MS and BA are measures of presence or absence (categorically nominal) and represent other microbial genera.

The cross-tabulations indicated that a higher percentage of marker agreement was recorded when neither of the markers were present in a sample. In fact, the BA and MS markers agreed more frequently than they disagreed. Differences between capillary

electrophoresis and gel electrophoresis were negligible when neither of the markers was present, but agreement between the methods was higher (80%) when the BA marker was analyzed. The MultiNA and the gel procedure were in agreement when the two markers showed different results (61%). In general, these results demonstrate that either marker can be used to evaluate the presence of human coastal water pollution and that either method can be used to generate the results. The advantage of the automated MultiNA method was its sensitivity to small concentrations of DNA in a sample and its ability to evaluate a large number of samples in a short time period. Further, gel staining is not required, avoiding the use of ethidium bromide. The digital gel picture which the instrument presented was a very high resolution image; typically, bands appeared during a MultiNA analysis where none could be seen on an agarose gel. The capillary electrophoresis method has the added advantage of presenting data on the base pair units for each band and the amount (ng/ul) of each DNA fragment in the sample. The instrument requires careful management during the analysis of environmental samples and chip cleaning is often necessary and time consuming. However, if the objective of analysis is to process numerous samples in an abbreviated timeframe, requiring minimal operator attention and inexpensive results, the capillary electrophoresis method would be an appropriate technology.

Despite the fact that these data were part of a local sample population, the conclusion that EN levels did not correlate from sampling site to sampling site nor was their correlation with the levels of two human markers is troubling. Marker analysis has been persistently studied by a variety of international researchers for at least a decade and was considered a complement to enterococcal analysis. However, the random nature of

the isolation of both the BA and the MS markers points to the fact that marker presence can be influenced by such factors as dilution, the salt water environment, tidal movements, the presence of sediment in the water column, resiliency to degradation, or other coastal features. This randomness suggests that the analysis of human markers and their relationship to the variable EN count cannot be used to identify and control pollution on coastal beaches.

In the future, a substitute for the measurement of indicator bacterial levels in coastal waters may be a dependable detection of specific microbial pathogens. Several viral pathogens are currently able to be detected by qPCR (McQuaig et al., 2006) and other bacterial and protozoal pathogens can be detected with molecular methods. For the time being, the use of the enterococcal count or the qPRC analysis of the level of this organism in coastal waters will continue, almost certainly in concert with data on one or more of the human markers.

Although enterococcal measurements are the current measure of human risk from contact with enteric pathogens in coastal waters, recent studies have indicated that there are factors that mitigate the value of these analyses. For example, enterococci are known to exist in many animal species, and to reproduce in the coastal environment. Furthermore, sediments and beach sand have been shown to harbor enterococci and allow them to persist in the environment.

Environmental Coastal Samples 5/2009 – 11/2009. During this study there were 14 coastal sampling sites analyzed for the presence of 4 human specific markers as well as enterococci and fecal coliforms. Of these, positive correlations were found between human specific *Bacteroides* and enterococci, *B. thetaiotaomicron* and fecal coliforms,

and *Fecalibacterium* and enterococci. *B. thetaiotaomicron* was found to be positive most often at 32% of the time. Of all the positives for all four organisms, 15% percent of those were found in the creek CC2, followed by 12% at CC1. This creek system is indicative of an area with a large number of anthropogenic inputs. It is also an area that is in close proximity of a sewage lift station. Except for 7A, which receives its effluent from another highly polluted creek system (7ACC & 7ACT), the marine sampling sites had a much lower percentage of positives (<9%) for the 4 organisms that were assayed. From all the statistical analyses performed there does seem to be correlations between 3 of the organisms and standard indicator bacteria. Further analyses were needed to further elucidate this possible relationship.

All sample sites and sub-groupings are the same as designated in the above results section for 5/12/2009-11/19/2009. Because the collected data violated the rules for normality, non-parametric tests were used. The Kruskal-Wallis ranking of the three water types, FWS>NSB>NSM and FWS>NSB≥NSM for EN/100mL and FC/100mL, respectively, was geographically intuitive; the probable abundance of each organism coincided with natural hydrological influences.

If sites were to be significantly different based on indicator organisms measured, this difference, as shown in the results, would be directly tied to presumed bacterial input (source), differences in survival in fresh and marine waters, or dilution factors, based on location. The exact differences from the Mann-Whitney test of indicator bacteria measured from each water type further supported this supposition. This test grouped the water types by two and tested for significant differences between En/100mL and FC/100mL.

The recovery of these two bacterial indicators differed significantly for the paired grouping of water types except for NSM and NSB where there was no significant difference for FC/100mL. For En/100mL the relationship again indicated that the major enterococci source of input originated from the freshwater creeks and directly influenced the counts at the terminal effluent sampling points in the NSB. Although it cannot be directly proven from this experimental design, the assumption is that the dilution factor of reaching the marine environment was the cause of the NSM variable being ranked lower. It is possible that enterococci are being harbored in sand and eventually re-suspended into the water column by tidal and wave action. With respect to tidally influenced systems and recreational water quality standards it has been documented that enterococci can be found in higher numbers and can actually multiply in the subsurface sediment and vegetation in the absence of fecal contamination (Desmarais et al., 2002). Because it was not directly measured, it is unclear if the large data set and robustness of the statistics were powerful enough to dwarf this conflicting variable. Further research is needed to quantify how extreme variations in uv exposure could affect enterococci survival. The ranking of the three water types based on FC/100mL yielded a slightly different response; unquestionably, the major source input was the freshwater creeks. However, the NSB environment was only ranked slightly higher than the NSM. One explanation for this is that the fecal coliforms do not share the exact same fate between these environments. The results of the Spearman's rho indicated that the highest correlation between En/100mL and FC/100mL was found in the commingling environment of NSB, indicating that as one increased so did the other. Within a geographical context this sampling point represents a confluence of all possible bacterial loads, point and non-point

sources, and complicating environmental variables. Proximity to possible fecal pollution inputs and variable temperature, turbidity, uv exposure, bacterial re-suspension, and dilution factors were all normalized at this point. The correlation of these two bacterial indicators could be explained by this global aggregation and mixing of variables. It is interesting to note, however, that the lowest correlation was obtained from the freshwater environment. This could be attributed to the differential input or survivability of these two bacterial indicators in this environmental sampling area. Salinity, turbidity, temperature, and UV exposure were the environmental variables applied to the statistical tests. En/100mL and FC/100mL were both inversely correlated with salinity. The effect of bacterial counts decreasing as salinity values increased could be attributed to their inabilities to mitigate the effects of osmotic pressures. In addition, increases in salinity for these sampling areas were also directly tied to increases in UV exposure and dilutions of nutrient availabilities and bacterial indicator communities in the water column. In an attempt to predict the distribution of the four proposed human specific markers based on salinity, a Spearman's test was run. The marker distribution was ranked by highest negative correlation and significance values in order of *B. thetaiotaomicron*, human *Bacteroides*, *M. smithii*, and *Fecalibacterium*. This further supported the data that as salinity increased in ppt, bacterial markers were less abundant. *B. thetaiotaomicron* did have the most significant correlation to salinity at 23%, but this physical variable could not account for the other 77% affecting the organism's presence or absence. The effects of temperature on marker presence showed *B. thetaiotaomicron* and *Fecalibacterium* were both positively correlated. *M. smithii* and human *Bacteroides* were both negatively correlated. As temperature increased at the study site, *Fecalibacterium* abundance

increased slightly but not significantly. The correlation for all the human markers was minimal and yielded essentially uninformative results. The relationship of En/100mL and FC/100mL was weak. The most significant relationship was from the En/100mL data set and it was inversely correlated. This weak correlation could be a result of the precision of the measurements or an artifact of the data and not a true correlation. These results were not shocking as temperature variations remained somewhat consistent during the sampling months. Multiple regression analyses, used to predict the presence of these markers based on current bacterial indicators (En/100mL and FC/100mL), yielded conflicting results. Based on the coefficient of non-determination ($1-R^2$); $R^2 = 0.07$ {$F(4,217) = 4.28$, $P = 0.002$}, 7% of CFU's for En/100mL can be predicted by the presence of *B. thetaiotaomicron*. However, there was no significant correlation for FC/100ml, indicating that none of the proposed human specific markers was predicting its presence in any of the coastal environments. It should be noted that if any of the markers were chosen it would have to *B. thetaiotaomicron* based on its Anova p-Value = 0.032. A Spearman's correlation was run to test the influence of turbidity on En/100mL, FC/100mL, and all four human specific markers. *M. smithii* and *Fecalibacterium* were the only organisms that did not have a significant correlation to turbidity. All other bacterial groups had a significantly low inverse correlation with turbidity, with *B. thetaiotaomicron* having a low-moderate correlation and En/100mL having a moderate correlation. Even though these correlations are statistically relevant, the calculations were performed in spite of very apparent disrupting outliers. Removing these outliers from the equation yielded essentially a moot turbidity affect. This was surprising considering that a more turbid environment would have provided UV shielding and

possibly more abundant nutrient sources. This event could have been related to the unmeasured variable of bacterial attachment and sedimentation. The effects of UV exposure on the bacteria assayed were also variable. Both En/100mL and FC/100mL had a moderate negative correlation to UV exposure, with FC/100mL being slightly higher. All four human specific markers had a negative correlation to UV exposure but the *B. thetaiotaomicron* marker was the only one to be significant and had the highest correlation. Considering that the UV measurements were collected in the field and were not continuously recorded on a data logger, the variability could be considered a grab sample of the total penetrating radiation for the sample site. A multiple regression analysis was used to determine if any of the human specific markers were recovered predominately from any one water source and a clear pattern emerged as the markers were recovered in greatest numbers in order of FWS, NSB, and NSM. *B. thetaiotaomicron* had the highest recovery rate and was directly correlated with the freshwater environment. This pattern was consistent with the rank order of the recovery rates for enterococci and fecal coliforms from different water types. From these data it was concluded that the human specific marker of *B. thetaiotaomicron* performed best for describing areas that seemed contaminated with fecal pollution. Of the environmental variables tested salinity emerged as the most robust factor influencing the presence or absence of either the bacterial indicators (En/100mL and FC/100mL) or the human specific markers. The primary source of these bacteria was the freshwater creeks that spill into the sound (Flood et al., 2011).

Environmental Coastal Samples 4/2010–7/2010. The data followed the same sub-groupings as stated above. Bacterial counts for this portion of the study followed a

pattern similar to the above section. Bacterial recovery rates for EN/100mL and FC/100mL were significantly different among the sampling areas and were rank ordered by FWS, NSB, and NSM. For the two bacterial indicator variables there was a difference in recovery rates between freshwater and marine sample sites. The enterococci were recovered in significantly different values between the NSB and FWS sites but fecal coliforms were not. Among the three water sources the highest correlation between these two bacterial indicators was found in the NSB environment. This mirrors the sentiment of this sampling area being a terminal site for all converging variables. Both En/100mL and FC/100mL were inversely impacted by increases in salinity with enterococci having the highest correlation. Between the human specific markers, *B. thetaiotaomicron* showed the highest inverse relationship and the most sensitivity to increases in salinity and temperature. En/100mL had a very high inverse correlation to salinity and a low to moderate correlation to temperature. FC/100mL yielded a moderate inverse relationship to both salinity and temperature. Both bacterial indicators had strong inverse relationships to turbidity when data outliers were calculated and graphed. The outliers were calculated due to insufficient reasons to remove them, i.e., there were no transcription errors from written to digital data sets. When these outlier data points were removed from the calculations the relationships became moot. This relationship has proven to be enigmatic when viewed in a purely biologically relevant context and probably needs further testing to draw any real concrete conclusions. *B. thetaiotaomicron* was significantly associated with En/100mL and FC/100mL bacterial counts but, based on their magnitude of effect, still failed as a good predictor for these variables.

Again, all three human specific markers were recovered more frequently from the freshwater environment. This is further supported by the ranking of recovery rates for the bacterial indicators, the ranking of recovery rates for each marker, and the inverse correlations for each marker with salinity. It is still unclear if the inverse correlations with salinity were due to the organism's ability to mitigate changes in osmotic pressure or if salinity is acting as a proxy for dilution within the sampling area. Salinity was almost never above zero for the FWS sites and remained consistent at 26-33ppt for the NSM and NSB sites. A multiple regression showed that *B. thetaiotaomicron*, the most prevalent marker, was recovered at a significant rate from the FWS, indicating that the creek systems are responsible for the majority of human fecal input into the study sites. The order of marker recovery (FWS>NSB>NSM) followed the same ranking order of the recovery rates for EN/100mL and FC/100mL. These analyses clearly supported the data indicated by the prior two studies. There was a prominent spatial trend for the presence of both the bacterial indicators and the human specific markers, thus, the geographical structure of a study site could be a valuable model parameter when trying to ascertain direct sources of input, probability of host source input, and proper sampling/remediation strategies.

For the three environmental studies described above (8/2007-7/2010) the results are consistent. The probability of recovering either a high bacterial count (CFU/100mL) or a human specific fecal marker can be directly tied to the sampling location and its respective water type. The recovery rate of these biological variables does not appear to be dependent on the presence or absence of one another. Recent MST research supports the opinion that using one bacterial genus to describe the probability of another or the

presence of pathogens is flawed. Bacterial communities are in constant flux in the environment; a flux that is directly tied to their host origin, spatial and temporal moments, ability to mitigate detrimental abiotic factors, nutrient requirements, selective predation, and genetic heterogeneity. Our understanding of how these bacteria are able to meliorate environmental stressors (UV damage, osmotic pressures, and temperature) is expanding with studies similar to this one. Carotenoid pigmentation may mitigate the effects of photo-damage by Reactive Oxygen Species (Maraccini et al., 2011). Regression analyses for human specific markers of fecal pollution and indicator bacteria from environmental samples have demonstrated that relationships can be significant, but have low correlations, for example, human specific *Bacteroides* marker HF183 were present at low concentrations of indicators (Bonkosky et al., 2009).

Gram (+) (EN) and Gram (–) (FC) bacteria respond differently to predation, osmotic pressure, and photo-inactivation (Solecki et al., 2011). The differential survival characteristics of both indicator bacteria and human specific markers do much to confound a researcher's ability to extrapolate valuable data for hypothesis testing. Under the most utopian settings, with variables scaling at rates which reflect their biological characteristics, choosing the wrong metric for data analysis can further distort experimental results. This is especially true for library-dependent mechanisms of MST. PFGE analysis of *E. coli* isolates revealed a 27% inconsistency between discriminate analysis and jackknife classification matrices (Lasalde et al., 2005). All of these weaken the efficacy of using a single indicator species collected from a single grab sample to ascertain the probability of detecting a possible pathogen in environmental waters.

Summary

This research encompassed 67 sampling trips and the collection and processing of 819 samples. It was our intention to test the efficacy of using published human specific markers to identify areas of fecal pollution along the Mississippi Gulf Coast. And, we endeavored to elucidate relationships of these markers with the current bacterial indicators of water quality and the physical variables that may have affected their presence or absence.

The experimental constructs described indicate, in our view, a logical progression of design and analyses, a progression that went from casting a wide net over many seasons to examining one swimming season with a temporally compressed multi-tiered approach. It is of particular interest that all of these studies resonated the same themes; the current standards for measuring bacterial water quality are failing and the major contributors of fecal bacteria were that of freshwater sources. This research, and that of others, found the same statistical discrepancies between correlations of standard indicator bacteria (enterococci and fecal coliforms) and human specific molecular markers in the environment.

Undeniably, the coastal creek systems tested during this study demonstrated a strong influence of fresh water effluents on the presence or absence of these human specific markers. In addition, there appears to be other variables influencing the ability to assay for the presence of each marker in the marine setting. The most simplistic explanation would simply be a dilution effect on these markers when they reach the marine environment. This research indicated that salinity and temperature were the two main variables influencing both bacterial counts and marker presence. Another

explanation could be differential survivorship of these markers/organisms in the natural environment. This concept is currently being vetted within the source tracking community.

Future projects will include performing multivariate analyses incorporating other environmental variables (rainfall, wind direction, tidal/wind action, salinity gradients, and solar exposure) measured at each sampling site in a more temporally and spatially compressed manner. Future investigations aimed at determining the relative differences in transport and fate of each of the indicator organisms, as well as the compliment of human markers, in both the marine and freshwater environments should shed new light on the value of microbial source tracking and its use in the marine environment.

Conclusions

2007-2009

- No correlation was found between enterococcal or fecal coliform counts and the presence or absence of two human markers at 12 coastal sampling sites.
- A higher occurrence of human markers was found in creek samples as compared to coastal waters.
- Enterococcal counts at coastal stations did not statistically correlate with counts at other coastal sampling stations, nor did the enterococcal counts at coastal stations correlate with enterococcal counts at creek sampling sites.
- The presence of the human markers in the freshwater creeks appears to indicate that they were a source of pollution for the coastal environment.
- The MultiNA method of DNA analysis is favored when many samples are to be analyzed in a short time period or when a sample has a very low

concentrations of a target amplicon; the method is not favored when low cost and ease of use are significant priorities.

05/12/2009-11/19/2009

- The freshwater creek systems contributed the highest number of positive markers as well as the highest number of En/100mL and FC/100mL bacterial counts.
- Coffee Creek and its associated effluent site was the most polluted.
- Enteroccoci were inversely correlated to salinity and temperature but was more significantly impacted by salinity.
- Of the four markers *B. thetaiotaomicron* was most affected by salinity and ultraviolet radiation.
- Enterococci and fecal coliforms were both inversely correlated to UV.
- Bacterial recovery significantly differed between sampling environments and were ranked in order of FWS > NSB > NSM.
- Human specific markers were ranked in order of recovery as FWS > NSB > NSM.
- The human specific makers and indicator bacteria did not correlate well enough to be considered good predictors of each other.

04/21/2010-07/22/2010

- Freshwater creek systems were the major contributors of enterococci, fecal coliforms, and the three human specific markers tested.
- Coffee creek was the main source of fecal pollution along the study area.

- *B. thetaiotamicron* was the most affected by environmental variables and was significantly, but not highly correlated, with enterococci and fecal coliforms

Overall conclusions

- For this study area, the freshwater creek systems are contributing to the indicator bacteria input of the Mississippi Gulf coast.
- The presence of any of the four markers discussed above and the bacterial counts (En/100mL and FC/100mL) do not statistically correlate well with each other.
- The abiotic variables that most affected the bacterial groups were salinity, UV, and temperature.
- Among the human specific markers, *B. thetaiotamicron* emerged as the best candidate for predicting the presence of human fecal pollution.
- This study utilized a multi-organism approach to ascertain bacterial water quality. The current standards need revision for determining the relationship of indicator bacteria and the probability of coming into contact with a disease causing pathogens.
- When examined in the appropriate geographical and biological context, human specific markers can valuable tools for defining an area under constant exposure to fecal pollution.
- The lack of correlation or low magnitude of effect between enterococcal counts and the presence of human specific fecal markers reported here is for coastal waters along the Northern Central Gulf of Mexico where the water is

generally warm and rich in organic material. However, additional research in other types of habitats and geographic areas in both the United States and in other countries is needed to develop a more comprehensive understanding of the types of environments in which correlation between these two assays of environmental water quality can be expected.

APPENDIX A

REVISED MULTINA OPERATIONAL PROTOCOLS AND SUPLEMENTAL GRAPHS

MultiNa Analyses: During the course of the first study (August 2007 – April 2009) the secondary component of testing the efficacy of using gel electrophoresis and/or capillary electrophoresis was scrutinized thoroughly. The results of these analyses only highlight a fraction of the total data output used to normalize MultiNA standard operating procedures. The steps outlined in the methods section for capillary gel electrophoresis were augmented from the manufacturer's instruction. There were several steps that were omitted. The instrument was extremely sensitive to salt concentrations and high PCR product concentrations. The salt concentrations remained fairly consistent between sample sets and became less of an issue throughout the course of the experiments. In addition to particular inconsistencies with the hardware of the platform, we experienced significant problems with the kits supplied by the manufacturer. Migration solutions had to be checked repeatedly for precipitation, even with newly ordered kits. The smallest amount of precipitation immediately caused high voltage errors and caused the cessation of the assay. These issues, especially since they were difficult to diagnose visually, often led to chip failure due to clogging. Complete chip clogging and high voltage errors were the two prominent issues associated with the machine. These failures inadvertently led to many replicates of the original assays and, as such, increased our confidence in assay reproducibility. Through many months of trial and error we reached several conclusions of why exactly these issues were arising, as well what steps could be taken to mitigate the

problem. Below is a brief description of protocol changes that allowed us to continue using this machine.

Before proceeding with the analysis of any sample we developed the habit of visually inspecting both the kit components and micro-capillary chips. The electrode surfaces of the chip “docks” were also inspected. Even though it was not adequately highlighted in the service manual, these electrode surfaces can lead to chip failures. It was concluded that these surfaces should be cleaned at the same time as the chips. Any dust or residual fluids left on these surfaces greatly affect chip performance. The chips were cleaned thoroughly before every experiment. We completely disregarded the manufacture’s protocol and developed our own. We found that the inherent design flaw of the chips, the 90° geometry of the capillary migration, served to concentrate both ambient dust and lint particles. This aggregation of foreign particles reduced the efficiency of the assays and eventually led to complete chip failure. To offset this problem, the chip surfaces were immediately washed after each assay, 3mL of molecular grade water was used to flush out the capillaries, and the surfaces were NOT dried using the lint free paper supplied by the manufacturer. The chips were allowed to dry under ambient temperatures in the enclosed environment of their respective storage cases. Once foreign particles are allowed to adhere to the surface walls of the capillaries there is almost no full recovery of the chip. This was prevented by the above chip cleaning procedure and proper storage procedures. Chips were not stored in the machine or left in their docks with the machine under power. If the chips remained under charge for any length of time they exhibit excessive clogging potentials, likely due to the adherence of foreign particles to the constantly drying inner capillary walls while under electrical

current. The Shimadzu corporation refused to divulge what polymer coated the interior of the chip walls, preventing us from fully understanding these electrochemical relationships. It also prevented us from designing any additional light solvent-based chip cleaning solution

The preparation of the kit components, especially that of the migration buffer solutions, involved absolutely no vortexing. This was in disagreement with the manual but was later adopted by the company. Vortexing caused micro-cavitations to form within solution which completely skewed the analyses. This was a rudimentary but extremely time consuming aspect of sample preparation.

All software updates were performed on site with the aid of a Shimadzu technician. It was noticed that these updates could augment the internal coordinate references for the plunger. If the Z-value (plunger depth) was not accurate fluids would leak onto the chip surface resulting in a high voltage chip failure. Plunger depth had to be visually inspected with a plastic depth gauge. This was the only way to accurately adjust the plunger Z-value.

Analyses of sample products were complicated because of the above issues. Once these issues were solved and sample analysis was allowed to proceed uninterrupted, the machine became a useful platform. Several experiments were run to validate the fidelity of sample analyses with regards to contamination between wells. It was concluded that this machine did an exceptional job preventing cross-contamination between samples. There was not a single contamination event among thousands of individual assays.

The accuracy of each sample output from this platform was dependent upon all of the above criteria. If there was a system malfunction all of the above issues were

addressed and rectified. This was an extremely time consuming and arduous process. It was complicated even more by the fact that if there was an issue the PCR product for each individual sample had to be reproduced. After the system was optimized and reproducible it became a valuable addition to our research. The most promising aspect of this platform was the accuracy of the base pair readouts. The manual allows for an error rate of 5% for the any PCR product ≤ 500bp and 10% for any product ≤ 1000bp. This relationship was experimentally proven to be inversely correlated to the concentration of the PCR amplicon. The range at which this machine was able to identify PCR products was between 0.10ng/µl – 50.00ng/µl. Samples at the upper end of this spectrum were often much less accurate and caused unreliable peak migrations. PCR products ≤ 20.0 ng/µl were extremely accurate and exhibited less than 1% error rate, if any.

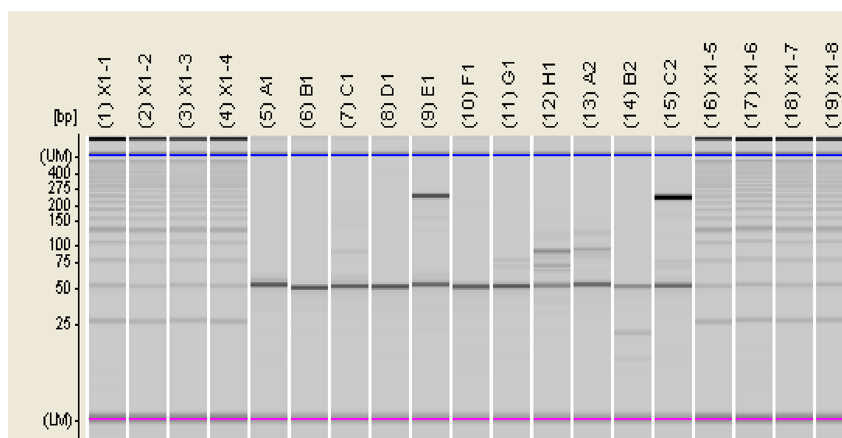

Figure 11. A representation of the digital gel image produced on the MultiNA Electrophoresis system. In this image there is a clear positive in lane E1 9 (sample CC2).

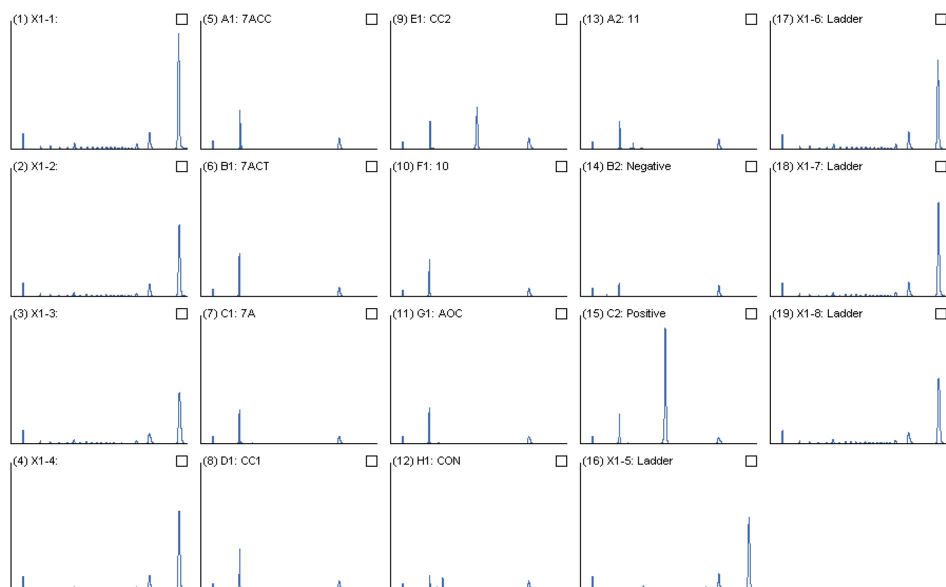

Figure 12. A multipeak display of all the electropherogram results corresponding to the above gel image. Of particular interest are the lanes E1, B2, and C2 which represent sample CC2, Negative control, and positive control respectively.

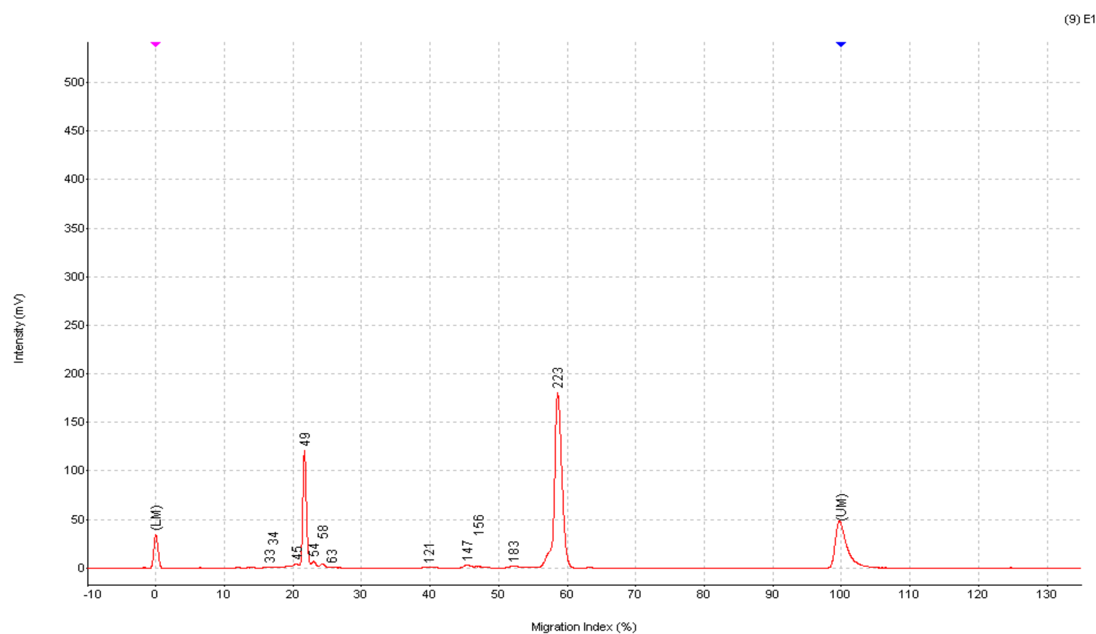

Figure 13. The above electropherogram peak display represents the single peak result of sample CC2 from figures 11 and 12. Notice the accuracy of the bp reading at 223bp when the expected was 222bp.

APPENDIX B

RAW DATA COLLECTED FOR EACH SAMPLING SITE

MS	7ACT	7ACC	7A	9	CC1	CC2
8/21/2007	0	0	1	0	0	Nd
8/28/2007	0	0	0	0	0	Nd
9/3/2007	0	0	0	0	0	Nd
9/10/2007	0	0	0	0	0	Nd
9/17/2007	0	0	0	0	0	Nd
9/24/2007	0	0	0	0	0	Nd
10/1/2007	0	0	0	0	0	Nd
10/8/2007	0	1	1	0	1	0
10/15/2007	0	0	0	0	1	1
10/22/2007	0	0	0	0	1	0
10/29/2007	0	0	0	0	1	0
11/5/2007	0	0	0	0	1	1
11/12/2007	1	0	1	0	1	1
11/26/2007	0	1	0	0	1	1
12/1/2007	1	0	0	1	0	1
1/7/2008	0	0	0	0	1	1
1/14/2008	1	1	1	1	1	1
1/28/2008	1	0	0	0	0	1
2/15/2008	0	0	0	0	0	1
3/17/2008	0	0	0	0	1	1
3/31/2008	0	0	0	0	1	1
4/28/2008	0	0	0	0	1	1
5/19/2008	0	0	0	0	1	1
5/27/2008	1	0	0	1	1	1
6/2/2008	0	0	0	0	0	0
6/23/2008	0	0	0	0	1	1
7/8/2008	0	0	0	0	0	0
7/14/2008	0	0	0	0	0	0
7/22/2008	0	0	0	0	0	0
8/12/2008	0	0	0	0	1	0
8/19/2008	0	0	0	0	0	0
9/8/2008	1	0	1	1	1	0
9/29/2008	0	1	0	0	1	0
10/20/2008	0	0	1	0	1	0
11/3/2008	0	1	0	0	1	0
11/17/2008	0	0	1	1	1	0

12/3/2008	0	0	1	1	0	0
1/5/2009	0	0	1	0	1	0
1/26/2009	0	1	0	1	1	1
2/16/2009	0	0	0	0	0	1
3/2/2009	0	0	1	0	1	0
4/20/2009	0	0	0	0	1	1

BA	7ACT	7ACC	7A	9	CC1	CC2
8/21/2007	1	1	0	0	1	
8/28/2007	0	0	0	0	0	
9/3/2007	0	0	0	0	1	
9/10/2007	0	1	0	0	1	
9/17/2007	1	0	0	0	1	
9/24/2007	0	1	0	1	1	
10/1/2007	0	0	0	0	1	
10/8/2007	0	0	1	0	1	1
10/15/2007	0	0	0	0	1	1
10/22/2007	1	1	1	1	1	1
10/29/2007	0	0	0	0	1	1
11/5/2007	0	0	0	0	1	1
11/12/2007	1	0	0	1	0	1
11/26/2007	0	0	0	0	0	1
12/1/2007	1	1	1	1	0	1
1/7/2008	1	1	1	1	0	1
1/14/2008	1	1	1	0	0	1
1/28/2008	1	0	1	0	0	1
2/15/2008	1	0	1	0	0	1
3/17/2008	0	0	1	1	1	1
3/31/2008	0	0	0	0	1	1
4/28/2008	0	0	0	0	1	1
5/19/2008	1	1	0	0	1	1
5/27/2008	1	1	1	0	1	1
6/2/2008	0	0	0	0	1	1
6/23/2008	0	0	0	0	1	1
7/8/2008	0	0	0	1	1	1
7/14/2008	0	0	0	0	1	0
7/22/2008	1	0	0	0	1	1
8/12/2008	0	0	0	0	1	1
8/19/2008	0	0	0	0	0	0
9/8/2008	0	1	0	0	0	0
9/29/2008	0	0	0	0	0	0

10/20/2008	0	0	0	0	0	0
11/3/2008	0	0	0	0	1	0
11/17/2008	0	0	0	0	0	1
12/3/2008	0	1	0	0	0	1
1/5/2009	0	1	0	0	1	1
1/26/2009	0	0	0	1	0	0
2/16/2009	0	0	0	1	1	1
3/2/2009	0	0	1	0	1	1
4/20/2009	1	1	1	1	1	1

EN	7ACT	7ACC	7A	9	CC1	CC2
8/21/2007	927	490	146	118	14350	
8/28/2007	2450	5380	11	4513	30550	
9/3/2007	550	1873	14	11	7250	
9/10/2007	660	1270	9	15	8000	
9/17/2007	8750	1070	11	53	13100	
9/24/2007	1360	870	1650	1593	4200	
10/1/2007	760	1107	21	63	30000	
10/8/2007	660	1780	21	867	12000	8967
10/15/2007	800	Tntc	50	100	7500	25000
10/22/2007	2420	3050	215	910	7150	9950
10/29/2007	1250	1350	190	200	13150	nd
11/5/2007	820	1280	17	40	37000	11000
11/12/2007	1500	1567	30	19	9500	39500
11/26/2007	58500	43000	20500	1200	31500	69000
12/1/2007	893	1600	0	0	40000	16500
1/7/2008	590	1187	20	0	4550	5250
1/14/2008	987	16000	19	4	62500	22000
1/28/2008	1800	500	0	48	1600	1200
2/15/2008	42000	1340	51	400	4050	910
3/17/2008	1000	1587	19	277	7867	17133
3/31/2008	40500	333	44	800		37500
4/28/2008		4750		90	23000	15900
5/19/2008	300	0	50	110	147000	14000
5/27/2008	1000	800	20	0	13000	19000
6/2/2008	1000	3600	145	0	13000	6500
6/23/2008	700	1550	95	18	23500	9500
7/8/2008	2150	4990	16	18	12000	6700
7/14/2008	600	2000	93	105	9500	1850
7/22/2008	510	5500	113	20	6000	1400
8/12/2008	2200	1900	25	201	25500	11000

8/19/2008	270000	210000	550	150	9400	11033
9/8/2008	600	100	600	203	2450	1600
9/29/2008	1320	1700	0	0	1350	793
10/20/2008	5200	4100	1253	0	12056	707
11/3/2008	1000	3300	210	0	1700	1480
11/17/2008	1053	1587	0	0	853	1280
12/3/2008	1393	9200	10	0	650	1250
1/5/2009	1180	500	43	0	1280	1460
1/26/2009	1500	693	0	40	4050	3550
2/16/2009	1300	4633	200	263	2100	9000
3/2/2009	1500	2500	13	158	1373	860
4/20/2009	1067	2160	465	8	9500	9250

Site	Date	*M. smithii*	*Bac*	*Btim*	*Fecali*	EN/100ml
7ACC	5/12/2009	0	1	0	0	4000
7ACT	5/12/2009	1	0	1	0	2267
7A	5/12/2009	0	0	0	0	25
9	5/12/2009	0	0	0	0	207
CC1	5/12/2009	0	0	1	0	4050
CC2	5/12/2009	0	0	1	0	5933
10	5/12/2009	0	0	1	0	0
10A	5/12/2009	0	0	1	0	375
AOC	5/12/2009	0	0	1	0	N/D
CON	5/12/2009	1	0	1	0	8850
11	5/12/2009	1	0	0	0	100
12A	5/12/2009	0	0	0	0	657
CTHC	5/12/2009	0	0	0	0	6000
CH	5/12/2009	1	0	0	0	287
7ACC	5/26/2009	1	1	0	0	1340
7ACT	5/26/2009	1	1	0	0	7850
7A	5/26/2009	0	0	0	0	124
9	5/26/2009	0	0	0	0	442
CC1	5/26/2009	1	0	1	1	3850
CC2	5/26/2009	1	1	1	1	3250
10	5/26/2009	0	1	0	0	141
10A	5/26/2009	1	0	0	0	910
AOC	5/26/2009	0	0	0	0	2000
CON	5/26/2009	0	0	0	0	6450
11	5/26/2009	0	1	0	0	705
12A	5/26/2009	0	0	0	0	523

CTHC	5/26/2009	1	0	0	0	12067
CH	5/26/2009	1	0	0	0	516
7ACC	6/1/2009	0	1	1	0	2300
7ACT	6/1/2009	0	1	1	0	2200
7A	6/1/2009	0	0	0	0	23
9	6/1/2009	0	0	0	0	N/D
CC1	6/1/2009	1	1	1	0	2500
CC2	6/1/2009	0	1	1	1	2600
10	6/1/2009	0	1	0	0	600
10A	6/1/2009	0	1	0	0	0
AOC	6/1/2009	0	1	1	0	1060
CON	6/1/2009	0	1	0	0	21000
11	6/1/2009	0	1	1	0	33
12A	6/1/2009	0	1	1	0	0
CTHC	6/1/2009	1	1	1	0	450
CH	6/1/2009	0	0	0	0	470
7ACC	6/8/2009	0	1	1	0	12500
7ACT	6/8/2009	0	0	1	0	525
7A	6/8/2009	0	0	0	0	52
9	6/8/2009	0	0	1	0	23
CC1	6/8/2009	0	1	1	0	1960
CC2	6/8/2009	1	1	1	0	950
10	6/8/2009	1	0	0	0	140
10A	6/8/2009	0	1	0	0	8
AOC	6/8/2009	0	1	1	0	1150
CON	6/8/2009	0	0	1	0	5600
11	6/8/2009	0	1	0	0	245
12A	6/8/2009	0	1	1	0	18
CTHC	6/8/2009	0	1	1	0	1240
CH	6/8/2009	0	1	0	1	243
7ACC	6/15/2009	0	0	0	0	228
7ACT	6/15/2009	0	1	0	0	23500
7A	6/15/2009	0	1	1	0	43
9	6/15/2009	0	0	1	0	0
CC1	6/15/2009	0	0	1	0	1160
CC2	6/15/2009	1	1	1	0	1060
10	6/15/2009	0	1	1	0	5
10A	6/15/2009	0	0	0	0	5
AOC	6/15/2009	0	0	0	0	1060
CON	6/15/2009	0	0	1	0	80000
11	6/15/2009	0	1	0	1	0

12A	6/15/2009	0	1	0	0	0
CTHC	6/15/2009	1	0	0	0	25
CH	6/15/2009	0	1	1	0	11467
7ACC	7/6/2009	1	1	1	1	29000
7ACT	7/6/2009	1	1	1	1	55000
7A	7/6/2009	1	0	1	1	125
9	7/6/2009	1	0	0	0	235
CC1	7/6/2009	1	1	1	1	6000
CC2	7/6/2009	1	1	1	1	2100
10	7/6/2009	1	1	1	0	83
10A	7/6/2009	0	0	0	0	440
AOC	7/6/2009	0	0	1	1	40000
CON	7/6/2009	1	0	1	1	36000
11	7/6/2009	0	1	1	0	950
12A	7/6/2009	0	0	0	0	32
CTHC	7/6/2009	1	1	1	0	700
CH	7/6/2009	0	1	1	0	1550
7ACC	7/14/2009	0	1	0	0	1840
7ACT	7/14/2009	1	0	1	1	1020
7A	7/14/2009	0	0	0	0	267
9	7/14/2009	0	0	0	0	42
CC1	7/14/2009	0	0	0	0	1800
CC2	7/14/2009	0	1	0	0	3500
10	7/14/2009	1	1	1	0	1660
10A	7/14/2009	0	0	0	0	460
AOC	7/14/2009	1	0	1	0	52
CON	7/14/2009	1	0	1	0	143
11	7/14/2009	0	0	1	0	240
12A	7/14/2009	1	0	0	0	60
CTHC	7/14/2009	0	0	1	0	780
CH	7/14/2009	0	0	0	0	420
7ACC	7/21/2009	0	0	1	0	270
7ACT	7/21/2009	0	0	0	0	1200
7A	7/21/2009	0	0	0	0	330
9	7/21/2009	0	0	0	0	245
CC1	7/21/2009	0	0	1	0	5200
CC2	7/21/2009	0	1	1	0	3800
10	7/21/2009	1	1	0	0	210
10A	7/21/2009	0	0	0	0	615
AOC	7/21/2009	1	0	1	0	840
CON	7/21/2009	0	0	1	0	2140

11	7/21/2009	0	0	0	0	210
12A	7/21/2009	0	0	1	0	1200
CTHC	7/21/2009	0	0	1	0	530
CH	7/21/2009	0	0	0	0	253
7ACC	7/28/2009	0	0	1	0	2450
7ACT	7/28/2009	0	0	1	0	510
7A	7/28/2009	0	0	0	0	35
9	7/28/2009	0	0	0	0	10
CC1	7/28/2009	1	1	1	0	8267
CC2	7/28/2009	1	1	1	0	860
10	7/28/2009	0	0	0	0	105
10A	7/28/2009	1	0	0	0	175
AOC	7/28/2009	0	0	1	0	4400
CON	7/28/2009	1	0	1	0	2000
11	7/28/2009	0	0	0	0	205
12A	7/28/2009	1	0	0	0	100
CTHC	7/28/2009	0	0	0	0	325
CH	7/28/2009	0	0	1	0	510
7ACC	8/11/2009	0	0	1	1	25000
7ACT	8/11/2009	0	0	1	1	1020
7A	8/11/2009	0	0	0	1	10
9	8/11/2009	0	0	0	1	0
CC1	8/11/2009	0	1	1	1	3100
CC2	8/11/2009	0	1	1	1	2500
10	8/11/2009	0	0	1	1	268
10A	8/11/2009	0	0	0	1	0
AOC	8/11/2009	1	0	1	1	2800
CON	8/11/2009	0	1	1	1	4900
11	8/11/2009	0	0	0	1	1060
12A	8/11/2009	0	0	1	1	0
CTHC	8/11/2009	1	0	0	1	72
CH	8/11/2009	0	0	0	1	150
7ACC	8/17/2009	0	0	0	1	1790
7ACT	8/17/2009	0	0	1	1	800
7A	8/17/2009	0	0	0	1	90
9	8/17/2009	0	1	0	1	240
CC1	8/17/2009	0	1	1	1	9000
CC2	8/17/2009	0	1	1	1	4000
10	8/17/2009	0	1	0	1	410
10A	8/17/2009	0	1	0	1	45
AOC	8/17/2009	0	0	1	1	3300

CON	8/17/2009	0	0	1	1	2600
11	8/17/2009	0	0	0	1	0
12A	8/17/2009	0	0	0	1	0
CTHC	8/17/2009	0	0	0	1	1100
CH	8/17/2009	0	1	0	1	700
7ACC	9/3/2009	1	0	0	1	3050
7ACT	9/3/2009	0	1	0	0	600
7A	9/3/2009	0	0	0	0	380
9	9/3/2009	0	0	0	1	298
CC1	9/3/2009	0	1	1	0	2500
CC2	9/3/2009	0	0	1	0	3100
10	9/3/2009	0	0	0	1	245
10A	9/3/2009	1	0	1	0	263
AOC	9/3/2009	0	1	0	0	5900
CON	9/3/2009	0	0	0	0	5400
11	9/3/2009	0	0	0	1	70
12A	9/3/2009	0	0	0	1	120
CTHC	9/3/2009	0	0	0	1	2300
CH	9/3/2009	0	0	0	1	145
7ACC	9/17/2009	0	1	1	1	1870
7ACT	9/17/2009	0	0	1	0	800
7A	9/17/2009	0	0	0	0	150
9	9/17/2009	0	0	0	0	32
CC1	9/17/2009	0	1	1	1	535
CC2	9/17/2009	0	1	1	1	4267
10	9/17/2009	0	1	0	1	430
10A	9/17/2009	0	0	1	1	88
AOC	9/17/2009	0	1	0	1	2100
CON	9/17/2009	0	0	1	0	4100
11	9/17/2009	1	0	0	1	1400
12A	9/17/2009	0	0	0	0	40
CTHC	9/17/2009	0	0	0	1	68
CH	9/17/2009	0	0	0	0	490
7ACC	10/1/2009	1	0	1	0	5000
7ACT	10/1/2009	1	0	1	1	1120
7A	10/1/2009	1	1	0	1	0
9	10/1/2009	1	0	0	1	20
CC1	10/1/2009	0	1	1	0	13500
CC2	10/1/2009	1	1	1	1	4800
10	10/1/2009	1	1	0	0	160
10A	10/1/2009	1	0	0	0	88

AOC	10/1/2009	1	1	1	0	1900
CON	10/1/2009	1	1	1	1	44000
11	10/1/2009	1	0	0	1	525
12A	10/1/2009	1	0	0	1	5
CTHC	10/1/2009	1	0	0	1	240
CH	10/1/2009	1	0	0	1	267
7ACC	11/5/2009	1	1	1	0	5600
7ACT	11/5/2009	1	1	0	0	378
7A	11/5/2009	1	0	0	0	0
9	11/5/2009	0	1	0	0	0
CC1	11/5/2009	0	0	1	0	5300
CC2	11/5/2009	1	1	0	0	2700
10	11/5/2009	0	1	0	0	22
10A	11/5/2009	1	0	0	0	0
AOC	11/5/2009	0	0	0	0	2000
CON	11/5/2009	0	0	0	0	4600
11	11/5/2009	0	0	1	0	1160
12A	11/5/2009	0	0	0	0	420
CTHC	11/5/2009	0	0	0	0	860
CH	11/5/2009	0	0	0	0	0
7ACC	11/19/2009	1	0	1	0	4700
7ACT	11/19/2009	1	0	1	0	1200
7A	11/19/2009	0	0	0	0	125
9	11/19/2009	0	0	0	0	27
CC1	11/19/2009	1	1	1	1	4800
CC2	11/19/2009	1	1	1	1	1750
10	11/19/2009	0	0	0	0	13
10A	11/19/2009	0	0	0	0	38
AOC	11/19/2009	0	0	0	0	5700
CON	11/19/2009	0	0	0	0	7100
11	11/19/2009	0	0	0	0	135
12A	11/19/2009	0	0	1	0	0
CTHC	11/19/2009	0	0	0	0	315
CH	11/19/2009	0	0	0	0	18

Site	Date	Turbidity (ntu)	High Tide	Height (ft)	Low Tide	Height (ft)
7ACC	5/12/2009	N/D	12:47PM	2.13	N/A	N/A
7ACT	5/12/2009	N/D	12:47PM	2.13	N/A	N/A
7A	5/12/2009	N/D	12:47PM	2.13	N/A	N/A
9	5/12/2009	N/D	12:47PM	2.13	N/A	N/A

CC1	5/12/2009	N/D	12:47PM	2.13	N/A	N/A
CC2	5/12/2009	N/D	12:47PM	2.13	N/A	N/A
10	5/12/2009	N/D	12:47PM	2.13	N/A	N/A
10A	5/12/2009	N/D	12:47PM	2.13	N/A	N/A
AOC	5/12/2009	N/D	12:47PM	2.13	N/A	N/A
CON	5/12/2009	N/D	12:47PM	2.13	N/A	N/A
11	5/12/2009	N/D	12:47PM	2.13	N/A	N/A
12A	5/12/2009	N/D	12:47PM	2.13	N/A	N/A
CTHC	5/12/2009	N/D	12:47PM	2.13	N/A	N/A
CH	5/12/2009	N/D	12:47PM	2.13	N/A	N/A
7ACC	5/26/2009	N/D	12:06PM	2.7	11:45PM	-0.81
7ACT	5/26/2009	N/D	12:06PM	2.7	11:45PM	-0.81
7A	5/26/2009	N/D	12:06PM	2.7	11:45PM	-0.81
9	5/26/2009	N/D	12:06PM	2.7	11:45PM	-0.81
CC1	5/26/2009	N/D	12:06PM	2.7	11:45PM	-0.81
CC2	5/26/2009	N/D	12:06PM	2.7	11:45PM	-0.81
10	5/26/2009	N/D	12:06PM	2.7	11:45PM	-0.81
10A	5/26/2009	N/D	12:06PM	2.7	11:45PM	-0.81
AOC	5/26/2009	N/D	12:06PM	2.7	11:45PM	-0.81
CON	5/26/2009	N/D	12:06PM	2.7	11:45PM	-0.81
11	5/26/2009	N/D	12:06PM	2.7	11:45PM	-0.81
12A	5/26/2009	N/D	12:06PM	2.7	11:45PM	-0.81
CTHC	5/26/2009	N/D	12:06PM	2.7	11:45PM	-0.81
CH	5/26/2009	N/D	12:06PM	2.7	11:45PM	-0.81
7ACC	6/1/2009	N/D	8:26AM	1.21	7:41PM	0.53
7ACT	6/1/2009	N/D	8:26AM	1.21	7:41PM	0.53
7A	6/1/2009	N/D	8:26AM	1.21	7:41PM	0.53
9	6/1/2009	N/D	8:26AM	1.21	7:41PM	0.53
CC1	6/1/2009	N/D	8:26AM	1.21	7:41PM	0.53
CC2	6/1/2009	N/D	8:26AM	1.21	7:41PM	0.53
10	6/1/2009	N/D	8:26AM	1.21	7:41PM	0.53
10A	6/1/2009	N/D	8:26AM	1.21	7:41PM	0.53
AOC	6/1/2009	N/D	8:26AM	1.21	7:41PM	0.53
CON	6/1/2009	N/D	8:26AM	1.21	7:41PM	0.53
11	6/1/2009	N/D	8:26AM	1.21	7:41PM	0.53
12A	6/1/2009	N/D	8:26AM	1.21	7:41PM	0.53
CTHC	6/1/2009	N/D	8:26AM	1.21	7:41PM	0.53
CH	6/1/2009	N/D	8:26AM	1.21	7:41PM	0.53
7ACC	6/8/2009	N/D	11:12AM	2.3	10:43PM	-0.4
7ACT	6/8/2009	N/D	11:12AM	2.3	10:43PM	-0.4
7A	6/8/2009	N/D	11:12AM	2.3	10:43PM	-0.4

9	6/8/2009	N/D	11:12AM	2.3	10:43PM	-0.4
CC1	6/8/2009	N/D	11:12AM	2.3	10:43PM	-0.4
CC2	6/8/2009	N/D	11:12AM	2.3	10:43PM	-0.4
10	6/8/2009	N/D	11:12AM	2.3	10:43PM	-0.4
10A	6/8/2009	N/D	11:12AM	2.3	10:43PM	-0.4
AOC	6/8/2009	N/D	11:12AM	2.3	10:43PM	-0.4
CON	6/8/2009	N/D	11:12AM	2.3	10:43PM	-0.4
11	6/8/2009	N/D	11:12AM	2.3	10:43PM	-0.4
12A	6/8/2009	N/D	11:12AM	2.3	10:43PM	-0.4
CTHC	6/8/2009	N/D	11:12AM	2.3	10:43PM	-0.4
CH	6/8/2009	N/D	11:12AM	2.3	10:43PM	-0.4
7ACC	6/15/2009	N/D	11:07AM	1.09	12:30AM	0.51
7ACT	6/15/2009	N/D	11:07AM	1.09	12:30AM	0.51
7A	6/15/2009	N/D	11:07AM	1.09	12:30AM	0.51
9	6/15/2009	N/D	11:07AM	1.09	12:30AM	0.51
CC1	6/15/2009	N/D	11:07AM	1.09	12:30AM	0.51
CC2	6/15/2009	N/D	11:07AM	1.09	12:30AM	0.51
10	6/15/2009	N/D	11:07AM	1.09	12:30AM	0.51
10A	6/15/2009	N/D	11:07AM	1.09	12:30AM	0.51
AOC	6/15/2009	N/D	11:07AM	1.09	12:30AM	0.51
CON	6/15/2009	N/D	11:07AM	1.09	12:30AM	0.51
11	6/15/2009	N/D	11:07AM	1.09	12:30AM	0.51
12A	6/15/2009	N/D	11:07AM	1.09	12:30AM	0.51
CTHC	6/15/2009	N/D	11:07AM	1.09	12:30AM	0.51
CH	6/15/2009	N/D	11:07AM	1.09	12:30AM	0.51
7ACC	7/6/2009	61.2	10:25AM	2.37	9:56PM	-0.26
7ACT	7/6/2009	9.11	10:25AM	2.37	9:56PM	-0.26
7A	7/6/2009	6.07	10:25AM	2.37	9:56PM	-0.26
9	7/6/2009	13.9	10:25AM	2.37	9:56PM	-0.26
CC1	7/6/2009	7.69	10:25AM	2.37	9:56PM	-0.26
CC2	7/6/2009	8.4	10:25AM	2.37	9:56PM	-0.26
10	7/6/2009	19.4	10:25AM	2.37	9:56PM	-0.26
10A	7/6/2009	66.7	10:25AM	2.37	9:56PM	-0.26
AOC	7/6/2009	46.3	10:25AM	2.37	9:56PM	-0.26
CON	7/6/2009	24.3	10:25AM	2.37	9:56PM	-0.26
11	7/6/2009	54.9	10:25AM	2.37	9:56PM	-0.26
12A	7/6/2009	61.2	10:25AM	2.37	9:56PM	-0.26
CTHC	7/6/2009	46.4	10:25AM	2.37	9:56PM	-0.26
CH	7/6/2009	96.9	10:25AM	2.37	9:56PM	-0.26
7ACC	7/14/2009	10.9	5:42AM	1.33	5:16PM	0.7
7ACT	7/14/2009	6.16	5:42AM	1.33	5:16PM	0.7

7A	7/14/2009	40.8	5:42AM	1.33	5:16PM	0.7
9	7/14/2009	17.5	5:42AM	1.33	5:16PM	0.7
CC1	7/14/2009	8.73	5:42AM	1.33	5:16PM	0.7
CC2	7/14/2009	11.5	5:42AM	1.33	5:16PM	0.7
10	7/14/2009	27.9	5:42AM	1.33	5:16PM	0.7
10A	7/14/2009	32	5:42AM	1.33	5:16PM	0.7
AOC	7/14/2009	16.7	5:42AM	1.33	5:16PM	0.7
CON	7/14/2009	17.3	5:42AM	1.33	5:16PM	0.7
11	7/14/2009	34.7	5:42AM	1.33	5:16PM	0.7
12A	7/14/2009	40.1	5:42AM	1.33	5:16PM	0.7
CTHC	7/14/2009	20.3	5:42AM	1.33	5:16PM	0.7
CH	7/14/2009	21.4	5:42AM	1.33	5:16PM	0.7
7ACC	7/21/2009	6.2	10:18AM	2.84	9:49PM	-0.48
7ACT	7/21/2009	3.71	10:18AM	2.84	9:49PM	-0.48
7A	7/21/2009	28.9	10:18AM	2.84	9:49PM	-0.48
9	7/21/2009	23.7	10:18AM	2.84	9:49PM	-0.48
CC1	7/21/2009	14.5	10:18AM	2.84	9:49PM	-0.48
CC2	7/21/2009	11.2	10:18AM	2.84	9:49PM	-0.48
10	7/21/2009	37.9	10:18AM	2.84	9:49PM	-0.48
10A	7/21/2009	46.6	10:18AM	2.84	9:49PM	-0.48
AOC	7/21/2009	19.4	10:18AM	2.84	9:49PM	-0.48
CON	7/21/2009	18.4	10:18AM	2.84	9:49PM	-0.48
11	7/21/2009	31.1	10:18AM	2.84	9:49PM	-0.48
12A	7/21/2009	37	10:18AM	2.84	9:49PM	-0.48
CTHC	7/21/2009	22.2	10:18AM	2.84	9:49PM	-0.48
CH	7/21/2009	84.7	10:18AM	2.84	9:49PM	-0.48
7ACC	7/28/2009	5.7	4:32AM	1.87	4:22PM	0.33
7ACT	7/28/2009	13.5	4:32AM	1.87	4:22PM	0.33
7A	7/28/2009	23.6	4:32AM	1.87	4:22PM	0.33
9	7/28/2009	1	4:32AM	1.87	4:22PM	0.33
CC1	7/28/2009	4.39	4:32AM	1.87	4:22PM	0.33
CC2	7/28/2009	5.3	4:32AM	1.87	4:22PM	0.33
10	7/28/2009	11.6	4:32AM	1.87	4:22PM	0.33
10A	7/28/2009	18.6	4:32AM	1.87	4:22PM	0.33
AOC	7/28/2009	12.1	4:32AM	1.87	4:22PM	0.33
CON	7/28/2009	19.2	4:32AM	1.87	4:22PM	0.33
11	7/28/2009	21.3	4:32AM	1.87	4:22PM	0.33
12A	7/28/2009	0	4:32AM	1.87	4:22PM	0.33
CTHC	7/28/2009	41.6	4:32AM	1.87	4:22PM	0.33
CH	7/28/2009	99.4	4:32AM	1.87	4:22PM	0.33
7ACC	8/11/2009	6.62	3:01AM	1.59	1:28PM	0.8

7ACT	8/11/2009	3.66	3:01AM	1.59	1:28PM	0.8
7A	8/11/2009	13.7	3:01AM	1.59	1:28PM	0.8
9	8/11/2009	8.62	3:01AM	1.59	1:28PM	0.8
CC1	8/11/2009	6.74	3:01AM	1.59	1:28PM	0.8
CC2	8/11/2009	18.4	3:01AM	1.59	1:28PM	0.8
10	8/11/2009	13.1	3:01AM	1.59	1:28PM	0.8
10A	8/11/2009	14.7	3:01AM	1.59	1:28PM	0.8
AOC	8/11/2009	12.6	3:01AM	1.59	1:28PM	0.8
CON	8/11/2009	16	3:01AM	1.59	1:28PM	0.8
11	8/11/2009	11.1	3:01AM	1.59	1:28PM	0.8
12A	8/11/2009	20.1	3:01AM	1.59	1:28PM	0.8
CTHC	8/11/2009	16.6	3:01AM	1.59	1:28PM	0.8
CH	8/11/2009	25.2	3:01AM	1.59	1:28PM	0.8
7ACC	8/17/2009	7.87	8:14AM	2.84	7:48PM	-0.21
7ACT	8/17/2009	8.2	8:14AM	2.84	7:48PM	-0.21
7A	8/17/2009	18.1	8:14AM	2.84	7:48PM	-0.21
9	8/17/2009	20.2	8:14AM	2.84	7:48PM	-0.21
CC1	8/17/2009	6.74	8:14AM	2.84	7:48PM	-0.21
CC2	8/17/2009	22.6	8:14AM	2.84	7:48PM	-0.21
10	8/17/2009	17	8:14AM	2.84	7:48PM	-0.21
10A	8/17/2009	18.3	8:14AM	2.84	7:48PM	-0.21
AOC	8/17/2009	13	8:14AM	2.84	7:48PM	-0.21
CON	8/17/2009	12.1	8:14AM	2.84	7:48PM	-0.21
11	8/17/2009	15.6	8:14AM	2.84	7:48PM	-0.21
12A	8/17/2009	20.1	8:14AM	2.84	7:48PM	-0.21
CTHC	8/17/2009	18.2	8:14AM	2.84	7:48PM	-0.21
CH	8/17/2009	17.1	8:14AM	2.84	7:48PM	-0.21
7ACC	9/3/2009	5.73	10:47am	2.03	9:05pm	0.91
7ACT	9/3/2009	4.5	10:47am	2.03	9:05pm	0.91
7A	9/3/2009	59.8	10:47am	2.03	9:05pm	0.91
9	9/3/2009	111	10:47am	2.03	9:05pm	0.91
CC1	9/3/2009	6.63	10:47am	2.03	9:05pm	0.91
CC2	9/3/2009	9.24	10:47am	2.03	9:05pm	0.91
10	9/3/2009	57.5	10:47am	2.03	9:05pm	0.91
10A	9/3/2009	113	10:47am	2.03	9:05pm	0.91
AOC	9/3/2009	20.2	10:47am	2.03	9:05pm	0.91
CON	9/3/2009	20.1	10:47am	2.03	9:05pm	0.91
11	9/3/2009	92.7	10:47am	2.03	9:05pm	0.91
12A	9/3/2009	102	10:47am	2.03	9:05pm	0.91
CTHC	9/3/2009	18.3	10:47am	2.03	9:05pm	0.91
CH	9/3/2009	19.7	10:47am	2.03	9:05pm	0.91

7ACC	9/17/2009	13.2	10:43am	1.98	7:44pm	1.13
7ACT	9/17/2009	8.22	10:43am	1.98	7:44pm	1.13
7A	9/17/2009	4.5	10:43am	1.98	7:44pm	1.13
9	9/17/2009	88.5	10:43am	1.98	7:44pm	1.13
CC1	9/17/2009	9.26	10:43am	1.98	7:44pm	1.13
CC2	9/17/2009	8.97	10:43am	1.98	7:44pm	1.13
10	9/17/2009	21.5	10:43am	1.98	7:44pm	1.13
10A	9/17/2009	55.9	10:43am	1.98	7:44pm	1.13
AOC	9/17/2009	21	10:43am	1.98	7:44pm	1.13
CON	9/17/2009	15.9	10:43am	1.98	7:44pm	1.13
11	9/17/2009	47.1	10:43am	1.98	7:44pm	1.13
12A	9/17/2009	23.4	10:43am	1.98	7:44pm	1.13
CTHC	9/17/2009	18.7	10:43am	1.98	7:44pm	1.13
CH	9/17/2009	67.3	10:43am	1.98	7:44pm	1.13
7ACC	10/1/2009	10.9	9:52am	1.74	6:36	1.18
7ACT	10/1/2009	13.8	9:52am	1.74	6:36	1.18
7A	10/1/2009	11.9	9:52am	1.74	6:36	1.18
9	10/1/2009	67	9:52am	1.74	6:36	1.18
CC1	10/1/2009	8.65	9:52am	1.74	6:36	1.18
CC2	10/1/2009	9.67	9:52am	1.74	6:36	1.18
10	10/1/2009	66.4	9:52am	1.74	6:36	1.18
10A	10/1/2009	93.6	9:52am	1.74	6:36	1.18
AOC	10/1/2009	63.1	9:52am	1.74	6:36	1.18
CON	10/1/2009	23.8	9:52am	1.74	6:36	1.18
11	10/1/2009	67	9:52am	1.74	6:36	1.18
12A	10/1/2009	37.6	9:52am	1.74	6:36	1.18
CTHC	10/1/2009	20.8	9:52am	1.74	6:36	1.18
CH	10/1/2009	37.4	9:52am	1.74	6:36	1.18
7ACC	11/5/2009	0	11:38 PM	2.71	10:26 AM	-0.48
7ACT	11/5/2009	4.03	11:38 PM	2.71	10:26 AM	-0.48
7A	11/5/2009	13.3	11:38 PM	2.71	10:26 AM	-0.48
9	11/5/2009	8.89	11:38 PM	2.71	10:26 AM	-0.48
CC1	11/5/2009	7.97	11:38 PM	2.71	10:26 AM	-0.48
CC2	11/5/2009	6.03	11:38 PM	2.71	10:26 AM	-0.48
10	11/5/2009	70.4	11:38 PM	2.71	10:26 AM	-0.48

10A	11/5/2009	25.3	11:38 PM	2.71	10:26 AM	-0.48
AOC	11/5/2009	18.1	11:38 PM	2.71	10:26 AM	-0.48
CON	11/5/2009	15.2	11:38 PM	2.71	10:26 AM	-0.48
11	11/5/2009	19.1	11:38 PM	2.71	10:26 AM	-0.48
12A	11/5/2009	25.4	11:38 PM	2.71	10:26 AM	-0.48
CTHC	11/5/2009	16.6	11:38 PM	2.71	10:26 AM	-0.48
CH	11/5/2009	71.3	11:38 PM	2.71	10:26 AM	-0.48
7ACC	11/19/2009	11	11:35 PM	2.28	10:21 AM	-0.4
7ACT	11/19/2009	7.91	11:35 PM	2.28	10:21 AM	-0.4
7A	11/19/2009	15.3	11:35 PM	2.28	10:21 AM	-0.4
9	11/19/2009	9.78	11:35 PM	2.28	10:21 AM	-0.4
CC1	11/19/2009	9.92	11:35 PM	2.28	10:21 AM	-0.4
CC2	11/19/2009	9.63	11:35 PM	2.28	10:21 AM	-0.4
10	11/19/2009	71.8	11:35 PM	2.28	10:21 AM	-0.4
10A	11/19/2009	4.29	11:35 PM	2.28	10:21 AM	-0.4
AOC	11/19/2009	26.8	11:35 PM	2.28	10:21 AM	-0.4
CON	11/19/2009	30.8	11:35 PM	2.28	10:21 AM	-0.4
11	11/19/2009	73.8	11:35 PM	2.28	10:21 AM	-0.4
12A	11/19/2009	106	11:35 PM	2.28	10:21 AM	-0.4
CTHC	11/19/2009	25	11:35 PM	2.28	10:21 AM	-0.4
CH	11/19/2009	37.5	11:35 PM	2.28	10:21 AM	-0.4

Site	DATE	*Btim*	*Bac*	*M.smithii*	*Fecali*	En/100ml
ACC	4/21/2010	0	0	0	0	980
ACT	4/21/2010	0	0	0	0	275

7A	4/21/2010	0	0	0	0	95
9	4/21/2010	0	1	0	0	20
CC1	4/21/2010	0	1	0	0	580
10	4/21/2010	1	1	0	0	190
CC2	4/21/2010	0	1	0	0	780
AOC	4/21/2010	1	0	0	0	4467
Con	4/21/2010	1	0	0	0	1060
11	4/21/2010	0	1	0	0	120
Cthc	4/21/2010	0	0	0	0	680
12A	4/21/2010	0	0	0	0	395
Ch	4/21/2010	0	0	0	0	170
ACT	5/26/2010	0	0	0	0	540
ACC	5/26/2010	0	0	1	0	16000
7A	5/26/2010	0	0	0	0	125
9	5/26/2010	1	1	0	0	154
CC1	5/26/2010	1	0	0	0	800
CC2	5/26/2010	1	0	0	0	—
10	5/26/2010	0	0	0	0	281
AOC	5/26/2010	1	0	0	0	460
Con	5/26/2010	0	0	0	0	950
11	5/26/2010	1	0	0	0	950
Cthc	5/26/2010	0	0	0	0	135
Ch	5/26/2010	0	0	0	0	235
12A	5/26/2010	0	0	0	0	263
ACC	6/2/2010	0	0	0	0	1400
ACT	6/2/2010	0	0	1	0	490
7A	6/2/2010	0	0	1	0	65
9	6/2/2010	0	0	1	0	5
10	6/2/2010	0	0	0	0	23
10A	6/2/2010	0	0	0	0	0
CC1	6/2/2010	0	0	1	0	2100
CC2	6/2/2010	1	0	1	0	1900
11	6/2/2010	0	0	1	0	213
CON	6/2/2010	0	0	0	0	12000
AOC	6/2/2010	0	0	0	0	4000
CTHC	6/2/2010	0	0	0	0	170
CH	6/2/2010	0	0	0	0	470
ACC	6/14/2010	0	0	0	0	569
12A	6/2/2010	0	0	0	0	55
ACT	6/14/2010	0	1	0	0	700
7A	6/14/2010	0	0	0	0	42

9	6/14/2010	0	0	0	0	20
CC2	6/14/2010	0	0	0	0	520
CC1	6/14/2010	0	0	0	0	1200
10	6/14/2010	0	1	0	0	71
CON	6/14/2010	0	1	0	0	6000
11	6/14/2010	0	1	0	0	620
AOC	6/14/2010	0	1	0	0	1300
10A	6/14/2010	0	0	0	0	—
12A	6/14/2010	0	0	0	0	0
ACC	6/9/2010	1	0	0	0	0
ACT	6/9/2010	0	0	0	0	0
7A	6/9/2010	0	0	0	0	—
9	6/9/2010	0	0	0	0	20
CC1	6/9/2010	0	1	1	0	0
CC2	6/9/2010	1	1	1	0	—
10	6/9/2010	1	1	0	0	0
10A	6/9/2010	0	0	0	0	35
AOC	6/9/2010	0	0	0	0	1000
CON	6/9/2010	0	0	0	0	2200
11	6/9/2010	0	0	0	0	630
Cthc	6/9/2010	1	0	0	0	25
Ch	6/9/2010	0	0	0	0	665
12A	6/9/2010	0	0	0	0	36
ACC	6/24/2010	0	0	0	0	760
ACT	6/24/2010	0	0	0	0	370
7A	6/24/2010	0	0	0	0	88
9	6/24/2010	0	0	0	0	230
CC1	6/24/2010	1	0	1	0	1800
CC2	6/24/2010	0	0	1	0	2100
10	6/24/2010	0	0	0	0	650
10A	6/24/2010	0	0	0	0	10
AOC	6/24/2010	0	0	0	0	34000
CON	6/24/2010	1	0	0	0	5000
11	6/24/2010	0	0	0	0	835
Cthc	6/24/2010	0	0	0	0	3400
Ch	6/24/2010	0	0	0	0	720
12A	6/24/2010	0	0	0	0	115
ACC	6/30/2010	1	0	0	0	25000
ACT	6/30/2010	1	1	0	0	22000
7A	6/30/2010	0	1	0	0	1800
9	6/30/2010	0	1	0	0	17000

CC1	6/30/2010	1	1	1	0	50000
CC2	6/30/2010	0	1	0	0	51000
10	6/30/2010	1	0	0	0	223
10A	6/30/2010	0	0	0	0	542
AOC	6/30/2010	0	0	0	0	54000
CON	6/30/2010	1	0	0	0	—
11	6/30/2010	1	1	0	0	15500
12A	6/30/2010	1	0	1	0	25000
ACC	7/7/2010	1	1	1	0	600
ACT	7/7/2010	1	1	0	0	960
7A	7/7/2010	0	0	0	0	50
9	7/7/2010	0	0	0	0	52
CC1	7/7/2010	1	1	1	0	3000
CC2	7/7/2010	1	1	1	0	555
10	7/7/2010	1	1	0	0	398
10A	7/7/2010	0	0	0	0	75
AOC	7/7/2010	1	1	0	0	1080
CON	7/7/2010	1	1	0	0	4900
11	7/7/2010	0	0	0	0	32
Cthc	7/7/2010	1	0	0	0	270
Ch	7/7/2010	1	0	0	0	318
12A	7/7/2010	0	0	0	0	10
ACC	7/22/2010	1	0	0	0	5267
ACT	7/22/2010	1	0	0	0	900
7a	7/22/2010	1	0	0	0	25
9	7/22/2010	0	0	0	0	98
CC1	7/22/2010	1	0	1	0	23000
CC2	7/22/2010	1	1	1	0	3000
10	7/22/2010	0	1	1	0	328
10A	7/22/2010	0	0	0	0	33
AOC	7/22/2010	1	0	0	0	1300
11	7/22/2010	0	0	0	0	430
CON	7/22/2010	0	0	0	0	16500
12A	7/22/2010	0	0	0	0	22

MS	10	10A	AOC	Condo	11
8/21/2007	1	0	nd	0	0
8/28/2007	0	0	nd	0	0

9/3/2007	0	0	nd	0	0
9/10/2007	0	0	nd	0	0
9/17/2007	0	0	nd	0	0
9/24/2007	0	0	nd	0	0
10/1/2007	0	0	nd	0	0
10/8/2007	0	0	0	0	0
10/15/2007	0	0	0	1	0
10/22/2007	0	0	0	0	1
10/29/2007	0	0	0	0	0
11/5/2007	1	1	0	0	0
11/12/2007	0	1	1	0	1
11/26/2007	0	1	0	1	1
12/1/2007	0	1	1	0	1
1/7/2008	0	1	1	1	1
1/14/2008	1	1	1	0	0
1/28/2008	0	0	0	0	0
2/15/2008	0	0	0	0	0
3/17/2008	0	0	0	0	0
3/31/2008	0	0	0	0	0
4/28/2008	0	0	0	0	0
5/19/2008	0	0	0	0	0
5/27/2008	1	1	0	0	1
6/2/2008	0	0	0	0	0
6/23/2008	0	0	0	0	0
7/8/2008	0	0	0	0	0
7/14/2008	0	0	0	0	0
	0	0	0	0	0
8/12/2008	0	0	0	0	0
8/19/2008	0	0	0	0	0
9/8/2008	0	0	1	nd	0
9/29/2008	0	0	1	nd	0
10/20/2008	nd	0	1	nd	0
11/3/2008	nd	0	0	nd	1
11/17/2008	0	1	1	nd	0
12/3/2008	0	0	0	nd	0
1/5/2009	0	1	1	0	0
1/26/2009	0	1	0	1	1
2/16/2009	0	0	0	0	0
3/2/2009	0	1	0	0	1
4/20/2009	0	0	1	1	1
BA	10	10A	AOC	Condo	11

8/21/2007	0	0		1	0
8/28/2007	0	0		0	0
9/3/2007	0	0		0	0
9/10/2007	1	0		0	1
9/17/2007	0	1		0	0
9/24/2007	0	0		0	1
10/1/2007	0	0		1	0
10/8/2007	0	1	0	0	1
10/15/2007	0	0	0	1	0
10/22/2007	1	1	1	0	1
10/29/2007	0	0	0	0	0
11/5/2007	0	0	0	0	0
11/12/2007	0	1	1	1	1
11/26/2007	0	0	1	0	0
12/1/2007	0	0	1	0	0
1/7/2008	0	0	1	0	0
1/14/2008	1	0	0	1	0
1/28/2008	0	0	0	0	0
2/15/2008	0	0	0	0	0
3/17/2008	1	1	0	0	0
3/31/2008	0	0	0	0	0
4/28/2008	1	1	0	0	0
5/19/2008	0	0	1	1	0
5/27/2008	1	1	1	1	1
6/2/2008	0	0	0	0	0
6/23/2008	0	0	0	0	0
7/8/2008	1	1	0	0	0
7/14/2008	1	0	0	0	0
7/22/2008	0	0	0	0	0
8/12/2008	0	1	0	0	0
8/19/2008	0	0	0	0	0
9/8/2008	0	0	0		0
9/29/2008	0	0	0		0
10/20/2008		0	0		0
11/3/2008		0	0		0
11/17/2008	0	0	0		0
12/3/2008	1	0	1		0
1/5/2009	0	1	0	0	0
1/26/2009	1	1	0	0	1
2/16/2009	1	0	0	1	1
3/2/2009	1	0	0	1	0

4/20/2009	1	1	1	1	1
EN	10	10A	AOC	Condo	11
8/21/2007	10			2940	10
8/28/2007	150			17750	630
9/3/2007	200			1653	120
9/10/2007	60			1640	0
9/17/2007	0			1587	0
9/24/2007	335			4600	421
10/1/2007	60			11250	20
10/8/2007	303		7550	900	30
10/15/2007	0	39	2100	6450	200
10/22/2007	293	425	9800	10100	800
10/29/2007	580	90	7900	4150	660
11/5/2007	98	35	2600	6540	150
11/12/2007	34	89	4950	3550	73
11/26/2007	30500	1487	6033	9500	9000
12/1/2007	0	550	1061	1463	0
1/7/2008	243	83	2650	1560	43
1/14/2008	0	0	8000	6530	0
1/28/2008	11	390	1200	300	145
2/15/2008	258	239	2673		264
3/17/2008	525	187	1360	933	19
3/31/2008	237	257	1700	2650	
4/28/2008	110	195	930	2950	920
5/19/2008	20	40	847	1647	0
5/27/2008	0	0	910	1200	700
6/2/2008	230	10	1300	3350	20
6/23/2008	10	20	1800		990
7/8/2008	170	0	7667		0
7/14/2008	400	34	1100		400
7/22/2008	0		4733		2900
8/12/2008		600	7000		760
8/19/2008	330	90	1500		60
9/8/2008	200	45	1140		0
9/29/2008	0	0	1900		233
10/20/2008	0	0	12700		0
11/3/2008	160	0	900		1390
11/17/2008	0	0	420		100
12/3/2008	77	0	337		0
1/5/2009	23	33	2000	640	34
1/26/2009	5	0	5300	1240	60

2/16/2009	8	116	6733	3400	12
3/2/2009	125	64	4800	1300	0
4/20/2009	420	20	2300	4100	4100

2010

Site	FC/100ml	Solar Intensity (klux)	Water temp (°C)	Salinity (ppt)
7ACC	3500	0	N/D	N/D
7ACT	7150	0	N/D	N/D
7A	1300	N/D	N/D	N/D
9	600	N/D	N/D	N/D
CC1	3600	N/D	N/D	N/D
CC2	3700	N/D	N/D	N/D
10	218	N/D	N/D	N/D
10A	910	N/D	N/D	N/D
AOC	N/D	N/D	N/D	N/D
CON	6850	N/D	N/D	N/D
11	300	N/D	N/D	N/D
12A	2190	N/D	N/D	N/D
CTHC	333	N/D	N/D	N/D
CH	4200	N/D	N/D	N/D
7ACC	15000	N/D	N/D	N/D
7ACT	3550	N/D	N/D	N/D
7A	1800	N/D	N/D	N/D
9	393	N/D	N/D	N/D
CC1	12000	N/D	N/D	N/D
CC2	48000	N/D	N/D	N/D
10	620	N/D	N/D	N/D
10A	900	N/D	N/D	N/D
AOC	1800	N/D	N/D	N/D
CON	7500	N/D	N/D	N/D
11	163	N/D	N/D	N/D
12A	100	N/D	N/D	N/D
CTHC	1400	N/D	N/D	N/D
CH	5867	N/D	N/D	N/D
7ACC	440	N/D	N/D	N/D
7ACT	483	N/D	N/D	N/D
7A	41	N/D	N/D	N/D
9	N/D	N/D	N/D	N/D
CC1	2700	N/D	N/D	N/D
CC2	680	N/D	N/D	N/D

10	4000	N/D	N/D	N/D
10A	0	N/D	N/D	N/D
AOC	590	N/D	N/D	N/D
CON	2250	N/D	N/D	N/D
11	110	N/D	N/D	N/D
12A	13	N/D	N/D	N/D
CTHC	465	N/D	N/D	N/D
CH	760	N/D	N/D	N/D
7ACC	1180	3.35	19	0
7ACT	700	10.5	25	0
7A	120	85	26	14
9	295	112	26	18
CC1	840	90.2	24	0
CC2	1200	90.6	26	0
10	740	95.2	27	13
10A	25	110.2	28	17
AOC	330	51	27	0
CON	291	20.6	27	0
11	57	113.2	28	17
12A	1110	110.7	29	20
CTHC	1650	100.5	24	0
CH	1060	103	28	11
7ACC	135	27.3	31	0
7ACT	160	102.2	27	0
7A	378	100.5	27	0
9	200	104.2	30	0
CC1	46	70.6	28	0
CC2	1160	111.2	29	0
10	150	108	33	0
10A	22	109	32	0
AOC	2400	9.1	30	0
CON	290	104	32	0
11	15	108	30	0
12A	5	112	33	0
CTHC	20	110	31	0
CH	355	104.7	26	0
7ACC	22000	55.2	29	0
7ACT	30000	7	29	0
7A	5300	11.4	29	0
9	3650	43.5	29	30
CC1	11800	8.3	29	0

CC2	22000	13.1	29	0
10	800	35.5	29	21
10A	3000	nd	nd	29
AOC	47000	nd	nd	0
CON	55000	nd	nd	0
11	430000	nd	nd	29
12A	3000	nd	nd	27
CTHC	6000	7.8	27	0
CH	3700	44.7	28	30
7ACC	960	12.4	27.5	0
7ACT	5600	20.2	28	0
7A	402	80.9	30.5	28
9	308	93.1	30	30
CC1	330	37.1	26	0
CC2	4400	29.7	26.5	0
10	3900	87.8	30	9
10A	139	102	29	27
AOC	4200	13.6	30	0
CON	5100	93.7	30	0
11	200	83.7	32	26
12A	37	98.3	30	29
CTHC	1500	86.3	29.5	0
CH	305	84	29.5	10
7ACC	5600	4.12	26	0
7ACT	240	8.02	27	0
7A	1243	82.5	27	29
9	530	89.9	27	31
CC1	620	92	26	0
CC2	525	93	27	1
10	120	100	29	30
10A	73	109.7	27	32
AOC	1090	2.5	30	0
CON	2900	102.4	31	0
11	1600	105.4	28	29
12A	430	115	29	31
CTHC	560	94.3	26	0
CH	640	104.1	26	23
7ACC	13200	11.5	27	0
7ACT	1060	34.4	27	0
7A	172	80	30	27
9	720	45.7	30	31

CC1	806	38.5	28	0
CC2	8000	32.1	27	0
10	185	82.2	31	28
10A	3300	94.5	31.5	26
AOC	123000	34.3	31	0
CON	4100	97.5	33	0
11	260	98.5	31.5	27
12A	200	0	0	0
CTHC	4700	63.4	27	0
CH	2300	80.2	30	10
7ACC	46000	8.9	27	0
7ACT	32000	38	28	0
7A	485	15.7	30	27
9	430	19.2	28	25
CC1	27000	17.3	28	0
CC2	47000	19.5	29	0
10	570	47.4	33	24
10A	45	79.5	34	29
AOC	26000	17.9	31	0
CON	17000	121.3	33	0
11	37000	113.7	33	11
12A	160	116.4	33	26
CTHC	5500	90.6	27	0
CH	20000	95.5	36	20
7ACC	135	27.6	27	0
7ACT	2290	15	28	0
7A	22000	38.72	30	30
9	1790	103	30	26
CC1	18000	10.37	26	0
CC2	12000	20.62	27	2
10	4800	111.3	30	24
10A	135	114.5	31	27
AOC	32000	20.5	30	0
CON	5400	108.5	30.5	0
11	100	115.5	31	27
12A	800	112.5	33	26
CTHC	6000	110.3	27	0
CH	300	110	31	26
7ACC	5500	9.23	24	0
7ACT	3700	13.4	24	0
7A	510	65	26	28

9	3450	72.02	25	30
CC1	44000	17.6	24	0
CC2	4767	17.4	24	0
10	283	93.2	25	27
10A	605	27.4	26	29
AOC	22000	9.2	27	0
CON	22000	32.11	27	0
11	47	22.2	26	28
12A	665	26.8	27	29
CTHC	2600	31.6	26	0
CH	940	109.4	26	28
7ACC	2200	3.36	24	0
7ACT	3400	3.62	24	0
7A	505	68.1	25	21
9	2250	77.7	24	24
CC1	530	5.12	24	0
CC2	3400	46.5	25	0
10	2300	102.4	27	16
10A	465	113.7	28	18
AOC	2100	5.43	28	0
CON	3100	89.2	27	0
11	150	91.3	27	10
12A	320	103.2	30	19
CTHC	2600	13.4	26	0
CH	4300	74.6	26	11
7ACC	12000	5.7	23	0
7ACT	2700	75.1	23	0
7A	18	72.7	26	25
9	92	78.1	24	23
CC1	750	6.1	22	0
CC2	3700	81	21	0
10	395	101.2	24	22
10A	860	103.2	26	23
AOC	740	83.6	25	0
CON	2500	102.7	25	0
11	225	102.7	24	23
12A	140	82.1	27	21
CTHC	1100	67.4	25	0
CH	1650	88.9	27	21
7ACC	59000	5.75	19	0
7ACT	29000	56.3	19	0

7A	13	67.3	20	24
9	251	75.6	20	24
CC1	1200	3.81	19	0
CC2	7967	73.6	20	0
10	165	78.6	21	20
10A	48	85.2	22	23
AOC	43000	68.4	23	0
CON	43000	74.5	24	0
11	5700	79.7	24	3
12A	0	88.8	23	21
CTHC	3300	37.5	22	0
CH	180	87.9	21	23
7ACC	2800	2.67	17	0
7ACT	827	55.6	15	0
7A	340	80.6	16	15
9	23	78.5	16	24
CC1	1680	3.12	15	0
CC2	820	65.7	15	0
10	80	0	0	22
10A	15	80	18	23
AOC	3100	77.4	21	0
CON	5267	78.2	19	0
11	76	88.7	18	21
12A	18	94.3	19	23
CTHC	860	63.7	20	0
CH	25	79.5	18	22

Site	Direction	F(1)/E(0)	Wind/Direction	Rainfall sum (-2,-1,0)
7ACC	Flow	1	4-17(ESE)	0,0,0
7ACT	Flow	1	4-17(ESE)	0,0,0
7A	Flow	1	4-17(ESE)	0,0,0
9	Flow	1	4-17(ESE)	0,0,0
CC1	Flow	1	4-17(ESE)	0,0,0
CC2	Flow	1	4-17(ESE)	0,0,0
10	Flow	1	4-17(ESE)	0,0,0
10A	Flow	1	4-17(ESE)	0,0,0
AOC	Flow	1	4-17(ESE)	0,0,0
CON	Flow	1	4-17(ESE)	0,0,0
11	Flow	1	4-17(ESE)	0,0,0
12A	Flow	1	4-17(ESE)	0,0,0
CTHC	Flow	1	4-17(ESE)	0,0,0

CH	Flow	1	4-17(ESE)	0,0,0
7ACC	Flow	1	6-13(SSW)	0, 0.27, 0
7ACT	Flow	1	6-13(SSW)	0, 0.27, 0
7A	Flow	1	6-13(SSW)	0, 0.27, 0
9	Flow	1	6-13(SSW)	0, 0.27, 0
CC1	Flow	1	6-13(SSW)	0, 0.27, 0
CC2	Flow	1	6-13(SSW)	0, 0.27, 0
10	Flow	1	6-13(SSW)	0, 0.27, 0
10A	Flow	1	6-13(SSW)	0, 0.27, 0
AOC	Flow	1	6-13(SSW)	0, 0.27, 0
CON	Flow	1	6-13(SSW)	0, 0.27, 0
11	Flow	1	6-13(SSW)	0, 0.27, 0
12A	Flow	1	6-13(SSW)	0, 0.27, 0
CTHC	Flow	1	6-13(SSW)	0, 0.27, 0
CH	Flow	1	6-13(SSW)	0, 0.27, 0
7ACC	Ebb	0	0-10(S)	0,0,0
7ACT	Ebb	0	0-10(S)	0,0,0
7A	Ebb	0	0-10(S)	0,0,0
9	Ebb	0	0-10(S)	0,0,0
CC1	Ebb	0	0-10(S)	0,0,0
CC2	Ebb	0	0-10(S)	0,0,0
10	Ebb	0	0-10(S)	0,0,0
10A	Ebb	0	0-10(S)	0,0,0
AOC	Ebb	0	0-10(S)	0,0,0
CON	Ebb	0	0-10(S)	0,0,0
11	Ebb	0	0-10(S)	0,0,0
12A	Ebb	0	0-10(S)	0,0,0
CTHC	Ebb	0	0-10(S)	0,0,0
CH	Ebb	0	0-10(S)	0,0,0
7ACC	Flow	1	0-12(SSW)	0,0,0
7ACT	Flow	1	0-12(SSW)	0,0,0
7A	Flow	1	0-12(SSW)	0,0,0
9	Flow	1	0-12(SSW)	0,0,0
CC1	Flow	1	0-12(SSW)	0,0,0
CC2	Flow	1	0-12(SSW)	0,0,0
10	Flow	1	0-12(SSW)	0,0,0
10A	Flow	1	0-12(SSW)	0,0,0
AOC	Flow	1	0-12(SSW)	0,0,0
CON	Flow	1	0-12(SSW)	0,0,0
11	Flow	1	0-12(SSW)	0,0,0
12A	Flow	1	0-12(SSW)	0,0,0

CTHC	Flow	1	0-12(SSW)	0,0,0
CH	Flow	1	0-12(SSW)	0,0,0
7ACC	Flow	1	3-10(SW)	0,0,0
7ACT	Flow	1	3-10(SW)	0,0,0
7A	Flow	1	3-10(SW)	0,0,0
9	Flow	1	3-10(SW)	0,0,0
CC1	Flow	1	3-10(SW)	0,0,0
CC2	Flow	1	3-10(SW)	0,0,0
10	Flow	1	3-10(SW)	0,0,0
10A	Flow	1	3-10(SW)	0,0,0
AOC	Flow	1	3-10(SW)	0,0,0
CON	Flow	1	3-10(SW)	0,0,0
11	Flow	1	3-10(SW)	0,0,0
12A	Flow	1	3-10(SW)	0,0,0
CTHC	Flow	1	3-10(SW)	0,0,0
CH	Flow	1	3-10(SW)	0,0,0
7ACC	Flow	1	4-13(WNW)	2.12, 0.19, 0.23
7ACT	Flow	1	4-13(WNW)	2.12, 0.19, 0.23
7A	Flow	1	4-13(WNW)	2.12, 0.19, 0.23
9	Flow	1	4-13(WNW)	2.12, 0.19, 0.23
CC1	Flow	1	4-13(WNW)	2.12, 0.19, 0.23
CC2	Flow	1	4-13(WNW)	2.12, 0.19, 0.23
10	Flow	1	4-13(WNW)	2.12, 0.19, 0.23
10A	Flow	1	4-13(WNW)	2.12, 0.19, 0.23
AOC	Flow	1	4-13(WNW)	2.12, 0.19, 0.23
CON	Flow	1	4-13(WNW)	2.12, 0.19, 0.23
11	Flow	1	4-13(WNW)	2.12, 0.19, 0.23
12A	Flow	1	4-13(WNW)	2.12, 0.19, 0.23
CTHC	Flow	1	4-13(WNW)	2.12, 0.19, 0.23
CH	Flow	1	4-13(WNW)	2.12, 0.19, 0.23
7ACC	Ebb	0	2-12(NNW)	0, 0.22, 0.08
7ACT	Ebb	0	2-12(NNW)	0, 0.22, 0.08
7A	Ebb	0	2-12(NNW)	0, 0.22, 0.08
9	Ebb	0	2-12(NNW)	0, 0.22, 0.08
CC1	Ebb	0	2-12(NNW)	0, 0.22, 0.08
CC2	Ebb	0	2-12(NNW)	0, 0.22, 0.08
10	Ebb	0	2-12(NNW)	0, 0.22, 0.08
10A	Ebb	0	2-12(NNW)	0, 0.22, 0.08
AOC	Ebb	0	2-12(NNW)	0, 0.22, 0.08
CON	Ebb	0	2-12(NNW)	0, 0.22, 0.08
11	Ebb	0	2-12(NNW)	0, 0.22, 0.08

12A	Ebb	0	2-12(NNW)	0, 0.22, 0.08
CTHC	Ebb	0	2-12(NNW)	0, 0.22, 0.08
CH	Ebb	0	2-12(NNW)	0, 0.22, 0.08
7ACC	Flow	1	2-12(S)	0,0,0
7ACT	Flow	1	2-12(S)	0,0,0
7A	Flow	1	2-12(S)	0,0,0
9	Flow	1	2-12(S)	0,0,0
CC1	Flow	1	2-12(S)	0,0,0
CC2	Flow	1	2-12(S)	0,0,0
10	Flow	1	2-12(S)	0,0,0
10A	Flow	1	2-12(S)	0,0,0
AOC	Flow	1	2-12(S)	0,0,0
CON	Flow	1	2-12(S)	0,0,0
11	Flow	1	2-12(S)	0,0,0
12A	Flow	1	2-12(S)	0,0,0
CTHC	Flow	1	2-12(S)	0,0,0
CH	Flow	1	2-12(S)	0,0,0
7ACC	Ebb	0	0-13(S)	0.02, 0, 0.3
7ACT	Ebb	0	0-13(S)	0.02, 0, 0.3
7A	Ebb	0	0-13(S)	0.02, 0, 0.3
9	Ebb	0	0-13(S)	0.02, 0, 0.3
CC1	Ebb	0	0-13(S)	0.02, 0, 0.3
CC2	Ebb	0	0-13(S)	0.02, 0, 0.3
10	Ebb	0	0-13(S)	0.02, 0, 0.3
10A	Ebb	0	0-13(S)	0.02, 0, 0.3
AOC	Ebb	0	0-13(S)	0.02, 0, 0.3
CON	Ebb	0	0-13(S)	0.02, 0, 0.3
11	Ebb	0	0-13(S)	0.02, 0, 0.3
12A	Ebb	0	0-13(S)	0.02, 0, 0.3
CTHC	Ebb	0	0-13(S)	0.02, 0, 0.3
CH	Ebb	0	0-13(S)	0.02, 0, 0.3
7ACC	Ebb	0	1-21(WNW)	0.12, 0, 0.01
7ACT	Ebb	0	1-21(WNW)	0.12, 0, 0.01
7A	Ebb	0	1-21(WNW)	0.12, 0, 0.01
9	Ebb	0	1-21(WNW)	0.12, 0, 0.01
CC1	Ebb	0	1-21(WNW)	0.12, 0, 0.01
CC2	Ebb	0	1-21(WNW)	0.12, 0, 0.01
10	Ebb	0	1-21(WNW)	0.12, 0, 0.01
10A	Ebb	0	1-21(WNW)	0.12, 0, 0.01
AOC	Ebb	0	1-21(WNW)	0.12, 0, 0.01
CON	Ebb	0	1-21(WNW)	0.12, 0, 0.01

11	Ebb	0	1-21(WNW)	0.12, 0, 0.01
12A	Ebb	0	1-21(WNW)	0.12, 0, 0.01
CTHC	Ebb	0	1-21(WNW)	0.12, 0, 0.01
CH	Ebb	0	1-21(WNW)	0.12, 0, 0.01
7ACC	Ebb	0	1-13(ESE)	0.02, 0.57, 0.71
7ACT	Ebb	0	1-13(ESE)	0.02, 0.57, 0.71
7A	Ebb	0	1-13(ESE)	0.02, 0.57, 0.71
9	Ebb	0	1-13(ESE)	0.02, 0.57, 0.71
CC1	Ebb	0	1-13(ESE)	0.02, 0.57, 0.71
CC2	Ebb	0	1-13(ESE)	0.02, 0.57, 0.71
10	Ebb	0	1-13(ESE)	0.02, 0.57, 0.71
10A	Ebb	0	1-13(ESE)	0.02, 0.57, 0.71
AOC	Ebb	0	1-13(ESE)	0.02, 0.57, 0.71
CON	Ebb	0	1-13(ESE)	0.02, 0.57, 0.71
11	Ebb	0	1-13(ESE)	0.02, 0.57, 0.71
12A	Ebb	0	1-13(ESE)	0.02, 0.57, 0.71
CTHC	Ebb	0	1-13(ESE)	0.02, 0.57, 0.71
CH	Ebb	0	1-13(ESE)	0.02, 0.57, 0.71
7ACC	Flow	1	6-12(NE	0,0,0
7ACT	Flow	1	6-12(NE	0,0,0
7A	Flow	1	6-12(NE	0,0,0
9	Flow	1	6-12(NE	0,0,0
CC1	Flow	1	6-12(NE	0,0,0
CC2	Flow	1	6-12(NE	0,0,0
10	Flow	1	6-12(NE	0,0,0
10A	Flow	1	6-12(NE	0,0,0
AOC	Flow	1	6-12(NE	0,0,0
CON	Flow	1	6-12(NE	0,0,0
11	Flow	1	6-12(NE	0,0,0
12A	Flow	1	6-12(NE	0,0,0
CTHC	Flow	1	6-12(NE	0,0,0
CH	Flow	1	6-12(NE	0,0,0
7ACC	Flow	1	3-10(S)	0.01,0,0
7ACT	Flow	1	3-10(S)	0.01,0,0
7A	Flow	1	3-10(S)	0.01,0,0
9	Flow	1	3-10(S)	0.01,0,0
CC1	Flow	1	3-10(S)	0.01,0,0
CC2	Flow	1	3-10(S)	0.01,0,0
10	Flow	1	3-10(S)	0.01,0,0
10A	Flow	1	3-10(S)	0.01,0,0
AOC	Flow	1	3-10(S)	0.01,0,0

CON	Flow	1	3-10(S)	0.01,0,0
11	Flow	1	3-10(S)	0.01,0,0
12A	Flow	1	3-10(S)	0.01,0,0
CTHC	Flow	1	3-10(S)	0.01,0,0
CH	Flow	1	3-10(S)	0.01,0,0
7ACC	Ebb	0	0-8(SSE)	0,0,0
7ACT	Ebb	0	0-8(SSE)	0,0,0
7A	Ebb	0	0-8(SSE)	0,0,0
9	Ebb	0	0-8(SSE)	0,0,0
CC1	Ebb	0	0-8(SSE)	0,0,0
CC2	Ebb	0	0-8(SSE)	0,0,0
10	Ebb	0	0-8(SSE)	0,0,0
10A	Ebb	0	0-8(SSE)	0,0,0
AOC	Ebb	0	0-8(SSE)	0,0,0
CON	Ebb	0	0-8(SSE)	0,0,0
11	Ebb	0	0-8(SSE)	0,0,0
12A	Ebb	0	0-8(SSE)	0,0,0
CTHC	Ebb	0	0-8(SSE)	0,0,0
CH	Ebb	0	0-8(SSE)	0,0,0
7ACC	Ebb	0	6-12(SSE)	0,0,0
7ACT	Ebb	0	6-12(SSE)	0,0,0
7A	Ebb	0	6-12(SSE)	0,0,0
9	Ebb	0	6-12(SSE)	0,0,0
CC1	Ebb	0	6-12(SSE)	0,0,0
CC2	Ebb	0	6-12(SSE)	0,0,0
10	Ebb	0	6-12(SSE)	0,0,0
10A	Ebb	0	6-12(SSE)	0,0,0
AOC	Ebb	0	6-12(SSE)	0,0,0
CON	Ebb	0	6-12(SSE)	0,0,0
11	Ebb	0	6-12(SSE)	0,0,0
12A	Ebb	0	6-12(SSE)	0,0,0
CTHC	Ebb	0	6-12(SSE)	0,0,0
CH	Ebb	0	6-12(SSE)	0,0,0
7ACC	Ebb	0	6-12(SSE)	0,0,0
7ACT	Ebb	0	6-12(SSE)	0,0,0
7A	Ebb	0	6-12(SSE)	0,0,0
9	Ebb	0	6-12(SSE)	0,0,0
CC1	Ebb	0	6-12(SSE)	0,0,0
CC2	Ebb	0	6-12(SSE)	0,0,0
10	Ebb	0	6-12(SSE)	0,0,0
10A	Ebb	0	6-12(SSE)	0,0,0

AOC	Ebb	0	6-12(SSE)	0,0,0
CON	Ebb	0	6-12(SSE)	0,0,0
11	Ebb	0	6-12(SSE)	0,0,0
12A	Ebb	0	6-12(SSE)	0,0,0
CTHC	Ebb	0	6-12(SSE)	0,0,0
CH	Ebb	0	6-12(SSE)	0,0,0

Site	FC/100ml	Salinity	Turbidity	UV
ACC(4/21)	3500	—	—	724
ACT	2900	—	—	1857
7A	800	—	—	>2000
9	21000	—	—	526
CC1	700	—	—	>2000
10	1000	—	—	>2000
CC2	2250	—	—	>2000
AOC	1650	—	—	>2000
Con	3900	—	—	>2000
11	4700	—	—	>2000
Cthc	3000	—	—	>2000
12A	540	—	—	>2000
Ch	2300	—	—	>2000
ACT(5/26)	15	0	11.7	597
ACC	165	0	19.8	584
7A	270	15	40.9	>2000
9	0	17	23.3	>2000
CC1	290	0	17.2	>2000
CC2	—	—	nd	>2000
10	660	17	45.1	>2000
AOC	110	0	29.5	>2000
Con	460	0	23.9	>2000
11	3100	7	43.4	>2000
Cthc	5500	0	24.7	>2000
Ch	13	11	25	>2000
12A	620	15	46.2	>2000
ACC(6/2)	2900	0	14.2	1056
ACT	165	0	9.47	1218
7A	430	10	13.1	>2000
9	25	21	13	>2000
10	45	16	10.2	>2000
10A	20	18	16.1	>2000
CC1	338	0	16.9	>2000

CC2	273	0	16.5	>2000
11	900	10	27.7	>2000
CON	3400	0	20.3	>2000
AOC	2000	0	32.4	>2000
CTHC	3900	0	22.5	>2000
CH	105	5	24.5	>2000
ACC(6/14)	—	0	15.4	437
12A(6/2)	200	15	33.2	>2000
ACT(6/14)	—	0	13.1	639
7A	25	12	8.96	>2000
9	363	15	14.5	>2000
CC2	358	0	18.1	>2000
CC1	1440	0	18	>2000
10	115	13	30.5	>2000
CON	2700	0	20.3	>2000
11	530	5	21.1	>2000
AOC	258	0	27.2	>2000
10A	—	—	—	>2000
12A	13	11	19.5	>2000
ACC(6/9)	1700	0	15.4	772
ACT	1600	0	20.3	1963
7A	—	15	6.7	>2000
9	23	15	9.4	>2000
CC1	—	0	16.3	870
CC2	—	2	17.5	1862
10	363	8	6.8	—
10A	8	13	11.3	>2000
AOC	4000	0	23	876
CON	1350	0	20.5	>2000
11	—	5	10.1	>2000
Cthc	172	12	7	>2000
Ch	700	0	19.3	>2000
12A	500	7	9.3	>2000
ACC(6/24)	0	0	15.4	650
ACT	5000	0	10.5	>2000
7A	433	16	12	>2000
9	105	16	10.2	>2000
CC1	22500	0	13.9	584
CC2	15000	0	15	567
10	10000	9	17.7	>2000
10A	320	15	9.59	>2000

AOC	—	0	26.3	>2000
CON	835	0	18.8	>2000
11	4500	8	14.9	>2000
Cthc	2000	0	23.8	>2000
Ch	20367	10	15.5	>2000
12A	200	12	13.8	>2000
ACC(6/30)	4200	0	—	52
ACT	4600	—	—	82
7A	3100	15	—	124
9	3940	14	—	52
CC1	21000	0	—	54
CC2	16800	—	—	55
10	430	—	—	184
10A	83	15	—	137
AOC	16000	—	—	—
CON	—	—	—	—
11	3700	12	—	—
12A	24000	10	—	—
ACC(7/7)	270	0	15.6	300
ACT	318	0	14.4	555
7A	338	16	30.2	>2000
9	110	12	28.1	>2000
CC1	267	0	29.8	>2000
CC2	480	0	15.1	>2000
10	297	5	32.6	>2000
10A	140	13	69.1	>2000
AOC	540	0	24	>2000
CON	3000	0	22	>2000
11	372	14	69.9	>2000
Cthc	110	0	25.8	>2000
Ch	1100	8	58.7	>2000
12A	20	11	26.7	>2000
ACC(7/22)	1950	2	17.8	452
ACT	440	0	11.4	1680
7a	60	18	19.2	>2000
9	275	18	30.2	>2000
CC1	45000	1	305	927
CC2	2300	1	38.8	690
10	4167	10	30.9	>2000
10A	135	16	40.9	>2000
AOC	8600	2	22.3	948

11	235	15	37.4	>2000
CON	3700	3	18.3	>2000
12A	120	15	10.7	>2000

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EVALUATION OF HUMAN FECAL POLLUTION IN MISSISSIPPI COASTAL

AND CREEK WATERS USING LIBRARY INDEPENDENT MARKERS

by

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ABSTRACT

EVALUATION OF HUMAN FECAL POLLUTION IN MISSISSIPPI COASTAL AND CREEK WATERS USING LIBRARY INDEPENDENT MARKERS

by Christopher John Flood

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The objective of this study was to determine whether statistically valid correlations could be elucidated between standard indicator bacteria (enterococci and fecal coliforms) from coastal creek and marine samples and the presence of four library independent molecular markers that are human or sewage specific. Eight hundred and nineteen samples were collected between August 2007 and July 2010 to determine enterococcal and fecal coliform counts and the presence of genetic markers for sewage indicator organisms *Methanobrevibacter smithii*, human specific *Bacteroides* sp., *Bacteroides thetaiotaomicron*, and *Fecalibacterium* sp. During the course of this study environmental parameters were measured and statistically analyzed to determine if there was any correlation for the presence of any one of these organisms and the environmental variables.