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ORIGINAL RESEARCH

Molecular Approach to Microbiological Examination of Water Quality in the Grand Bay National Estuarine Research Reserve (NERR) in Mississippi, USA

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Abstract: Grand Bay National Estuarine Research Reserve (NERR) is an important ecosystem in the Mississippi Gulf Coast. It serves as important nursery areas for juveniles of many species of fish. The bay is also used for fishing, crabbing, oyster togging, boating as well as recreation. Like in other aquatic environments, this bay may be contaminated by microorganisms including pathogenic bacteria. The objective of this study was to evaluate the microbiological quality of water in the Grand Bay NERR and determine the levels and potential source(s) of human fecal pollution. To achieve this goal, water samples were collected aseptically every month in Bayou Heron, Bayou Cumbest, Point Aux Chenes Bay and Bangs Lake. Enterococci were concentrated from water samples by membrane filtration according to the methodology outlined in USEPA Method 1600. After incubation, DNA was extracted from bacteria colonies on the membrane filters by using QIAamp DNA extraction kit. Water samples were also tested for the presence of traditional indicator bacteria including: heterotrophic plate count, total coliforms, fecal coliforms, and *Enterococcus* bacteria. The marker *esp* gene was detected in one site of Bayou Cumbest, an area where human populations reside. Data from this study indicates higher concentrations of indicator bacteria compared to the recommended acceptable levels. Presence of *esp* marker and high numbers of indicator bacteria suggest a public health concern for shellfish and water contact activities. Hence, control strategies should be developed and implemented to prevent further contamination of the Grand bay NERR waters.

Keywords: Grand Bay NERR, recreation, molecular probing, *esp* gene, bacteria, public health

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Introduction

The Grand Bay National Estuarine Research Reserve (NERR) is an important ecosystem in the Mississippi Gulf Coast that lies within the gently sloping, lower Gulf coastal plain. It serves as an important nursery ground for juveniles of many species of fish. The bay is also used for fishing, crabbing, oyster togging, boating as well as recreation activities. Water contact activities like bathing and swimming in recreational waters have been associated with a broad spectrum of illnesses.[1]

Fecal contamination is one of the major concerns in relation to water bodies used for public water supply, shellfish harvesting, and recreation activities due to a wide array of pathogenic bacteria, viruses, and protozoa.[2] Non-point sources such as domestic, wild animals and birds defecation, malfunctioning septic trenches,[3] defective on-site wastewater treatment systems, agricultural run-off, storm water drainage, and/or point sources such as municipal wastes and industrial effluents are known to be potential sources of such contamination.[4] Moreover, non-point sources of contamination are of significant concern with respect to the dissemination of pathogens and their indicators in the water systems.[5]

In general, human fecal wastes give rise to the highest risk of waterborne diseases, environmental degradation, and economic losses[6,7] due to closures of beaches and shellfish harvesting areas.[8] Because of the seriousness of the diseases caused by water borne-bacteria and the increasingly growing importance of water in human life, accurate and reliable methods for detecting fecal pollution are needed to reduce its occurrence and take legal measures. Frequent assessment of fecal indicator bacteria levels is recommended to ensure better understanding of microbial water quality and prevent human exposure to pathogenic bacteria. Fecal coliforms, enterococci, total coliforms, and *Escherichia coli* have traditionally been used as microbial fecal indicators in water.[6,9] The presence of these indicators in water bodies generally points to fecal pollution and potential public health concern.

Monitoring for indicator bacteria is less expensive and easier than monitoring pathogenic bacteria and while still providing useful indication of the relative safety for recreational use of water bodies.[10] However, these microorganisms fail to fulfill the criteria of an ideal microbial indicator.[11] For example, they have short survival rate in water bodies, non-fecal sources, low ability to multiply once released in to the water column, low levels of correlation with the presence of pathogens, low sensitivity to detections methods, susceptibility to disinfection processes, and inability to delineate the sources of fecal contamination in water into point and non-point classes.[5,9] Water resources can be impacted by multiple sources of fecal pollution making it extremely difficult to implement a robust management plan without understanding the potential sources of pollution.[2] Therefore to identify indicator bacteria point source is a vital component for effectively assessing health risks and pursuing remediation measures.[12]

In recent years, several library-independent microbial source tracking methods have been developed to distinguish the various sources of animal and/or human fecal contamination. These methods include F^+-specific RNA coliphages, antibiotic resistance, human-specific *Bacteroides* HF183, human-specific adenoviruses, human-specific polyomaviruses, and human-specific *Enterococcus faecium esp* gene.[13–15] Chemical methods such as the detection of caffeine and sterol have also been used to detect sources of fecal contamination in surface water, however it requires stringent sampling, can be expensive,[4] and is not sensitive enough to detect recent pollution.[8] Human-specific *esp* marker was used in this study because of its high specificity to distinguish between human and animal sources of fecal pollution.[16]

Bayou Cumbest and Bangs Lake in Grand Bay watershed were highly ranked on the Mississippi 1996 Section 303(d) List of water bodies based on elevated levels of fecal coliform bacteria. This watershed is classified for shellfish harvest but is often closed to harvest due to elevated levels of coliform bacteria. However, little is currently known about the microbiological quality of water in the Grand Bay NERR. Therefore, our goal for the present study was to evaluate its microbiological water quality and to subsequently determine the levels and potential source of fecal pollution.

Materials and Methods

Water quality monitoring data collected from permanent stations in various sites of the Grand Bay NERR (Fig. 1) were used for assessing concentrations

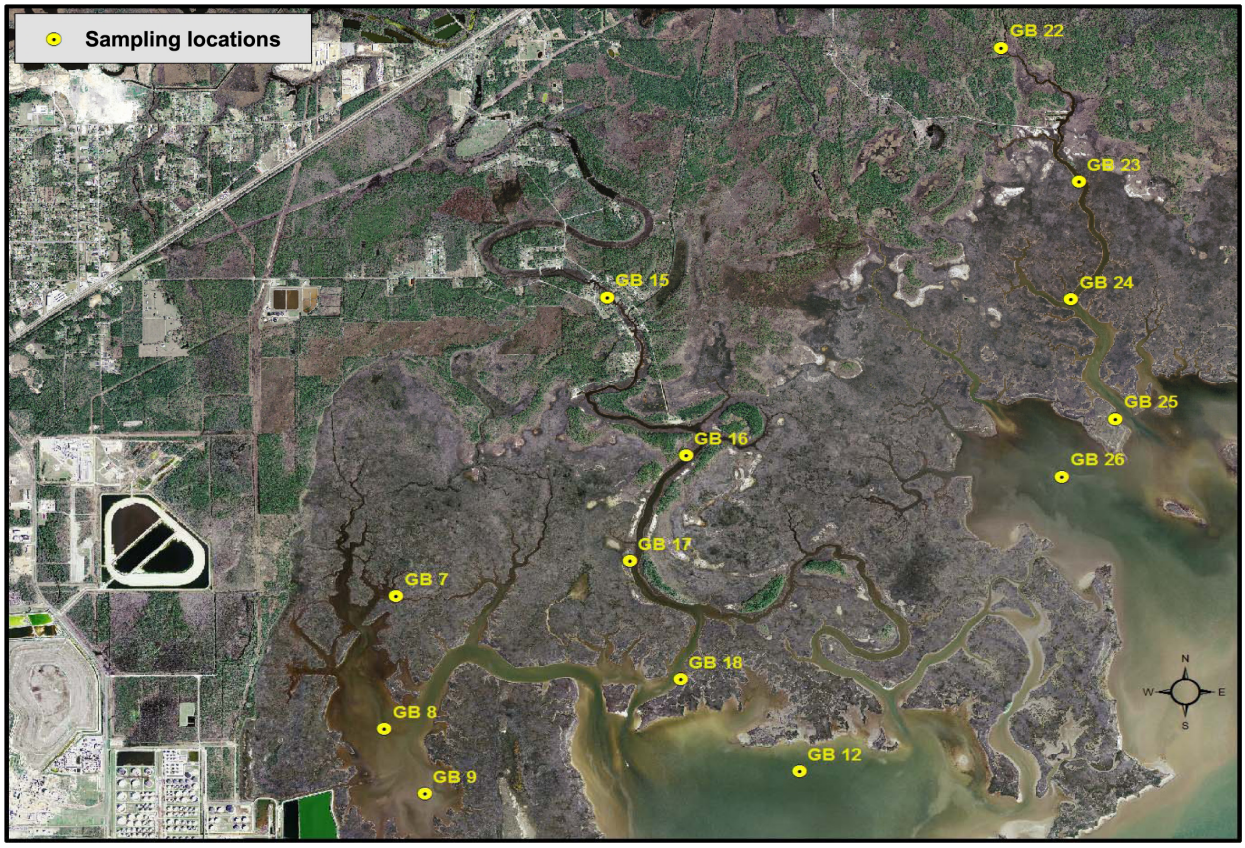

Figure 1. Presents a map of Grand Bay NERR showing the four sampling sites including Bayou Heron, Bayou Cumbest, Bangs Lake and Point Aux Chenes Bay.

of indicator bacteria (heterotrophic bacteria, total coliforms, fecal coliforms, and *Enterococcus* bacteria) and to evaluate the presence of *E. faecium esp* gene in the Grand Bay NERR waters. The presence of *esp* marker indicates human fecal contamination in the water.

Sampling stations and study sites

Figure 1 presents a map of Grand Bay NERR showing the four sampling sites including Bayou Heron, Bayou Cumbest, Bangs Lake, and Point Aux Chenes Bay. Four sites were randomly selected in Bayou Heron; the sites included GB22, GB23, GB24, and GB25. Although there are no major sources of freshwater entering Bayou Heron, it has however been concluded that some source of freshwater exists close to the bottom of the bayou (Grand Bay NERR reports). Bayou Cumbest (GB15, GB16, GB17, and GB18) is relatively small and has slow-moving, tea-colored waters that are rich in tannin, a natural by-product of decaying vegetation. This area is the most developed (human residing) in the Grand Bay. Bangs Lake is another location selected that is very close to a multinational oil refining company. Three sites were selected from the lake (GB7, GB8, and GB9) to assess the potential impact of the industry on the microbial water quality. Because of accessibility problem, only one site (GB12) was selected to represent Point Aux Chenes Bay.

Sample collection

Water samples were collected in duplicate at each site in sterile 250 mL screw-caped plastic bottles using an environmental water pump sampler (model 7518-02) from Cole-Parmer Instrument Company. Immediately after collection, samples were transported in a cooler with ice packs at 4 °C to the Environmental Microbiology Research Laboratory at Jackson State University, where they were processed.

Bacterial strains

E. faecium strain C68, which contains the marker *esp* gene was used as a positive control in all polymerase chain reaction (PCR) reactions, was kindly provided

by Dr. Troy M. Scott of the Biological Consulting Services of N. Florida, Inc.

Sample preparation for bacteriological assessment

Water samples were processed within eight hours of collection to determine the concentrations of heterotrophic bacteria (HPC), total coliforms (TC), fecal coliforms (FC), and enterococci (ENT). The samples were processed using membrane filtration method, protocol 9215D, 9222B, 9222D[17] and USEPA Method 1600 for testing HPC, TC, FC, and ENT, respectively. In these techniques, 10 to 100 mL of water samples was used to enumerate enterococci and fecal coliforms. Ten milliliters of the sample was used to enumerate total coliforms while one milliliter of the sample (1 mL of the sample was mixed with 10 mL of the distilled water to cover the whole surface of the membrane) was used for heterotrophic bacteria. The measured amount of water was passed through a membrane filter of 0.45 µm that trapped bacteria on its surface.[18]

Water samples were filtered under partial vacuum (using vacuum pressure station) through a sterilized glass microanalysis filter holder assembly (Fisher Scientific, USA). Sterilized 0.45 µm, 47 mm membrane filters mounted to the microanalysis filter holder assembly were used to trap bacteria. After filtration, the filters were removed from the holder using sterilized forceps, transferred to petri dishes and placed on the m-HPC, m-Endo, m-FC and mEI agar (Difco) for HPC, TC, FC, and ENT bacteria respectively. The petri dishes were then placed inverted in a plastic bag containing moistened paper towels and incubated in isotemp incubator (Fisher Scientific, USA) for 24 hrs at 35 °C for the total coliforms and heterotrophic bacteria. An ordinary Shelb-Lab water bath (Sheldon manufacturing Inc.) was used to incubate fecal coliforms at a temperature of 44.5 °C for 24 hrs.

Isolation of genomic DNA from gram-positive bacteria

Enterococci were concentrated from water samples by membrane filtration. Filters were incubated at 41 °C for 24 hrs on Difco-mEI Agar, according to the methodology outlined in USEPA Method 1600. After incubation, DNA was extracted from bacteria colonies on the membrane filters by using QIAamp DNA extraction kit according to manufacturer's instructions (Qiagen, Inc.).

PCR primers and reaction conditions

Primers specific for the *esp* gene in *E. faecium* have been previously developed and tested for specificity to human fecal pollution.[16] The forward primer designed in this study, which is specific for the *E. faecium esp* gene, was (5′-TAT GAA AGC AAC AGC ACA AGT T-3′) and conserved reverse primer (5′-ACG TCG AAA GTT CGA TTT CC-3′), developed previously by Hammerum and Jensen,[19] was used for all reactions. PCR reactions were prepared in a 25 µL reaction volume. The reaction mixture contained 12.5 µL Go Taq colorless master mix (2X) (Qiagen), 0.3 µM of each primer, and 5 µL of template DNA. Amplification was performed with an initial denaturing step at 95 °C for 2 min (to activate Taq polymerase), followed by 35 cycles of 94 °C for one min; 58 °C for one min was used to optimize annealing conditions and 72 °C for one min for extension. PCR products were separated on a 1% agarose gel stained with ethidium bromide and viewed under UV light. The PCR product from the *E. faecium* C68 was purified using a QIAquick PCR Purification Kit (Qiagen, Inc.).

Minimum detection limit and persistence of marker in the Grand Bay NERR water

Sewage sample was collected from the primary influent of Mississippi waste water treatment plant. A ratio of 1:1 sewage (50 mL) to distilled water/Grand Bay NERR water (50 mL) was used. Each sample type was serially diluted (10^{-1}–10^{-5}), and 1 mL of each dilution was passed through the membrane filters and placed on the mEI media as described above. Each filter was enumerated for enterococci and was prepared for analysis by PCR. Total viable enterococci were then compared to PCR results in order to estimate the colony densities that must be present to ensure detection of the human associated marker.

To determine persistence of the marker (*esp* gene) in the water, water samples from Grand Bay NERR (n = 3) and tap water (n = 3) were autoclaved at 121 °C for 30 min and exposed under UV light for 1 hr to minimize background target DNA that could be present due to fecal pollution.[16] 990 mL of each

type of sample were spiked with 10 mL of fresh sewage and were incubated in a water bath at 35 °C at intervals of 0, 3, 5, 7, and 10 days. Samples were then processed and analyzed for enterococci and the *esp* gene by plate counts and PCR respectively.

Statistical analysis

To enable meaningful statistical evaluations, all bacteriological data from Grand Bay NERR were transformed prior to statistical analyses by conversion to a logarithmic$_{10}$ scale.[20] This transformation is also used when the standard deviations of the data are proportional to the means.[18] Comparisons of the data among sites were made by the analysis of variance (ANOVA) and Tukey test. Statistical difference was assessed at $P < 0.05$ (95% probability). All statistical analyses were performed using SAS Computer Software Program.

Results

Microbiological assessment of water quality

Heterotrophic bacteria, total coliforms, fecal coliforms, and enterococci were assessed in Bayou Heron, Bayou Cumbest, Bangs Lake and Point Aux Chenes Bay sites. Assessment data were then compared to the recommended standards and guidelines set by Mississippi Department of Environmental Quality (MDEQ) and United States Environmental Protection Agency (USEPA). The USEPA[21] has set an acceptable HPC standard of 50,000 CFU/100 mL. The findings from this study exceed the standard in Bayou Heron, Bangs Lake, and Bayou Cumbest in 2009. Generally, when heterotrophic bacteria count data were pooled in all the sites of the Grand Bay, the counts significantly varied in Point Aux Chenes Bay ($P = 0.0002$) compared to the other sites of the Grand Bay NERR (Table 1). This study also indicates a significant difference in mean heterotrophic bacteria counts between Bayou Heron/Bayou Cumbest and Bangs Lake/Point Aux Chenes Bay ($P < 0.0001$) in 2010 (Table 2).

The mean concentrations of TC in all the sites exceeded the maximum recommended levels of <500 CFU/100 mL (Tables 1 and 2). As observed in heterotrophic bacteria counts, high concentrations of total coliforms were also observed in Bayou Cumbest and Bayou Heron followed by Bangs Lake and Point Aux Chenes Bay respectively. When the mean values from the four sites were analyzed, significant differences in the concentrations of total coliforms were observed between the sites ($P < 0.0001$).

The mean levels of FC concentrations were calculated in all the four sampling sites of the Grand Bay NERR (Tables 1 and 2). Bayou Cumbest showed high mean counts of FC (211 ± 260 CFU/100 mL) followed by Bayou Heron (151 ± 149 CFU/100 mL), Bangs Lake (49 ± 59 CFU/100 mL), and Point Aux Chenes Bay (31 ± 33 CFU/100 mL) (Table 1). The numbers of FC counts ranged from 0 to 1010 CFU/100 mL of water. The results of this study also indicated high numbers of FC in Bayou Cumbest (197 ± 156 CFU/100 mL) and Bayou Heron (181 ± 110 CFU/100 mL) in 2010 (Table 2).

Distribution of ENT in the Grand Bay NERR was analyzed in 2009 where the mean concentrations of *Enterococcus* were relatively high in Bayou Cumbest (92 ± 100 CFU/100 mL) compared to Bayou Heron (55 ± 64 CFU/100 mL), Bangs Lake (20 ± 20 CFU/100 mL), and Point Aux Chenes Bay (10 ± 10 CFU/100 mL), respectively (Table 1). Although the number of ENT was slightly lower in 2010, a similar trend was observed where the *Enterococcus* concentrations were higher in Bayou

Table 1. Concentration of indicator bacteria (CFU/100 mL) in the Grand Bay NERR.

Sampling sites	HPC	TC	FC	ENT
Bayou Heron	28575 ± 51290[a]	1608 ± 881[a,b]	151 ± 149[a]	55 ± 64[a]
Bayou Cumbest	24617 ± 56352[a]	1686 ± 2025[a]	211 ± 260[a]	92 ± 100[a]
Bangs Lake	31740 ± 54311[a]	992 ± 561[b]	49 ± 59[b]	20 ± 20[b]
Point Aux Chenes	18575 ± 2290[b]	575 ± 563[c]	31 ± 33[b]	10 ± 10[b]
P-value	0.0002	<0.0001	<0.0001	<0.0001
Recommended levels	<50,000	<500	<200	<35

Notes: Data are mean values (±standard deviations in 2009. Means with the same letter among the sites are not statistically different at $P < 0.05$.

Table 2. Concentration of indicator bacteria (CFU/100 mL) in the Grand Bay NERR.

Sampling sites	HPC	TC	FC	ENT
Bayou Heron	13700 ± 5260[a]	1964 ± 892[a]	181 ± 110[a]	75 ± 49[a]
Bayou Cumbest	16162 ± 23562[a]	2752 ± 5833[a]	197 ± 156[a]	87 ± 50[a]
Bangs Lake	7000 ± 5215[b]	928 ± 428[b]	28 ± 24[c]	22 ± 30[b]
Point Aux Chenes	8845 ± 6634[b]	443 ± 265[c]	45 ± 34[b]	7 ± 6[c]
P-value	<0.0001	<0.0001	<0.0001	<0.0001
Recommended levels	<50000	<500	<200	<35

Notes: Data are mean values (±standard deviations in 2010. Means with the same letter among the sites are not statistically different at $P < 0.05$.

Cumbest and Bayou Heron compared to Bangs Lake and Point Aux Chenes respectively (Table 2).

Presence of enterococcal surface protein gene (*esp*) in the Grand Bay NERR water, as an indicator of human fecal pollution

Primers specific for the *E. faecium esp* gene were used in this study as a marker of human derived fecal pollution. The human-specific (*E. faecium esp*) marker was detected only in water samples collected from Bayou Cumbest (Fig. 2) and not in any other sites of the Grand Bay NERR.

Minimum detection limit and persistence of the marker in the Grand Bay NERR

Membrane filtration, DNA extraction, and PCR were performed to determine limits of the naturally occurring *esp* gene in four separate sewage and water samples. As shown in Table 3, enterococci counts reached the $>10^3$ to $<10^4$ and 10 to 100 CFU/100 mL countable ranges in sewage and water samples, respectively. Additionally, enterococcal surface protein (*esp*) gene was detected from 10–100 plate countable range and above. On average 62 ± 26 CFU/100 mL of water were required to ensure detection of the *esp* gene and to determine if human fecal pollution is present in the Grand Bay NERR waters.

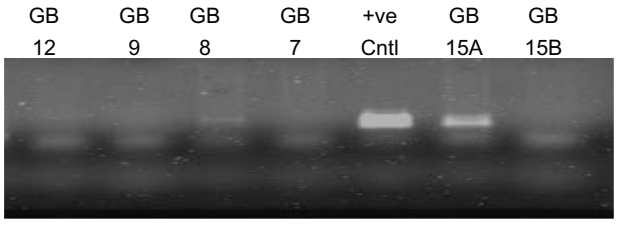

Figure 2. PCR profile showing the presence of *esp* gene in Bayou Cumbest (GB 15A), Grand Bay NERR.

Survival studies using naturally occurring enterococci in raw sewage inoculated into distilled water and Grand Bay NERR water were conducted, and the results are tabulated in Tables 4 and 5. High temperature (35 °C) was used to enhance die-off rates during incubation. The mean concentration of culturable enterococci in distilled water sharply declined in day 3 (167 CFU/mL) and the *esp* gene was no longer detectable in day 7 (Table 5). In the Grand Bay NERR water, the concentrations of culturable enterococci declined to 198 CFU/100 mL of water at day 5 to 92 CFU/100 mL of water at day 7 and were no longer detectable in day 10. In general, the *esp* gene was detectable up to day 7 in Grand Bay NERR waters when the levels of enterococci were between 83 and 100 CFU/100 mL and only to day 5 when the levels were between 51 and 72 CFU/100 mL of distilled water (Tables 4 and 5).

Discussion

Fecal bacteria have been used as an indicator of the possible presence of pathogens in surface waters and the risk of disease, based on epidemiological evidence of gastrointestinal disorders from ingestion of contaminated surface water or raw shellfish.[22] Results obtained from bacteriological analysis indicate a potential public health concern with respect to microbiological contamination of water. High numbers of fecal indicators were observed in Bayou Heron and Bayou Cumbest. The area around Bayou Heron is surrounded by many trees that harbor different species of animals and birds. Microbial source tracking studies have revealed that wildlife and waterfowl make important contributions to fecal counts.[23] Our results confirm the specificity of the

Table 3. PCR results (±) for the *esp* gene in diluted sewage and Grand Bay NERR water samples: data are numbers of *Enterococci* screened from a specific countable range (CFU/100 mL).

Sample type (# of samples)	$>10^3$ to $<10^4$	100 to 1000	10 to 100 ($\mu = 62 \pm 26$)	1 to 10 ($\mu = 6 \pm 2$)
Sewage (3)	+ (3)	+ (3)	+ (3)	–
Water samples (3)	NA	NA	+ (3)	–

Note: NA indicates counts were not in this range for any of these samples.

esp marker in differentiating the sources of fecal contamination from humans and animals, the marker was observed in Bayou Cumbest and not Bayou Heron. High population of humans and untreated sewage suggest being the contributing source of high concentration of indicator bacteria in Bayou Cumbest. Presence of microorganisms of fecal origin from mammals and birds in Grand Bay NERR water indicate fecal pollution and possible association with enteric pathogens.[9]

In most cases, the numbers of heterotrophic bacteria, total coliforms, and enterococci were generally high in all the sites while fecal coliforms were highly concentrated in Bayou Heron and Bayou Cumbest, leading to the violation of available State and Federal recreational water quality. Although HPC have not been associated with gastrointestinal infection,[24] they have been useful in operational monitoring as treatment and disinfectant indicator, especially where the objective is to keep their numbers as low possible. The U.S. Army recommended 500 CFU/100 mL as a standard for potable waters.[25] Results of this study occasionally show an exceedance of this standard by several orders of magnitude in all the sites during the investigation.

Recommended standard for fecal coliforms in water-contact sites is 200 CFU/100 mL.[21,26] The results from this investigation show that Grand Bay water occasionally failed to meet this standard, especially in Bayou Heron and Bayou Cumbest. These results also support the conclusions of the studies made in Ross Barnett Reservoir and Pearl River in Mississippi, indicating that fecal coliform bacteria occasionally exceeded state water quality standards.[1,27]

Spatial and temporal variations are also an important factor in water monitoring. In the present investigation, enterococci considered to be the best indicator of fecal pollution, consistently exceeded USEPA bacterial water quality standards (35 CFU/100 mL) in Bayou Cumbest and Bayou Heron while not in Point Aux Chenes and Bangs Lake. The consistent high counts in these sites could be due to the proximity to potential non-point sources of fecal pollution.[28] Similar variations were reported by Abdelzaher et al[29] who reported higher concentrations of *Enterococcus* (110 CFU/100 mL) in one water sample (WHa) than in the rest of samples 65 CFU/100 mL and 25 CFU/100 mL. USEPA set $<$ 104 CFU/100 mL of *Enterococcus* as the regulatory guideline for a single sampling event. Additionally, low numbers of microbial indicators, particularly fecal coliforms and *Enterococcus* in Point Aux Chenes and Bangs Lake, may not necessarily mean Grand Bay NERR water is free of pollution.[28]

Table 4. Descriptive statistics showing *Enterococcus* levels in distilled water and Grand Bay NERR water: data are means, standard deviations, minimum and maximum (CFU/100 mL).

Water type	Descriptive information	Day 0	Day 3	Day 5	Day 7	Day 10
Distilled	Mean	1897	167	62	17	4
	St. Dev.	346	49	11	6	4
	Minimum	1520	112	51	11	0
	Maximum	2200	208	72	23	7
Grand Bay	Mean	2533	630	198	92	18
	St. Dev.	643	267	59	9	9
	Minimum	1800	324	143	83	9
	Maximum	3000	820	260	100	27

Table 5. Persistence of *esp* gene in culturable *Enterococci* in distilled water and Grand Bay NERR water: data are mean *Enterococci* concentrations (CFU/100 mL).

Water type	Day 0	Day 3	Day 5	Day 7	Day 10
Distilled (n = 3)	1897[a]	167[a]	62[b]	17[b]	4[a]
PCR results (+/–)	+	+	+	–	–
Grand Bay (n = 3)	2533[a]	630[a]	198[a]	92[a]	18[a]
PCR results (+/–)	+	+	+	+	–

Notes: (+/–) indicates presence or absence of *esp* gene in the sample. Mean concentrations of enterococci in the same column followed by the same letter are not statistically different at $P < 0.05$.

The enterococcal surface protein (*esp*) of *E. faecium* is currently a choice by many investigators to address human fecal pollution in water bodies.[2,9,16,30,31] This choice is mainly based on the sensitivity and reliability of the *esp* gene to identify human fecal material outside the laboratory in which it was developed.[32] Therefore, the detection of *esp* gene in Bayou Cumbest supports the hypothesis that water in the Grand Bay NERR has been impacted by human fecal waste and also highlights a potential for human enteric viruses at a location.[7] These results also suggest that fecal wastes found in the Grand Bay area may originate either near shore from people and/or animals or offshore from boats that dump their waste.[29]

Although very little is known regarding the persistence of host-specific markers in the environment compared to traditional fecal indicator bacteria,[33] this parameter would be very helpful in understanding the rate of fecal contamination in the water if well characterized. Persistence of *E. faecium* C68, (*esp* gene) when spiked in distilled and Grand Bay NERR water were 5 days and 7 days respectively. There longer survival of *esp* marker in the Grand Bay NERR waters may be due to the presence of nutrients and other unknown conditions. More survival rates of *E. faecium* were observed by Scott et al[16] in simulated freshwater and seawater, where *esp* gene persisted for 9 and 10 days respectively. Environmental stresses such as temperature variations, radiation, salinity, and predators are among the factors that influence persistence *esp* gene outside the host.[13]

Conclusions

Monitoring for traditional indicators (heterotrophic bacteria, total coliforms, fecal coliforms, and enterococci) alone does not necessarily address the presence of human fecal pollution, but is one tool among many in microbial water quality assessment.[7] The data obtained in this study, which involved four indicator bacteria and a single biomarker of human fecal contamination *esp* gene, indicates that Bayou Cumbest, the area in Grand Bay NERR where humans reside, has been impacted by human fecal waste. However, a broad suite of potential new measurement methods and indicators,[32] as well as a comprehensive sanitary survey, should be used to help identify contributing sources of fecal contamination. The levels of traditional indicator bacteria in the Grand Bay waters frequently exceed the State and Federal recommended guidelines. It is therefore recommended to determine whether microbes that cause diseases are also present at the levels that pose a significant health concern to the people using Grand Bay waters. Although the disease risk of fecal contamination from wild animals and waterfowls in the Grand Bay NERR was not covered during this investigation, further research is also needed to enhance our understanding of its effects on ecosystem health.

Author Contributions

Conceived and designed the experiments: PBT, SSK, IOF. Analyzed the data: SSK. Wrote the first draft of the manuscript: SSK. Contributed to the writing of the manuscript: PBT, IOF. Agree with manuscript results and conclusions: PBT, IOF, SSK. Jointly developed the structure and arguments for the paper: PBT, SSK, IOF. Made critical revisions and approved final version: SSK, PBT. All authors reviewed and approved of the final manuscript.

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Competing Interests

Author(s) disclose no potential conflicts of interest.

Disclosures and Ethics

As a requirement of publication the authors have provided signed confirmation of their compliance with ethical and legal obligations including but not limited to compliance with ICMJE authorship and competing interests guidelines, that the article is neither under consideration for publication nor published elsewhere, of their compliance with legal and ethical guidelines concerning human and animal research participants (if applicable), and that permission has been obtained for reproduction of any copyrighted material. This article was subject to blind, independent, expert peer review. The reviewers reported no competing interests.

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