Analysis of water and sediments collected in coastal waters of the Gulf of Mexico potentially affected by Hurricanes Katrina and Rita to determine levels of human fecal indicators.

Mark E. Peterson, William B. Nilsson, Rohinee N. Paranjpye, and Mark S. Strom
Resource Enhancement and Utilization Technologies Division
Northwest Fisheries Science Center
NOAA Fisheries Service

Summary
We analyzed twenty-three water and fourteen sediment samples collected during the R/V NANCY FOSTER cruise of 28 September-2 October 2005. The results show that the numbers of microbial indicators of fecal contamination (*E. coli* and *Enterococcus*) in water samples did not exceed EPA guidelines for recreational waters (i.e., greater than 235 and 61 cells per 100 milliliters, respectively, EPA, 2004). *E. coli* and *Enterococci* were found in five and three sediment samples respectively, although numbers did not exceed 240 *E. coli* and 3200 *Enterococci* in 25 g sediment. No standards exist for determining health risks from *E. coli* and *Enterococci* in soil and sediments, including those from the marine environment (EPA, 2005). However, the presence of *E. coli* and *Enterococci* implies the presence of fecal contamination and people should limit their exposure to these sediments.

Introduction
Analyses have been completed on water and sediment sample that detect the presence of *E. coli* and *Enterococcus* species, two bacterial indicators of human and animal fecal contamination. The presence of fecal indicators is a tool to assess risk of contamination of recreational waters with pathogenic bacteria and viruses. These bacteria include species that may inhabit the intestines of warm-blooded animals or occur naturally in soil, vegetation, and water. Although these species are not usually pathogenic themselves, their identification in drinking or recreational waters indicates the possible presence of pathogens, and is often associated with disease outbreaks. *E. coli* is always found in feces and is therefore a more direct indicator of fecal contamination and the possible presence of enteric pathogens. Some strains of *E. coli* are also pathogenic. In addition, it has been shown that *Enterococcus* spp. provide a good indicator of fecal contamination in marine recreational waters (EPA Method 1600).

Methods.
Sampling methodology
Water samples for microbiological analyses were collected directly from CTD water bottles. At each station, 500 ml of water (into two sterile 250 ml Oakridge bottles) were collected from each depth that the CTD sampled. A total of twenty three water samples were collected from 12 stations (Stations 25, 27, 29, 32, 33, 34, 36, 37, 39, 41, 42, 43, see Figure 1).

Sediment samples for microbiological analyses were collected directly from the modified Van-Veen at the same time sediment composites for chemical analyses were collected. Duplicate samples were collected from each grab. If sediment was firm, a sterile polypropylene tube was pushed in from the surface to a depth of ~ 6 cm. A sterile stainless steel spoon was used to close the open end of the tube and the tube was withdrawn and capped. If sediment was soft, a sterile

stainless steel spoon was used to transfer sediment to a depth of ~ 6 cm into a sterile polypropylene tube. Tubes containing sediment were placed into a Ziplock bag and stored in an insulated box aboard the *Nancy Foster* at a temperature of 21-22°C. A total of 14 sediment samples were collected from 14 stations (Stations 29, 32, 33, 34, 35, 36, 38, 39, 40, 41, 43, 46, 47, see Figure 1).

Microbial analysis methodology

Escherichia coli and total coliforms were determined using EPA Membrane Filtration Method 1604 while Enterococci were enumerated using EPA Membrane Filtration Method 1600. 100 ml of water samples were analyzed undiluted as well as diluted by serial 10-fold dilution to $1x10^{-2}$. Results are reported as the total number of bacteria per 100 ml. Twenty-five grams of each sediment sample was resuspended in 25 ml PBS and allowed to settle. The diluent at this stage was considered undiluted and was analyzed directly as well as diluted 10-fold to $1x10^{-2}$, using the same methods as for the water samples. Results are reported as total CFU per 25 g sediment. All water samples were analyzed the same day as collection on board ship, while sediment samples were returned to the NWFSC Microbiology laboratory for analysis. Confirmation of *Enterococcus* species isolation on mEI medium was performed following protocols outlined in the same EPA Method 1600.

Results

The results are summarized in Table 1. The numbers of bacteria isolated from water samples are listed as colony forming units (CFU) per 100 milliliters of sample, while bacteria from sediments are listed as CFU per 25 g of sediment material. No water samples exceeded EPA guidelines for *E. coli* (greater than 235 CFU/100 ml) or *Enterococcus* (greater than 61 CFU/100 ml) in recreational waters. *E. coli* was isolated from five sediment samples (stations 29, 32, 33, 40, and 46) while *Enterococci* were isolated from three sediment samples (stations 32, 33, and 34). The presence of these indicators suggests these sites contain fecal contaminants. Water samples analyzed from these same sites did show the presence of low levels of *E. coli* and *Enterococci*, and it has been shown that fecal indicators can survive in marine sediments longer than in the overlaying water (Pianetti, et al, 2004). However, no data exist regarding the public health risk from sediments,

Conclusions

Water and sediment samples taken during the post-Katarina cruise of 28 September-2 October 2005 did not show significantly elevated or human health-threatening levels of bacterial indicators of fecal contamination.

References

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Pianetti, A., F. Bruscolini, L. Sabatini and P. Colantoni. 2004. Microbial characteristics of marine sediments in bathing area along Pesaro-Gabicce coast (Italy): a preliminary study. J. Appl. Microbiol. 97:682-689.

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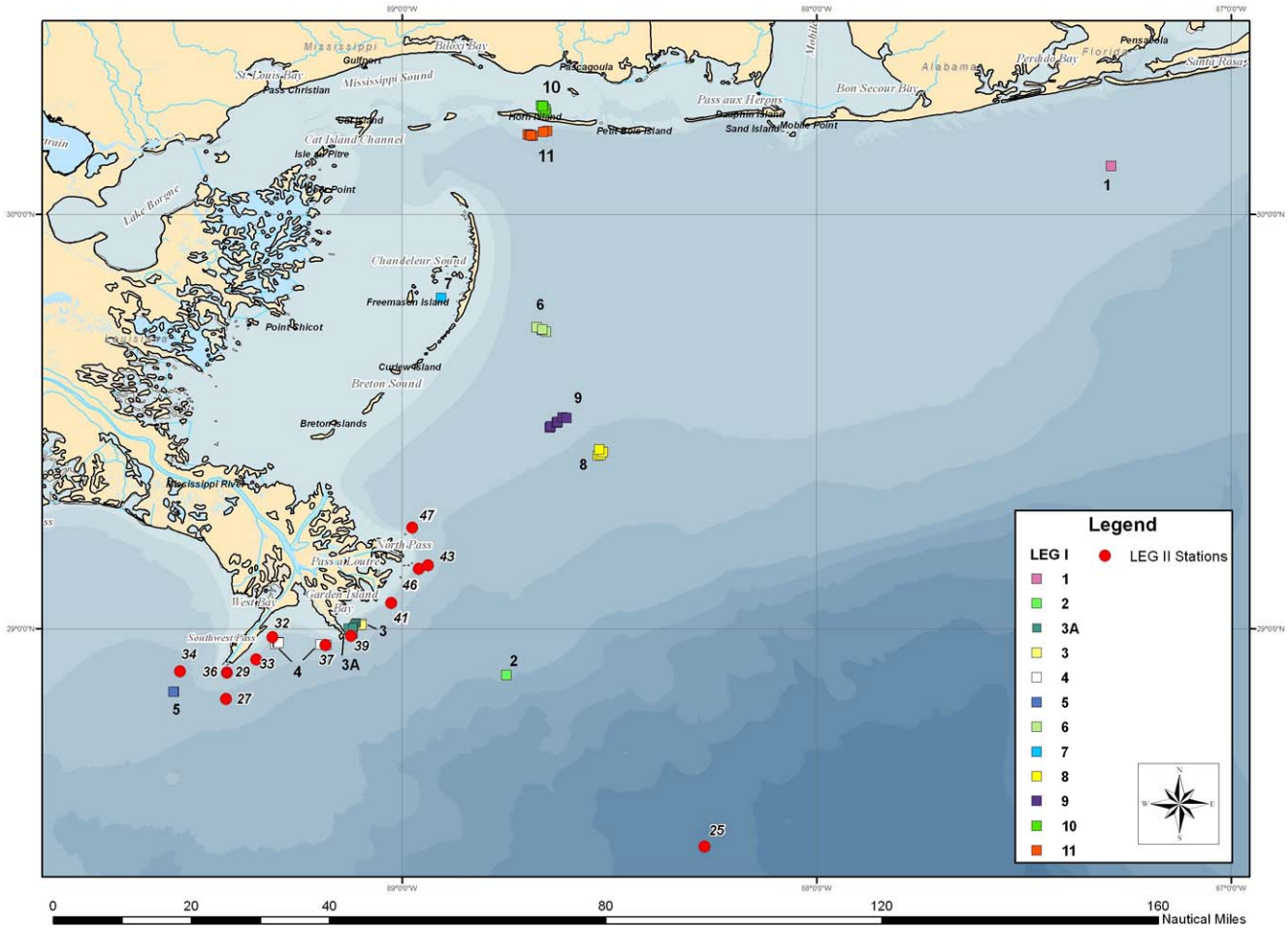
Table 1

Field ID	**Lab ID**	***E. coli***	**Total coliforms**	***Enterococcus***
Water samples (date, station-depth[1])		*CFU/100 ml*	*CFU/100 ml*	*CFU/100 ml*
9/29/05, 25-0	W23	not detected	20	1
9/29/05, 25-200	W24	not detected	15	not detected
9/30/05, 27-0A	W25	2	8	3
9/30/05, 27-50A	W26	1	2	not detected
9/30/05, 29-0B	W27	23	480	17
9/30/05, 29-10B	W28	12	not detected	5
9/30/05, 32-0	W29	39	320	12
9/30/05, 32-5	W30	4	not detected	not detected
9/30/05, 33-0	W31	10	57	12
9/30/05, 33-10	W32	15	37	12
9/30/05, 34-0	W33	2	9	1
9/30/05, 34-50	W34	3	7	not detected
9/30/05, 36-0	W35	37	760	not detected
9/30/05, 36-10	W36	1	10	not detected
10/1/05, 37-0	W37	18	220	not detected
10/1/05, 37-15	W38	8	7	not detected
10/1/05, 39-0	W39	2	32	not detected
10/1/05, 41-0	W40	11	15	not detected
10/1/05, 41-12	W41	43	9	not detected
10/1/05, 42-0	W42	7	42	not detected
10/1/05, 42-22	W43	2	13	not detected
10/1/05, 43-0	W44	Not detected	10	not detected
10/1/05, 42-22	W45	2	11	not detected
Sediment samples (date, station)		*CFU/25 g*	*CFU/25 g*	*CFU/25 g*
9/29/05, 29	S12	140	not detected	not detected
9/29/05, 32	S13	40	1100	3200
9/29/05, 33	S14	240	11600	240
9/29/05, 34	S15	not detected	240	440
10/1/05, 35	S16	not detected	not detected	not detected
10/1/05, 36	S17	not detected	620	not detected
10/1/05, 37	S18	not detected	1320	not detected
10/1/05, 38	S19	not detected	7840	not detected
10/1/05, 39	S20	not detected	1400	not detected
10/1/05, 40	S21	40	1400	not detected
10/1/05, 41	S22	not detected	780	not detected
10/1/05, 43	S23	not detected	1120	not detected
10/1/05, 46	S24	200	2200	not detected
10/1/05, 47	S25	not detected	5200	not detected

[1] depth, in meters

Figure 1


Legend
LEG I
LEG II Stations
1
2
3A
3
4
5
6
7
8
9
10
11
Nautical Miles
0
20
40
80
120
160