Analysis of water and sediments collected in coastal waters of the Gulf of Mexico potentially affected by Hurricane Katrina to determine levels of human fecal indicators and pathogenic *Vibrio* species.

Mark E. Peterson, Linda D. Rhodes, 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-two water and eight sediment samples collected during the R/V NANCY FOSTER cruise of September 12-16, 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). Total numbers in 25 g of three sediment samples did exceed these levels for *E. coli* but only one of these same three samples exceeded the level for *Enterococcus*. While no standards exist for determining health risks from *E. coli* in sediments or soils, particularly in the marine environment (EPA, 2005), the presence of *E. coli* implies the presence of fecal contamination and exposure to these sediments should be limited. The same samples were analyzed for the presence of naturally-occurring marine *Vibrio* species, some of which can be pathogenic to humans. Nine of twenty-two water samples cultured positive for *V. cholerae;* characterization of these isolates with the cholera toxin gene probe (*ctx*) demonstrated that these are *ctx* negative or non-toxigenic. Ten water samples tested positive for *V. vulnificus.* In addition, eight of the water samples showed very low levels of other *Vibrio* species. Six of eight sediment samples showed the presence of non-toxigenic *V. cholerae*, while *V. vulnificus* was isolated from all eight sediment samples. However, the total numbers of these naturally occurring *Vibrio* species were very low and were at levels considered to be normal for Gulf Coast marine waters and sediments.

Introduction
Analyses have been completed on water and sediment samples that detect the presence of *E. coli* and *Enterococcus* species, two bacterial indicators of human and animal fecal contamination. In addition, the same samples were analyzed for the presence of *Vibrio* species, particularly for *V. cholerae* and *V. vulnificus*, and total *Vibrios.*

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).

Many members of the bacterial genus *Vibrio* are naturally occurring microflora of the marine environment (Morris, 2003), and these include the potential human pathogens *V. cholerae, V. vulnificus,* and *V. parahaemolyticus.* All three species are common to marine waters in the Gulf Coast region of the United States. The great majority of free-living *V. cholerae* are non-toxigenic, lacking the gene (*ctx*) for cholera toxin. This toxin is required to cause the disease cholera (Morris, 2003). Toxigenic but non-epidemic *V. cholerae* has been isolated in the Gulf Coast region, with very few cases of cholera reported over the last decade and shown to be contracted by ingestion of raw or undercooked seafood (Morris, 2003). *V. vulnificus* and *V. parahaemolyticus* can also be found free-living in seawater and in sediments, but are more widely known for concentrating in molluscan shellfish. Human disease caused by these pathogens usually occurs by ingestion of raw shellfish such as oysters, but wound infections can also occur by contact with water containing high numbers of the bacteria. *V. parahaemolyticus* infections are typically self-limiting and manifest as a mild to severe gastroenteritis. On the other hand, *V. vulnificus* is capable of causing serious and life-threatening septicemias, although the at-risk population is very small (Strom and Paranjpye, 2000). The presence of *V. vulnificus* and *V. parahaemolyticus* in Gulf Coast water and sediments in the summer months is unremarkable, although their presence in fish and shellfish does pose a risk from undercooked or raw seafood. Likewise, the presence of non-toxigenic *V. cholerae* in the Gulf is expected. However, the isolation of toxigenic *V. cholerae* could be an indicator of a potential human health problem.

Methods.

Sampling methodology

Water samples for microbiological analyses were collected directly from CTD water bottles, except for Station 7 where surface water was sampled by hand dip. At each station, 500 ml of water (into two sterile 250 ml Oakridge bottles) were collected from each depth that the CTD sampled. One hundred ml of water was filtered through MES (mixed cellulose ester) membrane filters (0.45 µm pore, 47 mm diameter), and the filter was transferred to a polystyrene petri dish, sealed with Parafilm, and stored at -20°C. Bacterial DNA can be extracted from these filters for molecular (PCR) analysis for specific bacterial species, and are backups for the direct bacterial analysis of water samples to be carried out as described below. The remaining bottles containing 400 ml sample 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 22 water samples were collected from 11 stations (Stations 1, 2, 3, 4, 5, 6, 7, 8, 9, 11, and 12, 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 8 sediment samples were collected from 8 stations (Stations 1, 2, 3, 4, 5, 8, 9, and 11, see Figure 1).

Microbial analysis methodology

Aerobic plate counts were determined to indicate the level of microorganisms present in all samples according to the FDA/CFSAN Bacteriological Analytical Manual (BAM), Chapter 3, http://www.cfsan.fda.gov/~ebam/bam-3.htmlT.

For determination of *E. coli* and *Enterococci*, 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 were 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 $1x10^{-2}$. Results are reported as total CFU per 25 g sediment. *Escherichia coli* and total coliforms were determined using EPA Method 1604 while Enterococci were enumerated using EPA Method 1600.

Water and sediment samples were also tested for the three most common pathogenic *Vibrio* species, *V. cholerae*, *V. vulnificus* and *V. parahaemolyticus* according to procedures detailed in BAM, Chapter 9, http://www.cfsan.fda.gov/~ebam/bam-9.html. Water samples were diluted to 1×10^{-4} and 0.1 ml of each dilution plated on TCBS and VVA agar medium for determination of the *Vibrio* counts. The polymerase chain reaction (PCR) was used to verify preliminary identification by culture methods and, in addition, any strains of *V. cholerae* were tested for the presence of the cholera toxin gene (*ctx*) using published primers.

Results

The results are summarized in Tables 1 and 2. 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. Three sediment samples did exceed these levels on a per 25 gram basis for *E. coli* (Stations 8, 9, and 11) but only one of these same three sediment samples exceeded the level for *Enterococcus* (station 9), again in a total of 25 g of sediment. The presence of *E. coli* does imply that these sites contain fecal contaminants. Although no data exist regarding the public health risk from sediments from these sites, it has been shown that fecal indicators can survive in marine sediments longer than in the overlaying water (Pianetti, *et al.*, 2004). It will be important to revisit these sites on future cruises to determine if the levels detected in these sediments are transient.

Nine of twenty-two water samples cultured positive for *V. cholerae;* characterization of these isolates with the cholera toxin gene probe (*ctx*) demonstrated that these are *ctx* negative or non-toxigenic. Ten water samples tested positive for *V. vulnificus.* In addition, eight of the water samples showed very low levels of other *Vibrio* species. Six of eight sediment samples showed the presence of non-toxigenic *V. cholerae,* while *V. vulnificus* was isolated from all eight sediment samples. The numbers of these naturally occurring *Vibrio* species were at levels considered to be normal for Gulf Coast marine waters and sediments.

Conclusions

Water and sediment samples taken during the post-Katrina cruise of September 12-16, 2005, are not showing significantly elevated or human health-threatening levels of bacterial indicators of fecal contamination. In addition, while naturally occurring *Vibrio* species were found in water and sediment samples, their numbers were unremarkable. No toxigenic *V. cholerae* were isolated.

References

EPA Water Quality Standards for Coastal and Great Lakes Recreation Waters. Federal Register: November 16, 2004 (Volume 69, Number 220), http://www.epa.gov/fedrgstr/EPA-WATER/2004/November/Day-16/w25303.htm

EPA and LDEQ Report Potential Health Risks from Sediments, 9/16/2005, press release

EPA Method 1604: Total Coliforms and *Escherichia coli* in Water Using a Simultaneous Detection Technique (MI Medium), http://www.epa.gov/nerlcwww/1604sp02.pdf.

EPA Method 1600: Enterococci in Water by Membrane Filtration Using membrane-Enterococci Indoxyl–β–D-Glucoside Agar (mEI), http://www.epa.gov/nerlcwww/1600sp02.pdf

FDA Center for Food Safety and Applied Nutrition. Bacteriological Analytical Manual *Online*, Chapter 3, Aerobic Platte Count, http://www.cfsan.fda.gov/~ebam/bam-3.html

FDA Center for Food Safety and Applied Nutrition. Bacteriological Analytical Manual *Online*, Chapter 9, Vibrio. http://www.cfsan.fda.gov/~ebam/bam-9.html

Morris, J. G., Jr. 2003. Cholera and other types of vibriosis: a story of human pandemics and oysters on the half shell. Clin. Infect. Dis. 37:272-80.

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.

Strom, M. S., R. N. Paranjpye. 2000. Epidemiology and pathogenesis of Vibrio vulnificus. Microbes and Infection, 2:177-188.

Table 1

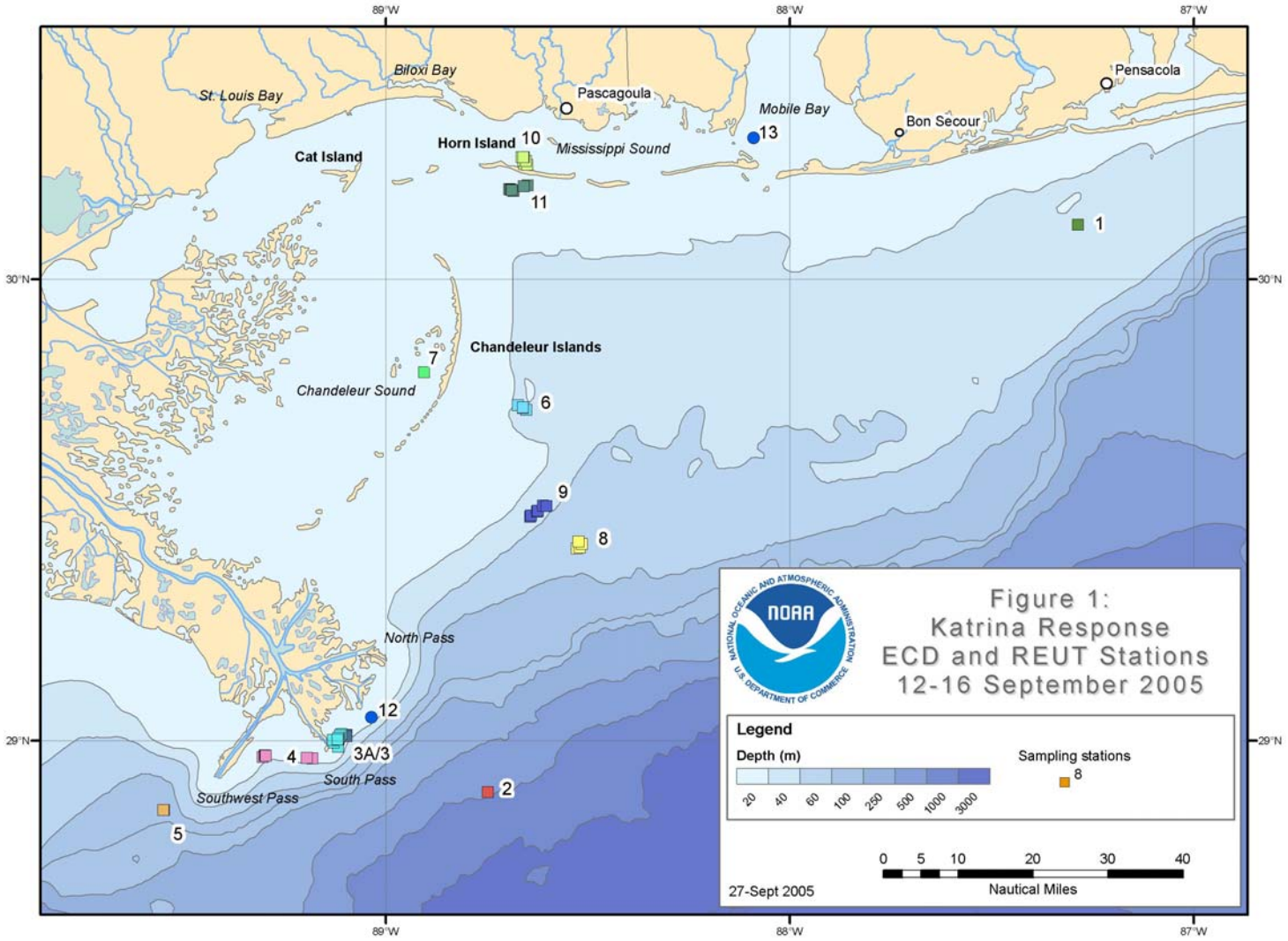
Field ID	**Lab ID**	***E. coli***	**Total coliforms**	***Enterococcoccus***
Water samples (date collected)		*CFU/100 ml*	*CFU/100 ml*	*CFU/100 ml*
Station 1, surface (9/12/05)	W1	not detected	4	20
Station 2, surface (9/13/05)	W2	not detected	not detected	not detected
Station 2, 64 m depth (9/13/05)	W3	not detected	6	not detected
Station 2, 300 m depth (9/13/05)	W4	not detected	2800	not detected
Station 3, surface (9/13/05)	W5	not detected	8	not detected
Station 3, 14 m depth (9/13/05)	W6	not detected	40	not detected
Station 4, surface (9/13/05)	W7	49	800	not detected
Station 4, 13 m depth (9/13/05)	W8	not detected	not detected	not detected
Station 5, surface (9/13/05)	W9	1	43	not detected
Station 5, 63 m depth (9/13/05)	W10	not detected	4	not detected
Station 6, surface (9/14/05)	W11	not detected	3	not detected
Station 6, 15 m depth (9/13/05)	W12	not detected	not detected	not detected
Station 7, surface (9/14/05)	W13	not detected	620	not detected
Station 8, surface (9/14/05)	W14	not detected	1	not detected
Station 8, 24 m depth (9/14/05)	W15	not detected	71	not detected
Station 8, 151 m depth (9/14/05)	W16	not detected	not detected	not detected
Station 9, surface (9/14/05)	W17	not detected	200	not detected
Station 9, 35 m depth (9/14/05)	W18	not detected	not detected	not detected
Station 11, surface (9/14/05)	W19	10	not detected	not detected
Station 11, 10 m depth (9/14/05)	W20	not detected	20	not detected
Station 12, surface (9/15/05)	W21	not detected	not detected	not detected
Station 12, 10 m depth (9/15/05)	W22	18	25	not detected
Sediment samples (date collected)		*CFU/25 g*	*CFU/25 g*	*CFU/25 g*
Station 1A	S1A	not detected	not detected	v
Station 2A	S2A	60	80	not detected
Station 3A	S3A	not detected	not detected	not detected
Station 4A	S4A	not detected	620	not detected
Station 5A	S5A	40	60	20
Station 8A	S8A	380	740	20
Station 9A	S9A	1840	9600	900
Station 11A	S11A	22600	14000	20

Table 2.

Field ID	Lab ID	non toxigenic *V. cholerae*	other *Vibrio* spp.	*V. vulnificus*
Water samples (date collected)		*CFU/100 ml*	*CFU/100 ml*	*CFU/100 ml*
Station 1, surface (9/12/05)	W1	not detected	not detected	not detected
Station 2, surface (9/13/05)	W2	not detected	not detected	not detected
Station 2, 64 m depth (9/13/05)	W3	<1[1]	<1	<1
Station 2, 300 m depth (9/13/05)	W4	not detected	not detected	not detected
Station 3, surface (9/13/05)	W5	not detected	not detected	not detected
Station 3, 14 m depth (9/13/05)	W6	6	3	17
Station 4, surface (9/13/05)	W7	<1	not detected	3
Station 4, 13 m depth (9/13/05)	W8	not detected	not detected	not detected
Station 5, surface (9/13/05)	W9	not detected	not detected	not detected
Station 5, 63 m depth (9/13/05)	W10	not detected	not detected	not detected
Station 6, surface (9/14/05)	W11	not detected	not detected	not detected
Station 6, 15 m depth (9/13/05)	W12	not detected	not detected	not detected
Station 7, surface (9/14/05)	W13	10	4	84
Station 8, surface (9/14/05)	W14	not detected	not detected	not detected
Station 8, 24 m depth (9/14/05)	W15	not detected	not detected	not detected
Station 8, 151 m depth (9/14/05)	W16	<1	not detected	3
Station 9, surface (9/14/05)	W17	1	2	4
Station 9, 35 m depth (9/14/05)	W18	3	3	10
Station 11, surface (9/14/05)	W19	not detected	not detected	1
Station 11, 10 m depth (9/14/05)	W20	<1	not detected	1
Station 12, surface (9/15/05)	W21	not detected	not detected	not detected
Station 12, 10 m depth (9/15/05)	W22	4	<1	12
Sediment samples (date collected)		*CFU/25 g*	*CFU/25 g*	*CFU/25 g*
Station 1A	S1A	20	5	1
Station 2A	S2A	67	not detected	31
Station 3A	S3A	not detected	not detected	1
Station 4A	S4A	1	10	50
Station 5A	S5A	<1	20	<1
Station 8A	S8A	18	20	28
Station 9A	S9A	12	20	64
Station 11A	S11A	not detected	not detected	20

[1] <1, number of CFU was less than 100 cfu/ml

Figure 1


89°W
88°W
87°W
30°N
29°N
St. Louis Bay
Biloxi Bay
Pascagoula
Mobile Bay
Bon Secour
Pensacola
Cat Island
Horn Island
Mississippi Sound
10
11
13
1
Chandeleur Islands
Chandeleur Sound
7
6
9
8
North Pass
12
4
3A/3
South Pass
Southwest Pass
2
5
Figure 1:
Katrina Response
ECD and REUT Stations
12-16 September 2005
Legend
Depth (m)
20
40
60
100
250
500
1000
3000
Sampling stations
8
0
5
10
20
30
40
Nautical Miles
27-Sept 2005