Analysis of fish and invertebrates collected in coastal waters of the Gulf of Mexico potentially affected by Hurricanes Katrina and Rita to determine levels of human fecal indicators and pathogenic Vibrios.

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

Summary
We analyzed 163 samples consisting of Atlantic croaker, big-eyed tuna, shrimp, blue crab, and two shellfish species (cockle and whelk) collected during the R/V NANCY FOSTER cruise of 12-16 September 2005 for *E. coli*, a microbial indicator of fecal contamination, and pathogenic *Vibrio* species. We similarly analyzed an additional 302 samples consisting of brown shrimp, Atlantic croaker, blue crab, spot, and hard-headed catfish collected during the R/V NANCY FOSTER and PATRICIA JEAN cruises of 28 September-2 October 2005. In summary, none of the samples gathered during both cruises harbored *E. coli*, and none harbored toxigenic or non-toxigenic *V. cholerae.* Other vibrios were isolated from some samples including toxigenic and non-toxigenic *V. parahaemolyticus* and *V. vulnificus.* However, since these species normally inhabit the marine environment, their presence only reinforces the standard FDA recommendation that fish, crab, and shrimp species be thoroughly cooked prior to consumption (FDA, Fish and Fisheries Products: Hazards and Controls Guidance, 2001).

Introduction
Analyses have been completed on fish and invertebrate samples that detect *E. coli*, a bacterial indicator of human and animal fecal contamination. The presence of fecal indicators is generally used as a tool to assess risk of contamination of seafood 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 most of these species are usually not pathogenic themselves, their identification in seafood indicates the possible presence of pathogens that may be capable of causing disease outbreaks in raw or undercooked seafood. Total coliforms (a group of lactose-fermenting bacteria that includes *E. coli*) are also often used as an indicator of fecal contamination, yet not all coliforms originate in feces. However, *E. coli* is always found in feces and is therefore a more direct indicator of fecal contamination and the possible presence of enteric pathogens. In a previous report on the first cruise sample set, we demonstrated that no fish and invertebrate samples harbored *E. coli*. The analysis of these samples was repeated along with those from the second cruise using a different and more definitive methodology.

Analyses have also been completed on fish and invertebrate samples for the presence of pathogenic *Vibrio* species, specifically toxigenic *V. cholerae, V. vulnificus,* and *V. parahaemolyticus. V. cholerae* and *V. parahaemolyticus* were further analyzed for the presence of genes that confer toxigenicity. Non-toxigenic *V. cholerae* and *V. vulnificus,* and both non-toxigenic and toxigenic *V. parahaemolyticus* are often found in the Gulf of Mexico and their presence is not necessarily indicative of increased microbial contamination.

Methods.

Sampling methodology

Fish tissues for microbiological analyses were the gill (1 or 2 arches) and the entire gastrointestinal tract with spleen ("guts"), collected into sterile Whirl-pak or Stomacher bags. Samples from Atlantic croaker were combined with an equal volume of sterile phosphate-buffered saline (PBS) and glycerol (final glycerol concentration 15%) and macerated with a handheld hard roller for at least 30 seconds. The liquid eluate was divided into 2 Whirl-Pak bags and stored at -20°C in a freezer aboard the NANCY FOSTER. After addition of PBS-glycerol, samples from big-eye tuna were blended in a Stomacher paddle blender for 2 minutes, and the liquid was divided into 2 Whirl-Pak bags and stored at -20°C in a freezer aboard the NANCY FOSTER. Tissues were collected from all fish necropsied for chemical analyses.

Invertebrate tissues for microbiological analyses were combined hepatopancreas and gill for blue crab. Crab tissues were processed as described above for Atlantic croaker. Invertebrate sampling corresponded to that for chemical analyses.

Table 1 summarizes the results from the 12-16 September 2005, while Table 2 summarizes the results from the 28 September-2 October 2005 cruises. Stations and total number of samples analyzed (as composites) from each station are included in the tables. Station locations are shown in Figure 1.

Microbial analysis methodology

Samples for each species and type of tissue sampled at each station, were combined for analysis (Table 1). Samples were thawed at room temperature and serial 10-fold dilutions of 1 g of each tissue sample were prepared to 1×10^{-X} and used for all bacteriological growth and enumeration analyses described below.

Total coliforms and *E. coli* analysis

Identification and enumeration of *E. coli* and total coliforms were performed using the 3M™ Petrifilm ™ *E. coli* Count Plate method (AOAC International validated method; AOAC Official Method 998.08). Briefly, 1.0 ml of the appropriate sample dilutions were placed on the Petrifilm plates and incubated 24 h ± 2h at 35^{o} C. Red colonies were counted as coliforms, and blue colonies as *E. coli*.

Analysis for *Vibrio cholerae, Vibrio parahaemolyticus* and *Vibrio vulnificus*

Vibrio cholerae

V. cholerae: A one gram portion each composite sample was inoculated into 10 mls of Alkaline Peptone water (APW) and incubated at 35°C for 24h. Enrichments were plated on TCBS and mCPC agar and incubated at 35°C for 24h. Typical yellow colonies on TCBS were tested for the presence of the cholera toxin gene (*ctx*), the extracellular protein secretion gene (*epsM*)(Gubala 2005; Overbye et al., 1993; Sandvist et al., 1997) by PCR. Suspect colonies were also tested for their ability to grow with and without the presence of NaCl in tryptose medium.

V. parahaemolyticus and ***V. vulnificus***: Green colonies on TCBS agar were tested for the presence of pathogenic (*tdh*), pandemic (*orf8*) and *V. parahaemolyticus* species specific (*tl*)

genes by PCR (FDA/CFSA BAM, Chapter 9), while yellow colonies on mCPC agar were tested for the presence of the cytolysin gene (*vvhA*) by PCR (FDA/CFSA BAM, Chapter 9).

In all analyses, positive control DNA from toxigenic *V. cholerae*, toxigenic and non-toxingenic *V. parahaemolyticus,* and *V. vulnificus* was included to confirm the fidelity of the PCR reactions.

Results

The results are summarized in Tables 1 and 2. The numbers of *E. coli* and total coliforms isolated are listed as colony forming units (CFU)/g of sample. No *E. coli* was isolated from any of the samples. Total coliforms were generally low, with only 8 samples greater than 1000 CFU/g. However, by themselves coliforms are not indicators of fecal contamination and the absence of *E. coli* suggests little or no contamination of the fish and shellfish samples tested.

No toxigenic or non-toxigenic *V. cholerae* was isolated from any of the samples (Tables 1 and 2). APW enrichment followed by plating on TCBS agar yielded yellow colonies but did not form purple colonies on mCPC agar. In addition these isolates were *ctx* and *epsM* negative (by PCR) and did not grow in tryptose broth without NaCl. The presence of the gene *epsM* (an extracellular secretion protein gene) has been used to speciate *V. cholerae* (toxigenic and non toxigenic) from other Vibrios, but it remains possible that some *V. cholerae* strains posses variants of *epsM* that are not detected by the PCR primers used.

Conclusions

Fish and invertebrate samples taken during the post Katrina cruise of 12-16 September 2005, and the post Katrina and Rita cruise of 28 September-2 October 2005 are not showing the presence of *E. coli* contamination. While we did not specifically look for pathogenic bacterial species in these samples, the results indicate that any fecal contamination at these sampling sites did not enter these organisms. It should be pointed out that with the exception of oysters (not included in the NOAA Fisheries Service sampling), all fish and most crustaceans are generally cooked prior to eating, a practice that should continue to be encouraged (FDA, Fish and Fisheries Products: Hazards and Controls Guidance, 2001).

References

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Acknowledgements

We gratefully thank Asta Johnson, Anne Mataia, and Surafel Gezahegne for their invaluable technical assistance in these analyses.

Table 1: Results from Cruise 1 (12-16 September 2005)

Station	# of samples[1]	Sample type	Total coliforms CFU / g	*E. coli* CFU / g	Non toxigenic *V. cholerae*[2]	*V. vunificus*[3]	*V. parahaemolyticus*[4]
3A	3	Atlantic croaker gills	7000	Not detected	Not detected	not detected	Not detected
3A	3	Atlantic croaker guts	Not detected	Not detected	Not detected	not detected	Not detected
3A	3	Blue crab hepatopancreas+gills	Not detected	Not detected	Not detected	not detected	Not detected
4	1	Blue crab hepatopancreas+gills	3200	Not detected	Not detected	not detected	Not detected
4	20	Atlantic croaker gills	Not detected	Not detected	Not detected	not detected	Not detected
4	20	Atlantic croaker guts	72	Not detected	Not detected	not detected	Not detected
8	9	Shrimp heads	16	Not detected	Not detected	not detected	Not detected
8	5	Big eye tuna gills	20	Not detected	Not detected	not detected	Not detected
8	5	Big eye tuna guts	Not detected	Not detected	Not detected	not detected	Not detected
9	8	Atlantic croaker gills	Not detected	Not detected	Not detected	not detected	Not detected
9	8	Atlantic croaker guts	Not detected	Not detected	Not detected	not detected	Not detected
9	6	Shrimp heads	Not detected	Not detected	Not detected	not detected	Not detected
9	4	Blue crab hepatopancreas+gills	Not detected	Not detected	Not detected	not detected	Not detected
10	13	Atlantic croaker gills	Not detected	Not detected	Not detected	not detected	Non toxigenic Vp
10	13	Atlantic croaker guts	4	Not detected	Not detected	not detected	Not detected
11	20	Atlantic croaker gills	Not detected	Not detected	Not detected	not detected	Not detected
11	20	Atlantic croaker guts	Not detected	Not detected	Not detected	not detected	Not detected
11	1	Cockle	Not detected	Not detected	Not detected	not detected	Non toxigenic Vp
11	1	Whelk	4	Not detected	Not detected	not detected	Not detected

[1]Where sample number is greater than 1, composite samples were prepared from the number of samples shown.

[2]Non toxigenic *V. cholerae*: Vc *ctx* negative, *epsM* positve, growth in Tryptose medium without NaCl.

[3]*V. vulnificus*: *vvhA* positive

[4]Toxigenic *V.parahaemolyticus*: *tdh* positive, orf8 negative; Non toxigenic *V. parahaemolyticus*: *tl* positive, *tdh* negative

Table 2: Results from Cruise 2 (28 September-2 October 2005)

Station	# of samples[1]	Sample type	Total coliforms CFU / g	*E.coli* CFU / g	Non toxigenic *V. cholerae*[2]	*V. vulnificus*[3]	*V. parahaemolyticus*[4]
32	8 composites	Brown shrimp	32	Not detected	Not detected	Not detected	Non toxigenic Vp
37	5 composites	Brown shrimp	68	Not detected	Not detected	Not detected	Non toxigenic Vp
43	5 composites	Brown shrimp	56	Not detected	Not detected	Not detected	Not detected
48	7 composites	Brown shrimp	8	Not detected	Not detected	Not detected	Non toxigenic Vp
49	5 composites	Brown shrimp	300	Not detected	Not detected	Not detected	Non toxigenic Vp
32	30	Atlantic croaker gills	Not detected	Not detected	Not detected	Not detected	Not detected
32	30	Atlantic croaker guts	Not detected	Not detected	Not detected	Not detected	Not detected
32	2 composites	Blue crab gills + hepatopancreas	Not detected	Not detected	Not detected	Not detected	Not detected
37	25	Atlantic croaker gills	Not detected	Not detected	Not detected	Not detected	Not detected
37	25	Atlantic croaker guts	Not detected	Not detected	Not detected	Not detected	Not detected
42	25	Atlantic croaker gills	Not detected	Not detected	Not detected	Not detected	Not detected
42	25	Atlantic croaker guts	Not detected	Not detected	Not detected	Not detected	Not detected
43	15	Atlantic croaker gills	Not detected	Not detected	Not detected	Not detected	Non toxigenic Vp
43	15	Atlantic croaker guts	4	Not detected	Not detected	Not detected	Not detected
48	25	Spot gills from Patricia Jean	Not detected	Not detected	Not detected	Not detected	Not detected
48	25	Spot guts from Patricia Jean	Not detected	Not detected	Not detected	Not detected	Not detected
49	15	Spot gills from Patricia Jean	12	Not detected	Not detected	Not detected	Not detected

49	15	Spot guts from Patricia Jean	Not detected	Not detected	Not detected	Not detected	Toxigenic Vp
49	10	Atlantic croaker gills	Not detected	Not detected	Not detected	Not detected	Not detected
49	10	Atlantic croaker guts	44	Not detected	Not detected	positive	Not detected
49	5	Hard headed catfish gills	Not detected	Not detected	Not detected	positive	Toxigenic Vp
49	5	Hard headed catfish guts	116	Not detected	Not detected	Not detected	Not detected

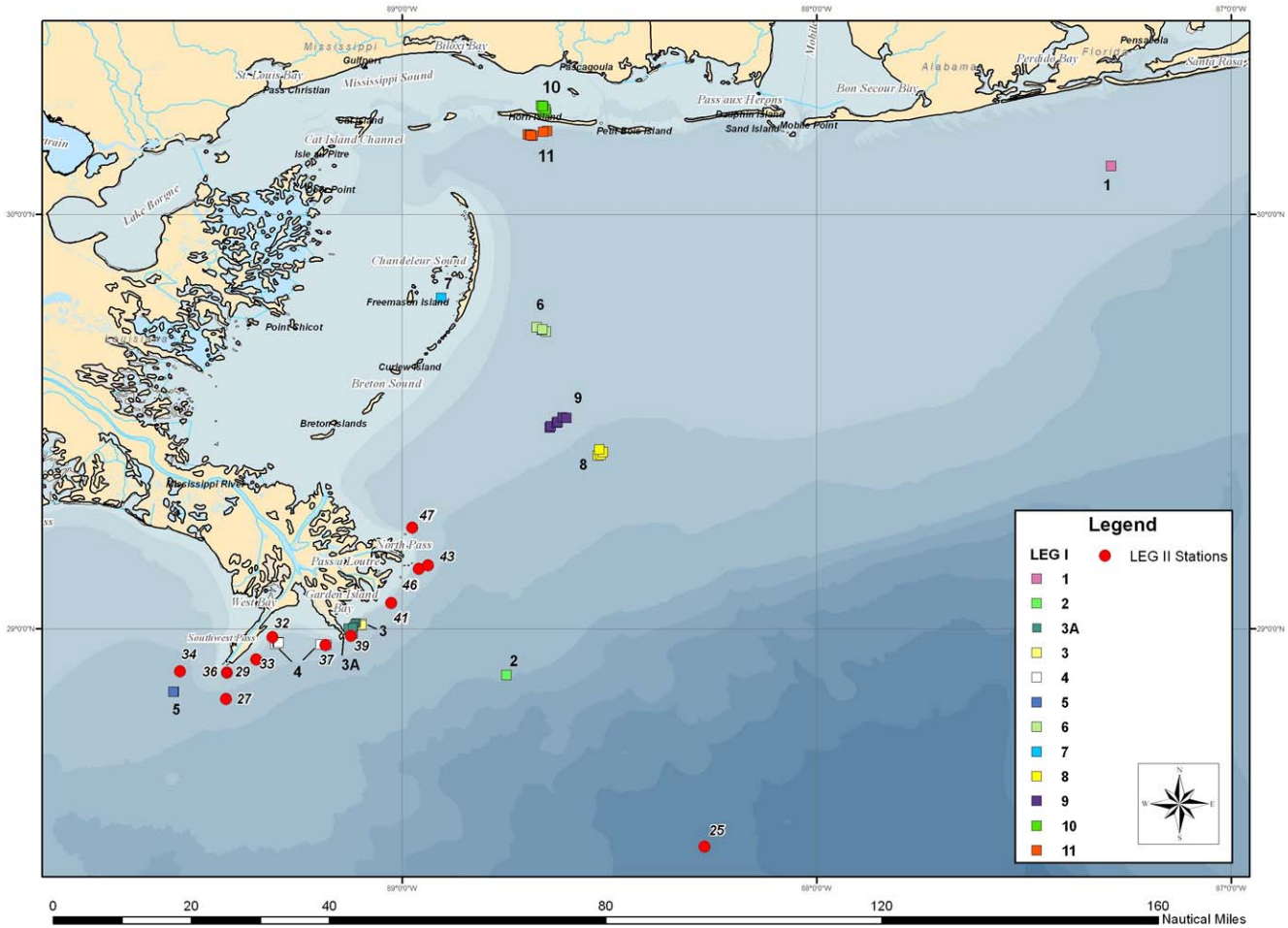
[1]Where sample number is greater than 1, composite samples were prepared from the number of samples shown.

[2]Non toxigenic *V. cholerae*: Vc *ctx* negative, *epsM* positve, growth in Tryptose medium without NaCl.

[3]*V. vulnificus*: *vvhA* positive

[4]Toxigenic *V.parahaemolyticus*: *tdh* positive, orf8 negative; Non toxigenic *V. parahaemolyticus*: *tl* positive, *tdh* negative

Figure 1


Legend
LEG I
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