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

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Summary

We analyzed 154 samples consisting of Atlantic croaker, big-eyed tuna, and blue crab collected during the R/V NANCY FOSTER cruise of September 12-16, 2005. The results demonstrate that no sample harbored *E. coli*, a microbial indicator of fecal contamination.

Introduction

Analyses have been completed on fish 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 not usually 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.

Methods.

Sampling methodology

Fish tissues for microbiological analyses were collected into sterile Whirl-pak or Stomacher bags. Tissues analyzed were the gill (1 or 2 arches) and the entire gastrointestinal tract with spleen ("guts"). Samples from Atlantic croaker were combined with an equal volume of sterile phosphate-buffered saline (PBS) and glycerol (final glycerol concentration 15 percent) 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 two 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; a total of 68 Atlantic croaker from 5 stations (Stations 3A, 4, 9, 10, and 11) and 5 big-eye tuna from Station 8 were sampled.

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. A total of 8 blue crab were sampled from 3 stations (Stations 3A, 4, and 9). All invertebrate tissue samples were held at -20°C in a freezer aboard the NANCY FOSTER. Station locations are shown in Figure 1.

Microbial analysis methodology

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.

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.

Identification and enumeration of *E. coli* and total coliforms were performed following the FDA/CFSAN BAM, Chapter 1, http://www.cfsan.fda.gov/~ebam/bam-4.html. Briefly, 1.0 ml of the appropriate sample dilutions were plated in a violet red bile agar (VRBA) overlay and incubated 18-24 h at 35° C. Purple-red colonies 0.5 mm in diameter or larger and surrounded by a zone of precipitated bile salts were counted as coliforms. To differentiate *E. coli* from other coliform colonies, the VRBA overlay included 100 µg/ml 4-methyl-umbelliferyl-β–D-glucuronide (MUG). After incubation, *E. coli* colonies can be distinguished by the appearance of a bluish fluorescence around the colonies under longwave UV light.

Results

The results are summarized in Table 1. The numbers of bacteria 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.

Conclusions

Fish and invertebrate samples taken during the post Katrina cruise of September 12-16, 2005, are not showing significantly elevated or human health-threatening levels of *E. coli* contamination. While the analysis presented here did not specifically look for pathogenic bacterial species, 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 R/V NANCY FOSTER 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
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 4, Enumeration of *Escherichia coli* and the Coliform Bacteria. http://www.cfsan.fda.gov/~ebam/bam-4.html.

FDA, Center for Food Safety and Applied Nutrition. 2001. Fish and Fisheries Products: Hazards and Controls Guidance, 3rd Edition.

Table 1

Field ID	Sample type[1]	Station	Total coliforms CFU/g	E. coli CFU/g	APC[2], CFU/g
#05-8501	gills-fish Ac	3A	not detected	not detected	720
#05-8501	guts-fish Ac	3A	360	not detected	12800
#05-8502	gills-fish Ac	3A	not detected	not detected	not done
#05-8502	guts-fish Ac	3A	5160	not detected	18400
#05-8503	gills-fish Ac	3A	40	not detected	160
#05-8503	guts-fish Ac	3A	360	not detected	35600
#05-8507	blue crab gills +hepatopancreas	3A	not detected	not detected	1880
#05-8508	blue crab gills +hepatopancreas	3A	404	not detected	2220
#05-8509	blue crab gills +hepatopancreas	3A	10	not detected	8560
#05-8510	gills-fish Ac	4	not detected	not detected	440
#05-8510	guts-fish Ac	4	400	not detected	4640
#05-8511	gills-fish Ac	4	not detected	not detected	720
#05-8511	guts-fish Ac	4	80	not detected	6600
#05-8512	gills-fish Ac	4	not detected	not detected	300
#05-8512	guts-fish Ac	4	360	not detected	7280
#05-8513	gills-fish Ac	4	not detected	not detected	286
#05-8513	guts-fish Ac	4	80	not detected	6720
#05-8514	blue crab gills +hepatopancreas	4	not detected	not detected	1760
#05-8516	gills-fish Ac	4	not detected	not detected	972
#05-8516	guts-fish Ac	4	not detected	not detected	4400
#05-8517	gills-fish Ac	4	not detected	not detected	629
#05-8517	guts-fish Ac	4	40	not detected	6520
#05-8518	gills-fish Ac	4	not detected	not detected	3560
#05-8518	guts-fish Ac	4	not detected	not detected	9520
#05-8519	gills-fish Ac	4	not detected	not detected	360
#05-8519	guts-fish Ac	4	not detected	not detected	7800
#05-8520	gills-fish Ac	4	not detected	not detected	720
#05-8520	guts-fish Ac	4	400	not detected	7720
#05-8521	gills-fish Ac	4	not detected	not detected	not detected

#05-8521	guts-fish Ac	4	120	not detected	18320
#05-8522	gills-fish Ac	4	not detected	not detected	3200000
#05-8522	guts-fish Ac	4	57	not detected	685
#05-8523	gills-fish Ac	4	not detected	not detected	not detected
#05-8523	guts-fish Ac	4	not detected	not detected	12640
#05-8524	gills-fish Ac	4	not detected	not detected	360
#05-8524	guts-fish Ac	4	not detected	not detected	7880
#05-8525	gills-fish Ac	4	not detected	not detected	not detected
#05-8525	guts fish Ac	4	not detected	not detected	7400
#05-8526	gills-fish Ac	4	36000	not detected	1040
#05-8526	guts-fish Ac	4	not detected	not detected	3000
#05-8527	gills-fish Ac	4	not detected	not detected	1714
#05-8527	guts-fish Ac	4	not detected	not detected	16400
#05-8528	gills-fish Ac	4	not detected	not detected	60000
#05-8528	guts-fish Ac	4	not detected	not detected	667
#05-8529	gills-fish Ac	4	not detected	not detected	2000
#05-8529	guts-fish Ac	4	not detected	not detected	38000
#05-8530	gills-fish Ac	4	6723	not detected	1786
#05-8530	guts-fish Ac	4	not detected	not detected	13404
#05-8531	gills-fish Ac	4	not detected	not detected	4857
#05-8531	guts-fish Ac	4	200	not detected	40400
#05-8532	gills-fish Ac	4	67	not detected	1467
#05-8532	guts-fish Ac	4	not detected	not detected	4840
#05-8533	gills-fish Ac	4	65	not detected	5097
#05-8533	guts-fish Ac	4	not detected	not detected	6280
#05-8534	gills-fish Ac	4	438	not detected	4500
#05-8534	guts-fish Ac	4	3760	not detected	6120
#05-8535	gills-fish Ac	4	not detected	not detected	2300
#05-8535	guts-fish Ac	4	40000	not detected	27600
#05-8536	gills-fish Ac	8	not detected	not detected	160
#05-8536	guts-fish BET	8	not detected	not detected	880
#05-8537	guts-fish BET	8	400	not detected	920
#05-8538	guts-fish BET	8	not detected	not detected	600

#05-8538	guts-fish BET	8	not detected	not detected	40
#05-8539	guts-fish BET	8	1320	not detected	720
#05-8539	guts-fish BET	8	not detected	not detected	80
#05-8540	guts-fish BET	8	40	not detected	120
#05-8540	guts-fish BET	8	80	not detected	not done
#05-8541	gills-fish Ac	9	not detected	not detected	not done
#05-8541	guts-fish Ac	9	not detected	not detected	200
#05-8542	gills-fish Ac	9	80	not detected	160
#05-8542	guts-fish Ac	9	80	not detected	1680
#05-8543	gills-fish Ac	9	not detected	not detected	not done
#05-8543	guts-fish Ac	9	120	not detected	200
#05-8544	gills-fish Ac	9	not detected	not detected	not done
#05-8544	guts-fish Ac	9	40	not detected	40
#05-8545	gills-fish Ac	9	not detected	not detected	1360
#05-8545	guts-fish Ac	9	12	not detected	4000
#05-8546	gills-fish Ac	9	not detected	not detected	100
#05-8546	guts-fish Ac	9	not detected	not detected	3533
#05-8547	gills-fish Ac	9	80	not detected	40
#05-8547	guts-fish Ac	9	40	not detected	not done
#05-8548	gills-fish Ac	9	not detected	not detected	200
#05-8548	guts-fish Ac	9	10	not detected	1200
#05-8549	blue crab gills +hepatopancreas	9	218	not detected	5200
#05-8550	blue crab gills +hepatopancreas	9	720	not detected	15200
#05-8551	blue crab gills +hepatopancreas	9	66	not detected	3200
#05-8552	blue crab gills +hepatopancreas	9	112	not detected	8000
#05-8553	gills-fish Ac	10	507	not detected	3200
#05-8553	guts-fish Ac	10	606	not detected	15400
#05-8554	gills-fish Ac	10	not detected	not detected	3800
#05-8554	guts-fish Ac	10	300	not detected	3920
#05-8555	gills-fish Ac	10	50	not detected	160
#05-8555	guts-fish Ac	10	109	not detected	4280

#05-8556	gills-fish Ac	10	28	not detected	not done
#05-8556	guts-fish Ac	10	93	not detected	4075
#05-8557	gills-fish Ac	10	not detected	not detected	1080
#05-8557	guts-fish Ac	10	87	not detected	3275
#05-8558	gills-fish Ac	10	not detected	not detected	560
#05-8558	guts-fish Ac	10	10	not detected	5440
#05-8559	gills-fish Ac	10	not detected	not detected	250
#05-8559	guts-fish Ac	10	32	not detected	3520
#05-8560	gills-fish Ac	10	not detected	not detected	200
#05-8560	guts-fish Ac	10	24	not detected	1240
#05-8561	gills-fish Ac	10	133	not detected	700
#05-8561	guts-fish Ac	10	10	not detected	1875
#05-8562	gills-fish Ac	10	47	not detected	5048
#05-8562	guts-fish Ac	10	10	not detected	2860
#05-8563	gills-fish Ac	10	80	not detected	not done
#05-8563	guts-fish Ac	10	20	not detected	3760
#05-8564	gills-fish Ac	10	not detected	not detected	400
#05-8564	guts-fish Ac	10	30	not detected	5500
#05-8565	gills-fish Ac	10	not detected	not detected	10
#05-8565	guts-fish Ac	10	20	not detected	1180
#05-8566	gills-fish Ac	11	147	not detected	800
#05-8566	guts-fish Ac	11	227	not detected	4833
#05-8567	gills-fish Ac	11	20	not detected	1200
#05-8567	guts-fish Ac	11	60	not detected	3200
#05-8568	gills-fish Ac	11	40	not detected	3080
#05-8568	guts-fish Ac	11	40	not detected	360
#05-8569	gills-fish Ac	11	67	not detected	333
#05-8569	guts-fish Ac	11	4	not detected	1640
#05-8570	gills-fish Ac	11	8	not detected	300
#05-8570	guts-fish Ac	11	not detected	not detected	3425
#05-8571	gills-fish Ac	11	not detected	not detected	233
#05-8571	guts-fish Ac	11	16	not detected	6640
#05-8572	gills-fish Ac	11	3	not detected	533

#05-8572	guts-fish Ac	11	7	not detected	1700
#05-8573	gills-fish Ac	11	40	not detected	171
#05-8573	guts-fish Ac	11	30	not detected	1900
#05-8574	gills-fish Ac	11	not detected	not detected	350
#05-8574	guts-fish Ac	11	2000	not detected	1400
#05-8575	gills-fish Ac	11	4	not detected	not done
#05-8575	guts-fish Ac	11	10	not detected	440
#05-8576	gills-fish Ac	11	not detected	not detected	356
#05-8576	guts-fish Ac	11	12	not detected	1133
#05-8577	gills-fish Ac	11	not detected	not detected	286
#05-8577	guts-fish Ac	11	4	not detected	844
#05-8578	gills-fish Ac	11	not detected	not detected	350
#05-8578	guts-fish Ac	11	20	not detected	1600
#05-8579	gills-fish Ac	11	8	not detected	680
#05-8579	guts-fish Ac	11	not detected	not detected	2178
#05-8580	gills-fish Ac	11	not detected	not detected	100
#05-8580	guts-fish Ac	11	5	not detected	1947
#05-8581	gills-fish Ac	11	not detected	not detected	80
#05-8581	guts-fish Ac	11	not detected	not detected	1000
#05-8582	gills-fish Ac	11	15	not detected	300
#05-8582	guts-fish Ac	11	31	not detected	3323
#05-8583	gills-fish Ac	11	not detected	not detected	200
#05-8583	guts-fish Ac	11	32	not detected	350
#05-8584	gills-fish Ac	11	53	not detected	150
#05-8584	guts-fish Ac	11	48	not detected	2905
#05-8585	gills-fish Ac	11	7	not detected	150
#05-8585	guts-fish Ac	11	5	not detected	1800

[1]Ac, Atlantic croaker; BET, big-eyed tuna
[2]APC, aerobic plate counts

Figure 1