Analysis of Bile of Fish Collected in Coastal Waters of the Gulf of Mexico Potentially Affected by Hurricane Katrina to Determine Recent Exposure to Polycyclic Aromatic Compounds (PACs)

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Summary
We analyzed bile samples from fish sampled during the R/V NANCY FOSTER cruise of 12-16 September 2005. The results indicate that Atlantic croaker and bigeye tuna collected soon after Hurricane Katrina are showing no greater exposure to polycyclic aromatic compounds (PACs, which are indicative of petroleum products) than was found for croaker that were collected in 1990 under the National Oceanic and Atmospheric Administration's (NOAA) National Benthic Surveillance Project.

Introduction
Analyses have been completed that screen for metabolites of PACs in bile from Atlantic croaker and one juvenile bigeye tuna captured from coastal waters of the Gulf of Mexico following Hurricane Katrina. Atlantic croaker is a widely distributed, commercially important species in the Gulf of Mexico that serves as a good indicator species for the tests that were conducted.

PACs are chemical contaminants that are prevalent in urban embayments, largely derived from petroleum products or their combustion products, that can alter normal physiological function in marine biota (Varanasi *et al*. 1989; Stein *et al*. 1992). Concerns have been raised over the effects of exposure to PACs, alone or in combination with other toxic contaminants, on marine organisms because of the worldwide use of fossil fuels (Geraci and Aubin 1990; Peterson *et al*., 2004) and the occurrence of oil spills in areas that support populations of marine fish. Marine fish can be exposed to PACs by various routes (e.g., consumption of contaminated prey, direct contact via gills).

Vertebrates (e.g., fish and marine mammals) rapidly take up PACs present in their food and the environment and quickly metabolize these compounds to more polar compounds. The more polar PAC metabolites are then secreted into the bile for elimination (Varanasi *et al*., 1989). As a result, fish exposed to high levels of PACs contain relatively low or undetectable levels of these compounds in their muscle (Varanasi et al., 1989). Therefore, bile can be analyzed for metabolites to demonstrate that vertebrates have been exposed to PACs.

Methods
The fish capture sites and corresponding station identification numbers are shown in Figure 1. Because each Atlantic croaker contained a small volume of bile (<25µL), bile samples from 3 to 8 fish were composited in the field (Table 1) to provide adequate bile

volume for high-performance liquid chromatography/fluorescence (HPLC/fluorescence) analysis. Bile of Atlantic croaker and a single juvenile bigeye tuna was analyzed for metabolites of PACs using an HPLC/fluorescence method described by Krahn *et al.* (1984).

Bile was injected directly onto a C-18 reverse-phase column (Phenomenex Synergi Hydro) and eluted with a linear gradient from 100% water (containing a trace amount of acetic acid) to 100% methanol at a flow of 1.0 mL/min. Chromatograms were recorded at the following wavelength pairs: 1) 293/335 nm where many 2-3 benzene ring aromatic compounds (e.g., naphthalene) fluoresce, 2) 260/380 nm where several 3-4 ring compounds (e.g., phenanthrene) fluoresce and 3) 380/430 nm where 4-5 ring compounds (e.g., benzo[a]pyrene) fluoresce. Peaks eluting after 5 minutes were integrated and the areas of these peaks were summed. The concentrations of fluorescent PACs in the bile samples of croaker and tuna were determined using naphthalene (NPH), phenanthrene (PHN) or benzo[a]pyrene (BaP) as external standards and converting the fluorescence response of bile to phenanthrene (ng PHN equivalents/g bile), naphthalene (ng NPH equivalents/g bile) or benzo(a)pyrene (ng BaP equivalents/g bile) equivalents.

To ensure that the HPLC/fluorescence system was operating properly, a NPH/PHN/BaP calibration standard was analyzed numerous times (n = 5) until a relative standard deviation < 15% was obtained for each PAC. As part of our laboratory quality assurance (QA) plan, two QA samples [a method blank and a fish bile control sample (bile of Atlantic salmon exposed to 25 µg/mL of Monterey crude oil for 48 hours)] were analyzed with the fish bile samples (see Table 3) (Krahn *et al*., 1988).

Results

The concentrations of fluorescent PACs (based on wet weight) measured in the bile samples of Atlantic croaker and bigeye tuna collected near coastal regions impacted by Hurricane Katrina are reported in Table 1. The NPH equivalents measured in the croaker bile samples ranged from 55,000 – 270,000 ng/g bile, wet weight and are lower or comparable to the levels (120,000 – 730,000 ng/g bile, wet weight) measured in bile of Atlantic croaker collected in waters of Louisiana and Alabama in 1990 as part of NOAA's National Benthic Surveillance Project (see Table 1). Similarly, BaP equivalents measured in Atlantic croaker in the current study (620 – 3,100 ng/g bile, wet weight) are lower or in the same range as those measured in croaker collected in 1990 from Louisiana and Alabama (1,400 – 7,500 ng/g bile, wet weight). Although the NPH level (96,000 ng/g bile, wet weight) in bile of the juvenile bigeye tuna was in the same range as the levels measured in the 2005 croaker, the biliary BaP equivalents measured in the tuna (270 ng/g bile, wet weight) were lower than those determined in bile of Atlantic croaker collected in 2005. The levels of NPH and BaP equivalents measured in bile of Atlantic croaker collected in 1990 and 2005 are similar to those measured in bile of fish collected in urbanized and semi-urbanized waterways of the U.S. (Myers et al., 1994, Stehr et al., 2000). Assessment of bile for PACs provides information on recent input and exposure to these compounds in fish. Because fish can rapidly metabolize and eliminate these compounds, routinely consumed tissues of fish (e.g., muscle) contain PAC levels that are generally undetected and, therefore, are not of concern for human consumption.

Conclusions

The Atlantic croaker and bigeye tuna captured following the hurricane are showing no greater exposure to PACs than was found for croaker that were collected in 1990 by the Environmental Conservation Division for NOAA's National Benthic Surveillance Project. The levels of PAC metabolites measured in bile of Atlantic croaker collected in 1990 and in the current study are similar to those measured in bile of fish collected in urbanized and semi-urbanized waterways of the U.S.

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Table 1. Concentrations of metabolites of polycyclic aromatic compounds measured in bile of Atlantic croaker and bigeye tuna collected in coastal waters of the Gulf of Mexico affected by Hurricane Katrina.

					Equivalents of fluorescent aromatic compounds (ng/g bile, wet weight)		
Species	Samples per Composite	Station Number	Collection Site	Collection Year	NPH equivalents[1]	PHN equivalents[2]	BaP equivalents[3]
Atlantic croaker	3	3A	Mississippi River Delta, Head of Passes, LA	2005	170,000	51,000	770
Atlantic croaker	3	4	Southwest Pass, LA (composite #1)	2005	170,000	72,000	940
Atlantic croaker	8	4	Southwest Pass, LA (composite #2)	2005	230,000	70,000	730
Atlantic croaker	7	4	Southwest Pass, LA (composite #3)	2005	270,000	83,000	830
Atlantic croaker	4	9	SE of Chandeleur Islands, LA	2005	140,000	21,000	620
Atlantic croaker	8	10	North of Horn Island, AL	2005	160,000	42,000	3,100
Atlantic croaker	6	11	South of Horn Island, AL (composite #1)	2005	55,000	13,000	740
Atlantic croaker	6	11	South of Horn Island, AL (composite #2)	2005	70,000	22,000	2,200
Bigeye tuna	1	8	Southeast of Chandeleur Islands, LA	2005	96,000	6,100	270

[1]Concentrations in part per billion (ng/g) based on total area compared to the fluorescence of naphthalene standard at 293/335 nm wavelengths

[2]Concentrations in part per billion (ng/g) based on total area compared to the fluorescence of phenanthrene standard at 260/380 nm wavelengths

[3]Concentrations in part per billion (ng/g) based on total area compared to the fluorescence of benzo[a]pyrene standard at 380/430 nm wavelengths

Table 2. Concentrations of metabolites of polycyclic aromatic compounds measured previously in bile of Atlantic croaker collected in the Gulf of Mexico region as part of NOAA's National Benthic Surveillance Project.

					Equivalents of fluorescent aromatic compounds (ng/g bile, wet weight)		
Species	Samples per Composite	Station Number	Collection Site	Collection Year	NPH equivalents[1]	PHN equivalents[2]	BaP equivalents[3]
Atlantic croaker	10	12	Mississippi River Delta, Southeast Pass, LA	1990	200,000	not analyzed	1,400
Atlantic croaker	10	off map[4]	Calcasieu River, Prien Lake, LA	1990	730,000	not analyzed	5,700
Atlantic croaker	7	off map[4]	Calcasieu River, West Cove, LA	1990	510,000	not analyzed	7,500
Atlantic croaker	10	13	Mobile Bay, North Point, AL	1990	120,000	not analyzed	1,400

[1]Concentrations in part per billion (ng/g) based on total area compared to the fluorescence of naphthalene standard at 293/335 nm wavelengths

[2]Concentrations in part per billion (ng/g) based on total area compared to the fluorescence of phenanthrene standard at 260/380 nm wavelengths

[3]Concentrations in part per billion (ng/g) based on total area compared to the fluorescence of benzo[a]pyrene standard at 380/430 nm wavelengths

[4]Station located in western Louisiana, near the border with Texas

Table 3. Concentrations of metabolites of polycyclic aromatic compounds measured in bile reference material and method blanks analyzed as part of the Hurricane Katrina Response 2005.

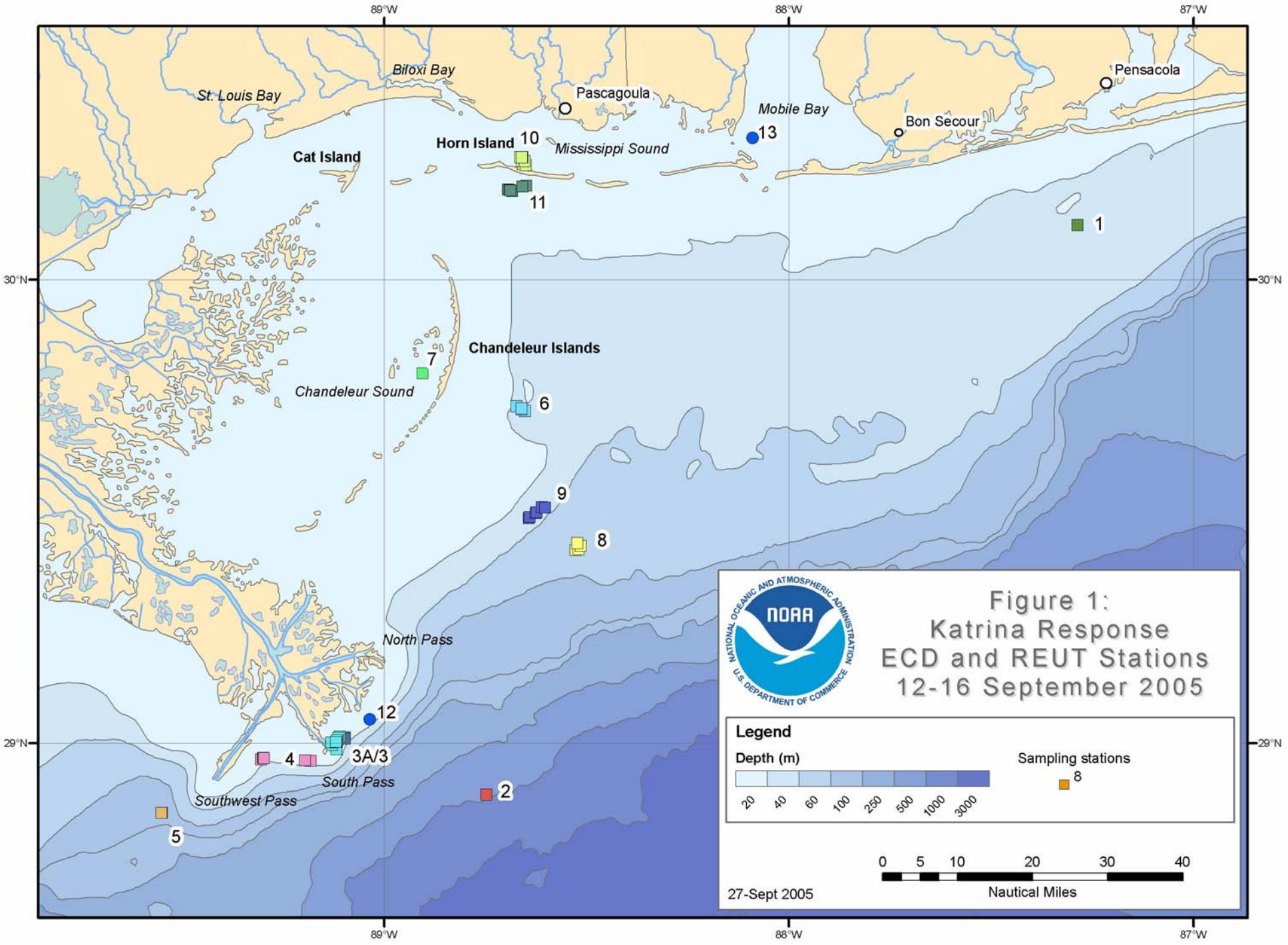
		Equivalents of fluorescent aromatic compounds (ng/g bile, wet weight)		
QA Sample Type	**QA Sample Information**	**NPH equivalents[1]**	**PHN equivalents[2]**	**BaP equivalents[3]**
ASMBC2 09/22/05 A	Atlantic salmon exposed to Monterey Bay crude oil for 48 hrs.	120,000	37,000	900
ASMBC2 09/22/05 A	Atlantic salmon exposed to Monterey Bay crude oil for 48 hrs.	120,000	37,000	1,000
Method blank	Method blank	0	0	0
Method blank	Method blank	0	0	0

Bile Reference Material ASMBC2:	Equivalents of fluorescent aromatic compounds (ng/g bile, wet weight)		
	NPH equivalents[1]	**PHN equivalents[2]**	**BaP equivalents[3]**
Mean	150,000	50,000	1,300
SD	20,000	5,300	250
Upper Control Limit	190,000	60,000	1,800
Lower Control Limit	110,000	39,000	800

[1]Concentrations in part per billion (ng/g) based on total area compared to the fluorescence of naphthalene standard at 293/335 nm wavelengths

[2]Concentrations in part per billion (ng/g) based on total area compared to the fluorescence of phenanthrene standard at 260/380 nm wavelengths

[3]Concentrations in part per billion (ng/g) based on total area compared to the fluorescence of benzo[a]pyrene standard at 380/430 nm wavelengths


89°W
88°W
87°W
30°N
29°N
Biloxi Bay
St. Louis Bay
Pascagoula
Mobile Bay
Pensacola
Bon Secour
Horn Island
Mississippi Sound
Cat Island
Chandeleur Islands
Chandeleur Sound
North Pass
South Pass
Southwest Pass
1
2
3A/3
4
5
6
7
8
9
10
11
12
13
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