Petroleum hydrocarbons, fluorescent aromatic compounds in fish bile and organochlorine pesticides from areas surrounding the spill of the Kab121 well, in the Southern Gulf of Mexico: A case study

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Abstract
In October 2007, a light crude oil spill took place in the off shore Kab121 oil well, 32 km north of the mouth of the Grijalva River, Tabasco, Mexico. In order to estimate the possible effects of oil spill on the biota in the area surrounding the spilled well, the level of different fractions of petroleum hydrocarbons were measured in fish, as well as the concentration of some chlorinated hydrocarbons and PCBs. The organisms examined were cat fish (Ariopsis felis), in addition fluorescent aromatic compounds in bile, the contaminants above mentioned and their relationship with cytochrome P-450 and Ethoxyresorufin-O-deethylase, Glutathione-S-Transferase and catalase activities in liver were determined. The concentration of most pollutants were low, except PAHs. Spatial distribution of these compounds, as well as most biomarkers, reflected the highest exposure of fish to pollutants in the area adjacent to well, as well as in the proximity of rivers. The profile of exposure to this environment was chronic in nature and not temporary.

Key words
Ariopsis felis, Biomarkers, Oil pollution, PCB

Introduction
The Gulf of Mexico is an area with many natural resources, oil being one of the most important. Mexico ranks sixth worldwide in oil production, and the marine area of the Gulf of Mexico contributes more than 76% of domestic production. The process of drilling and oil extraction are some of the activities that release significant amount of oil into the sea, and spills are other sources of petroleum to the environment that cause problems to the environment and organisms of the affected area. On October 2007, due to an accident there was a gas and light crude oil leak in the valve shaft of Kab-121 well, located in the Kab-101 platform 22 km north of Tabasco, Mexico. The leak was controlled 52 days later, but during this period it was estimated that approximately 16, 500 barrels were spilled into the sea.

The area of the spill is in front of the estuary of the Grijalva river, which represents about 30 to 35% of all freshwater runoff in Mexico. The plume of the river covered the spill area during the rainy season, complicating the interpretation of results. Chlorinated compounds, including polychlorinated biphenyls (PCBs), were determined to assess the influence of land-based sources of pollutants.

The aim of this study was to determine the possible effects of spill on fish of the surrounding area of the well Kab121, through identification of petroleum hydrocarbons as well as metabolites of polycyclic hydrocarbons and other biomarkers in cat fish (Ariopsis felis). Due to the agricultural activities conducted in coastal area, the concentrations of some organochlorine pesticides and PCBs were determined to assess the influence of...
land-based sources of pollutants, and also considering that these compounds have negative effects on the organisms.

Materials and Methods

Sampling was done twice, once in March and other in June 2008. The sampling network consisted of 21 stations located around the Kab-101 platform, in which the Kab-121 well is located (Fig. 1). Five fish per station were collected by trawling and dissected to extract the bile and liver. Liver and bile samples were preserved in liquid nitrogen and later transported to the laboratory for further analysis.

Hydrocarbons and organochlorine compounds in liver were determined according to Wade et al. (1993). Prior to extraction, liver samples were homogenized and 100 µl of the following surrogate standards were added: biphenyl d10, phenanthrene d10, chrysene d12, benzo[a]pyrene d12 (10 mg ml⁻¹), and o-terphenyl (200 mg ml⁻¹). Samples were extracted with hexane in a tissue homogenizer, and extracts were purified and separated into fractions using silica gel-alumina columns. Identification and quantification of the compounds was carried out with standards from Ultra Scientific in the case of the PAHs and organochlorine pesticides; Accu Standard for PCBs and from Chiron for deuterated PAHs. Total hydrocarbons were analyzed with an Agilent 5890 gas chromatograph equipped with a FID detector. PAHs were analyzed with a Perkin-Elmer gas chromatograph equipped with a Clarus 500 mass selective detector using a 30 m × 0.25 mm (i.d.) x 0.25 DB-5 MS fused silica capillary column (J & W Scientific) and operating in the selected ion monitoring (SIM) mode. For quality control/quality assurance in each set of samples a procedural blank and a duplicate sample were added; surrogate standards were used to evaluate recovery. Equipment calibrations were verified daily, and calibration curves were done for each set of samples analyzed. PAHs were reported as total PAHs, low molecular weight PAHs (two and three benzene ring compounds) and high molecular weight PAHs (four or more benzene rings), respectively.

Biliary metabolites (FACs) were analyzed according to Aas et al. (1998) using fixed-wavelength spectrofluorometry. Ethoxyresorufin-O-deethylase (EROD) activity was measured fluorometrically in the microsomal suspension in the presence of NADPH using ethoxyresorufin (ethoxyphenoxazone) as substrate. Enzyme activity was estimated from the resorufin formed per unit of time, which was monitored in a spectrofluorometer using excitation and emission wavelengths of 530 nm and 572 nm, respectively. A resorufin molar extinction coefficient of 73.2 nM cm⁻¹ was used. Results were expressed per mg of microsomal protein using bovine serum albumin as standard, according to the method described by Burke et al. (1974). Glutathione-S-transferase (GST) activity was determined according to Habig et al. (1974). Briefly, 1 mM CDNB was added to buffer containing 1 mM GSH and an aliquot of sample to be tested. Upon addition of CDNB, the change in absorbance at 340 nm was measured as a function of time. The extinction coefficient for this reaction was 9.6 mM cm⁻¹. GST activities with 1-chloro-2, 4-dinitrobenzen (CDNB) and 3, 4-dichloronitrobenzene (DCNB) were assayed spectrophotometrically at room temperature by monitoring the change in absorbance at 344, 270, and 344 nm, respectively by the method of Habig (1974). Activity with CDNB was carried out in the presence of 0.1 M HEPES (pH 7.6), 1 mM GSH, 1 mM CDNB, 1 mg purified protein or 50 mg cytosolic protein. GST–DCNB activity was measured using 0.1 M HEPES (pH 7.6), 5 mM GSH, 1 mM DCNB, 0.1–0.2 mg purified protein or 1.0–1.5 mg cytosolic protein. GST activity toward EA was measured in 0.1 M sodium phosphate (pH 6.5), 0.25 mM GSH, and 0.2 mM NaEA (freshly prepared from equimolar amounts of EA and sodium bicarbonate in deionized water). Activities were calculated using absorptivities of 9.6 mM cm⁻¹, 8.5 mM cm⁻¹ and 5.0 mM cm⁻¹, respectively.

Catalase (CAT) activity was evaluated according to the method proposed by Aebi (1984) in which the disappearance of H₂O₂[initial concentration: 0.04% (v/v)] in a phosphate buffer (0.0068 g l⁻¹KH₂PO₄, 0.0175 g l⁻¹ Na₂HPO₄, pH 7.0) was monitored at 240 nm. The reaction mixture contained 30 mM H₂O₂ in 50 mM phosphate buffer pH 7.0 and 0.1 ml enzyme in a total volume of 3 ml. CAT activity was estimated by decrease in absorbance of H₂O₂ at 240 nm. The enzyme activity (I.U.) was defined as 1 m mol of H₂O₂ decomposed per minute. The final activity was expressed in.
units per milligram of total soluble proteins. Total protein was quantified by the method of Lowry et al. (1954). Fish livers were homogenized in a boron buffer (pH 8.7), in a refrigerated centrifuge at 5000 g for 15 min. The absorbance of the solution was determined in the presence of Folin reagent at 750 nm wavelength. The protein content was determined following a standard curve obtained using bovine serum albumin as standard.

Statistical analyses: Data that did not meet the assumptions of normality and homogeneity of variance, non-parametric analyses were carried out using Mann-Whitney U test and Spearman correlation. All statistical analyses were done with R version 3.0.0 (http://www.r-project.org/).

Results and Discussion

Tables 1, 2 shows the average concentration of hydrocarbon fractions in the liver and metabolites of PAHs in bile of catfish from the study area. Low molecular weight PAHs showed a mean of 39.7 ± 24.6 µg g⁻¹ and 11.3 ± 7.9 µg g⁻¹ for first and second samplings. High molecular weight PAHs showed a mean of 1.47±1.67 µg g⁻¹ for first sampling and 1.19±1.21 µg g⁻¹ for second sampling. Total hydrocarbons showed a mean of 133.5 ± 84.9 µg g⁻¹ and 75.9 ± 50.2 µg g⁻¹ in the first and second sampling, respectively.

Low molecular weight PAHs concentrations were significantly higher (Mann-Whitney U = 22.2; P = 2.4 X 10⁻¹⁶) during the first sampling as compared to second (Fig. 2), probably due to metabolism and excretion, and also because sediment concentrations also decreased (data not shown); enough time passed between the two samplings to detect the decrease in the level of hydrocarbons. High molecular weight PAHs showed no significant differences (Mann-Whitney U = 0.11; P = 0.74) between samplings, probably due to lower contribution of these compounds in the oil spilled in the study area, because the oil spilled is light and high molecular weight PAHs are less abundant compounds in petroleum than low molecular weight PAHs (De Luca et al., 2005).

Fig. 3 shows the spatial distribution of low molecular weight PAHs in liver, which shows influence of spill and contribution of Grijalva river. It should be noted that the point of intersection of the transects (station 6) is the area where the spill originated. For total PAHs, significantly higher concentrations in the first sampling were found, where more than 90% of these compounds consisted of low molecular weight PAHs. This indicated that source of these hydrocarbons was petrogenic (originating from petroleum), but it is possible that high molecular weight PAHs were metabolized to low molecular weight PAHs (Gagnon and Holdway, 2002). Al-Hassan (2000) found that two, three and four benzene ring compounds were more common in sharks from Arabian Gulf, compared to higher-membered ring compounds. This may be due to relatively higher solubility of compounds in petroleum than low molecular weight PAHs (De Luca et al., 2005).

Table 1 : Average concentrations of hydrocarbons (µg g⁻¹ wet weight) in Ariopsis felis liver and metabolites in bile of PAHs (µg ml⁻¹) collected during first sampling

<table>
<thead>
<tr>
<th>Station</th>
<th>Low molecular weight PAHs</th>
<th>High molecular weight PAHs</th>
<th>PAHs</th>
<th>Total HC</th>
<th>OH-P</th>
<th>BaP</th>
<th>OH-N</th>
<th>PHE</th>
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<td>91.627</td>
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Total HC = Total hydrocarbons, OH-P = Hydroxipyrene, BaP = Benzo a pyrene, OH-N = Hydroxi naphthalene, PHE = Phenantrene ND = Not detected
these compounds in water, which facilitates their uptake by marine animals (Baumard et al., 1999). It is difficult to determine the origin of hydrocarbons in catfish, because these compounds are rapidly metabolized by fish (Malins and Hodgins, 1981) and therefore the level found in organisms reflect only part of the concentration present in sediments.

The level of PAHs in the liver of catfish in the present study were higher than those reported by Al-Hassan (2001) in the liver of other species of catfish (Arius bilineatus Val.) in the Arabian Gulf (0.0078 – 0.126 µg g⁻¹), a system characterized by intense oil activity; likewise the concentrations found in this study were higher than the values reported by Swapan et al. (2000) for fish obtained from Hiroshima Bay (0.25 – 13.6 µg g⁻¹), which is reported to be of toxicological concern to other trophic components of the coastal ecosystem.

Total hydrocarbons were significantly (Mann-Whitney U = 6.02; P = 0.014) higher during first sampling than second (Fig. 2); UCM contributed approximately 90% of hydrocarbons, which indicates petrogenic sources since UCM is a common feature of oil, certain refined products, and extracts of samples.

**Table 2**: Average concentration of hydrocarbons (µg g⁻¹ wet weight) in *Ariopsis felis* liver and metabolites in bile of PAHs (µg ml⁻¹) collected during second sampling

<table>
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<th>Station</th>
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<th>HPAHs</th>
<th>PAHs</th>
<th>THC</th>
<th>OH-P</th>
<th>BaP</th>
<th>OH-N</th>
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Total HC = Total hydrocarbons, OH-P = Hydroxy pyrene, BaP = Benzo a pyrene, OH-N = Hydroxy naphthalene, PHE = Phenanthrene ND = No detected

**Fig. 2**: Medium concentrations of low molecular weight PAHs and total hydrocarbons in liver of *A. felis* per cruise
contaminated by oil, and also an indication of weathering of oil.

Table 3 and 4 shows the concentration of organochlorine pesticides in the liver of catfish. Total organochlorine pesticides showed a mean of 19.9 ± 14.9 ng g⁻¹ and 40.1 ± 27.1 ng g⁻¹ in the first and second samplings, respectively. PCBs showed a mean of 18.1± 15.8 ng g⁻¹ for the first and 24.7 ± 14.5 ng g⁻¹ for the second sampling.

All groups of organochlorine pesticides showed higher concentration in the second sampling, and with exception to chlorobenzenes and PCBs, all showed significant differences (Fig. 4). The second sampling was conducted during the rainy season, and increased discharge from the rivers could have provided these pollutants. Cyclopentadienic pesticides (“Drins”) were the most abundant group.

Fig. 5 shows the spatial distribution of organochlorine pesticides in liver, showing the influence provided by the contribution of river Grijalva. Predominant direction of sea currents was East-West during the sampling period.

Comparing these results with those reported by Colombo et al. (2011) for fish from Rio de la Plata, Argentina, which varied from 10 to 100 ng g⁻¹, it was determined that concentration of these compounds in fish in the study area were low. Also, organochlorine pesticide level in fish of this study were lower than those reported by Adu-Kumi et al. (2010) in fish from a lagoon in Ghana, whose concentrations were considered low (average 442 ng g⁻¹).

Fig. 3 : Spatial distribution of low molecular weight PAHs in the liver A. felis

Fig. 4 : Concentration of chlorobenzenes and PCBs in liver of A. felis per cruise
The four metabolites tested had higher concentrations in fish from the first sampling, which is similar pattern than hydrocarbons in liver, and thus reflects exposure to hydrocarbons from the spill (Fig. 6). Statistical differences were highly significant for Benzo (a) Pyrenes (Mann-Whitney U = 30.8; P = 2.9 X 10^-8), for Pyrenes (Mann-Whitney U = 15.5; P = 8.3 X 10^-5), Fenanthrenes (Mann-Whitney U = 30.8; P = 2.9 X 10^-8) and Naphthalenes (Mann-Whitney U = 17.1; P = 3.5 X 10^-8). The concentration of all metabolites decreased for the second sampling, possibly because PAH metabolites were excreted from the fish.

Over all there was a predominance of low molecular weight metabolites (2 and 3 benzene rings) compared to high molecular weight (4 and more benzene rings), which is consistent and statistically correlated with the presence of more hydrocarbons of low molecular weight than high molecular weight found in the sediments. Krahn et al. (1992) reported the same relationship in areas where low molecular weight hydrocarbons were predominant, and hydrocarbons were supplied from petrogenic sources. High proportions of naphthalenes indicate exposure to petroleum products or crude oil, while low proportions of naphthalenes indicate contribution of pyrogenic hydrocarbons, such as the combustion of petroleum, wood, etc. (Krahn et al., 1992; Aas et al., 2000; Gagnon and Holdway, 2002). McDonald et al. (1995) presented a very similar value of low and high molecular weight FACs in the fish Nototheniogibberifrons exposed to diesel oil from the Arctic to those reported in this study, indicating that the source of FACs is petrogenic.

Fig. 7 shows the significant positive correlation (Spearman
Tables 5 and 6 shows the result of biomarkers for cruises 1 and 3, respectively. Catalase activity showed a mean of 36.3 ± 6.8 pmol mg⁻¹ protein in the first cruise and 53.5 ± 9.4 pmol mg⁻¹ protein in the second cruise. The difference was highly significant (Mann-Whitney U = 22.7; P = 1.9 X 10⁻⁴). EROD had a mean activity of 43.1 ± 10 pmol min⁻¹ mg⁻¹ protein in the first cruise, and 61.1 ± 14 pmol min⁻¹ mg⁻¹ protein in the second cruise, respectively. The difference was highly significant (Mann-Whitney U = 14.9; P = 0.00011). GST showed a mean activity of 33.2 ± 3.9 pmol mg⁻¹ protein in the first and 64.2 ± 7.5 pmol mg⁻¹ protein in the second cruise. The differences were highly significant (Mann-Whitney U = 30.8; P = 2.9 X 10⁻⁴). Since hydrocarbon concentration decreased in the second cruise, but organo chlorine compounds increased, it seems that biomarkers were responding to these latter compounds. Low molecular weight PAHs showed a significant negative correlation with CAT activity (Spearman Rho = -0.64; P = 4.5 X 10⁻⁸), which may reflect the interaction between hydrocarbons and organo chlorine compounds in the liver.

**Table 3**: Concentration of organochlorinated hydrocarbons (ng g⁻¹ wet weight) in the liver of *Ariopsis felis* during first sampling

<table>
<thead>
<tr>
<th>Station</th>
<th>Chlorobenzenes</th>
<th>HCHs</th>
<th>Drines</th>
<th>DDTs</th>
<th>Chorinated pesticides</th>
<th>PCBs</th>
</tr>
</thead>
<tbody>
<tr>
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<tr>
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<td>18.068</td>
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<tr>
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<td>ND</td>
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<tr>
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</table>

HCHs = Hexachlorocyclohexanes, Drins = Aldrin + Endrin + Dieldrin, DDTs = DDT + DDD + DDE
Table 4: Concentration of organochlorinated hydrocarbons (ng g⁻¹ wet weight) in the lives of Ariopsis felis during second sampling

<table>
<thead>
<tr>
<th>Station</th>
<th>Chlorobenzenes</th>
<th>HCHs</th>
<th>DDTs</th>
<th>Chorinated pesticides</th>
<th>PCBs</th>
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HCHs = Hexachlorocyclohexanes, Drins = Aldrin + Endrin + Dieldrin, DDTs = DDT + DDD + DDE

Table 5: Activities of biomarkers in the liver of Ariopsis felis during first sampling

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<th>CAT**</th>
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Table 6: Activities of biomarkers in the liver of Ariopsis felis during second sampling

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*Units for EROD are pmol min⁻¹ mg⁻¹ protein; **Units for GST and CAT are pmol mg⁻¹ protein.
be due to the metabolic breakdown of high molecular weight PAHs (Oliva et al., 2010). A similar profile was observed in a correlation between Benzo (a) pyrene metabolites and CAT activity. A significant negative correlation (Spearman Rho = -0.51; P = 0.00054) between Naphthalene metabolites with CAT was found. Part of the variation recorded for CAT activity could be linked to modification of food availability, spawning period, kind of pollution or environmental factors as season and temperature (Sheehan and Power, 1999).

GST activity in this study showed a significant correlation with HCHs in liver (Spearman Rho = 0.33; P = 0.034). Hepatic GST activity appeared to be induced in rainbow trout exposed to other halogenated compounds, such as PCB 153 or DDE, but was not induced after 2,3,7,8-TCDD exposure. This observation indicates that this parameter may be a suitable biomarker for exposure to nonplanar PCBs and organochlorines (Machala et al., 1998). GST activities reveal that the responses to pollutant mixtures are complex, since absence of a specific effect does not always reflect the absence of contamination.

As expected, EROD was highly correlated with low molecular weight PAHs (Spearman Rho = -0.55; P = 0.00018), since low molecular weight PAHs are classic inducers of EROD (Krahm et al., 1982; Machala et al., 1998; Sheehan and Power, 1999; Barra et al., 2001; Jewett et al., 2002; Oliva et al., 2010).

Hydrocarbon concentrations and particularly PAHs concentrations were higher in the first sampling, which together with their spatial distribution show that the spill is the source of these pollutants. The fact that level of organochlorinated with their spatial distribution show that the spill is the source of concentrations were higher in the first sampling, which together 1999; Barra 2001; Jewett 2002; Oliva 2010).

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