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Distribution of Polycyclic Aromatic Hydrocarbons in Edible Fish from Gomti River, India

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Abstract This study reports the levels and distribution patterns of selected polycyclic aromatic hydrocarbons (PAHs) in fish samples of the Gomti river, India, collected from three sites during the pre- and post-monsoon seasons of the years 2004–2005. In the fish muscles, $\sum$PAHs ranged between 12.85 and 34.89 ng g^{-1} wet wt (mean value: 23.98 ± 6.70 ng g^{-1}). Naphthalene was the most prevalent compound both in terms of detection as well as levels, while, benzo[k]fluoranthene, benzo(a)pyrene, and indeno(123-cd)pyrene + benzo(ghi)perylene could not be detected in any of the sample. Low-molecular weight PAHs were observed dominating over the high molecular weight PAHs.

Keywords PAHs · *Channa punctatus* · Fish muscles · POPs

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous contaminants in the environment and are classified as persistent organic pollutants (POPs). PAHs are a class of diverse organic compounds, which are highly stable in the environment and have the potential to manifest the eco-toxicological activity. They are the product of incomplete combustion of organic material and are introduced into the environment by anthropogenic as well as natural sources. The primary sources of PAHs in the environment include petroleum related activities as well as combustion of various fossil fuels, natural fires, and road runoff/street dust. Other sources of more localized significance includes domestic and industrial waste waters and sewage (Bouloubassi and Saliot 1991). PAHs in the aquatic environment, due to their hydrophobic nature, bind rapidly with particles and deposited sediments serve as their primary reservoirs (Latimer and Zheng 2003). PAHs may pose toxicity in fish and birds (Payne et al. 2003), by interfering with cellular membrane function and the associated enzyme systems (Neff 1985). Further, metabolites of PAHs may bind to proteins and DNA, which causes biochemical disruptions and cell damage in animals (Varanasi et al. 1989). The carcinogenic properties of some PAHs coupled with their stability during atmospheric and aquatic transport and widespread occurrence have, in recent years, generated interest in studying their sources, distribution, transport mechanisms, environmental impact, and fate (Bouloubassi and Saliot 1993). Levels of PAHs commonly found in many aquatic environments are among important risk factor for various health aspects of fish (Payne et al 2003). The water and bed sediments of the Gomti river (India), one of the major tributaries of the river Ganga, have been reported contaminated with PAHs (Malik et al. 2004).

The Gomti river, flowing through eight districts in Uttar Pradesh, drains a catchments area of about 25,000 km^2 and traverses a total distance of about 730 km. Throughout its stretch, there are a few small tributaries (Kathna, Sarayan, Reth, Kalyani, and Sai) originating within short distances and carrying the wastewater and industrial effluents from different towns and industrial units in the basin. On the banks of the river, Lucknow, Sultanpur, and Jaunpur, are the three major urban settlements. The river serves as one of the major source of drinking water for the Lucknow City, the State capital of Uttar Pradesh, with a population of about 3.5 million. The river, subsequently, receives the

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untreated wastewater and effluents from Lucknow, Jagdishpur, Sultanpur, and Jaunpur directly in its course through more than 40 wastewater drains.

However, there is no information available on levels of PAHs in the biota of the river. This study was undertaken to examine the levels and distribution patterns of 16 PAHs, identified as priority pollutants by the United States Environmental Protection Agency (USEPA) (UNEP 2003), in the fish samples (*Channa punctatus*, known locally as 'Bloch') of the Gomti river system, India.

Materials and Methods

The fish samples were collected during 2004–2005, in the pre- and post-monsoon seasons, from three selected sites (Fig. 1). The sampling sites are located at upstream (Site-1), middle (Site-2), and downstream of the Lucknow City (Site-3). As much as it was possible, similar sized fish samples were collected. Each fish was measured (cm), tagged, and placed on ice and later frozen until they were analyzed. In the laboratory, each fish was weighed (g) and the skin was removed with steel knife and steel tweezers from muscle in the middle of the fish. Then a sub-sample (~20 g) was cut from the muscle. These sub-samples were analyzed for the 16 USEPA PAHs viz. naphthalene (Nap), acenaphthylene (Acy), acenaphthene (Ace), fluorene (Fl), phenanthrene (Phen), anthracene (Anthra), fluoranthene (Fluo), pyrene, benzo[a]anthracene (B(a)A), chrysene (Chr), benzo[b]fluoran-thene (B(b)F), benzo[k]fluoranthene (B(k)F), benzo[a]pyrene (B(a)P), indeno[1,2,3-cd]pyrene (IP), dibenzo[ah]anthracene (D(ah)A), and benzo[ghi]-perylene (B(ghi)P) were used in this study The analysis of PAHs was conducted according to standard procedures (USEPA 1996a). The sub-samples were soxhlet extracted using an acetone and dichloromethane mixture (1:1) for at least 16 h, and triplicate extracts for each of the sample were prepared. All extracts were dried using anhydrous sodium sulfate and concentrated by a rotary evaporator. The concentrated extracts were diluted by adding 10 mL hexane and then concentrated to 2 mL. Freshly prepared copper granules were added to the

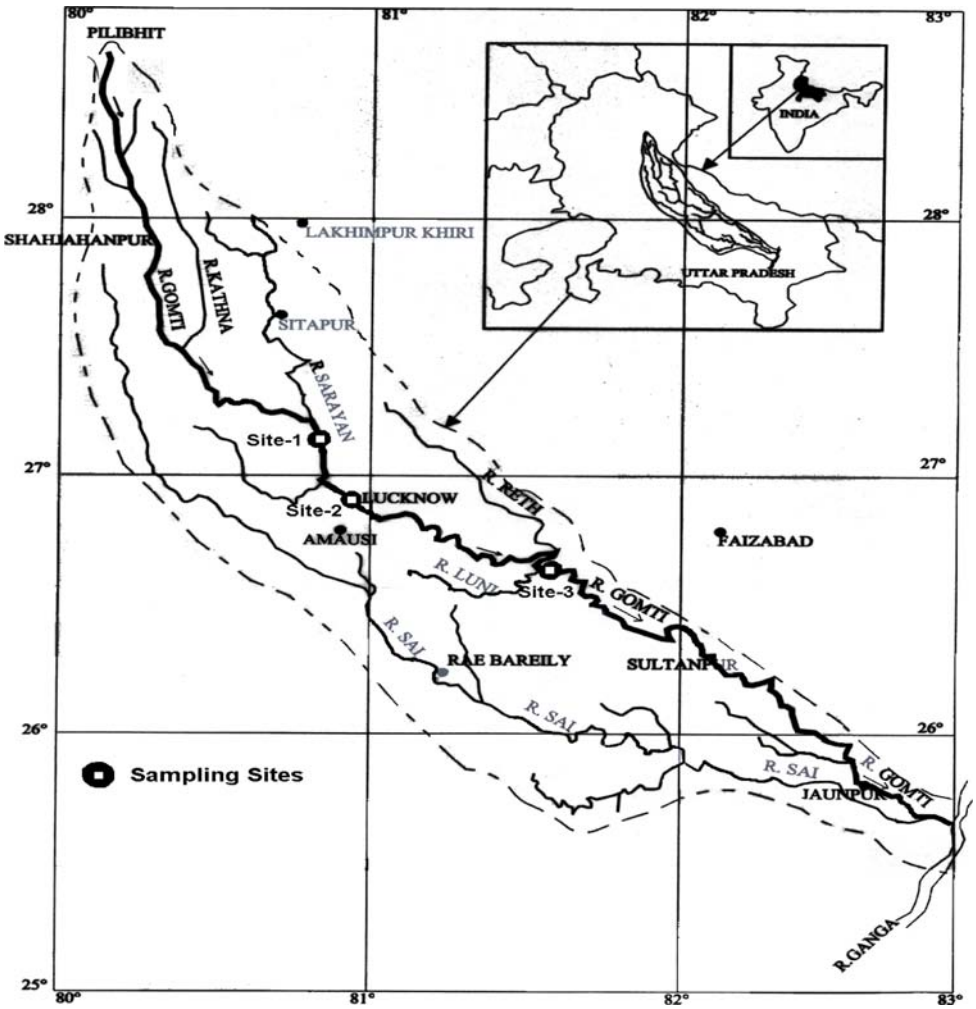

Fig. 1 Map of the Gomti river showing sampling locations

extracts for sulfur removal (USEPA 1996b). The removal of co-extracted contaminants from PAHs was achieved by florisil adsorption in which concentrated extracts were added to hexane pre-washed activated micro-florisil columns (USEPA 1996c). The columns were rinsed with at least 15 mL dichloromethane and hexane mixture (2:8) and the collected extracts were concentrated to 1 mL. The extracts were finally made up to 1 mL with acetonitrile in a volumetric flask. Then the extracts were analyzed for PAHs using water–acetonitrile solvent system on HPLC (Metrohm, Switzerland) equipped with a Metrohm IC-709 programmable pump, Metrohm IC-733 separation center, and Metrohm IC-753 UV–vis detector. The separation was achieved on a C-18 column (4.6 × 75 mm^2). The flow rate was adjusted to 1 mL min^{-1}. Injected volume for, both sample and standard (mixture) were 100 µL. The selected PAHs were identified by retention time comparison with reference to the corresponding standards. The detection limit for all the PAHs was 1 ng L^{-1}. The quality assurance measures included rigorous contamination control (strict washing/cleaning procedures), monitoring of blank levels of solvents, equipment and other materials, analysis of procedural blanks, recovery of spiked standards, monitoring of detector response, and linearity. The PAH standards (99.9% purity) were supplied by Sigma-Aldrich, USA. All analysis was carried out in duplicate and the recoveries of individual compounds were determined through spiked sample method, which were found between 80 and 95%. Recovery correction factors were applied to the final results. Results are presented as range (minimum–maximum) and mean values along with the standard deviation (SD). Means were the concentrations of the non-detected analytes treated as zero. Values below detection limits were assigned as a non-detectable (ND).

Results and Discussion

The concentration levels of PAHs detected in the fish muscles are summarized in Table 1. In the muscles of the fish, ∑PAHs (sum of 16 PAHs) ranged between 12.85 and 34.89 ng g^{-1} wet wt with a mean value of 23.98 ± 6.70 ng g^{-1} wet wt. To compare our results with those reported in the literature in areas of variable PAH contamination, the reported PAH concentrations were converted from ng g^{-1} dry wt to ng g^{-1} wet wt. For this purpose, a factor corresponding to an average reduction of weight of 75% during drying was used in the conversion (DouAbul et al. 1997). The ∑PAHs concentration observed in the fish muscles in the present study are lower than that reported in the muscles of the fish, from fishpond of Pearl river delta (mean value: 49.59 ng g^{-1} wet wt, Kong et al. 2005) and Mai Po Marshes Nature Reserve of

Table 1 Detection frequency (DF), range, mean, and standard deviation (SD) of PAHs in the fish samples (ng g^{-1} wet wt) collected from Gomti river

PAH	DF (%)	Range	Mean	SD
Nap	100.00	5.63–19.48	13.50	4.12
Acy	66.67	ND–4.47	1.92	1.66
Fl + Ace	50.00	ND–3.95	0.92	1.31
Phen	50.00	ND–6.24	1.61	2.10
Anthra	25.00	ND–3.24	1.15	1.44
Fluo	50.00	ND–5.88	1.36	1.81
Pyrene	50.00	ND–4.68	1.34	1.63
B(a)A + Chr	8.33	ND–1.05[a]	–	–
B(k)F	0.00	ND	–	–
B(b)F	58.33	ND–6.61	2.01	2.79
B(a)P	0.00	ND	–	–
D(ah)A	8.33	ND–3.36[a]	–	–
IP + B(ghi)P	0.00	ND	–	–
ΣPAHs	–	12.85–34.89	23.98	6.70

ND = not detected

[a] Single value detected

Hong Kong (mean value: 497.00 ng g^{-1} wet wt, Liang et al. 2007), however, these are relatively higher than that reported in the fish muscles from Red Sea Coast (mean value: 12.29 ng g^{-1} wet wt, DouAbul et al. 1997). From Table 1, it is apparent that naphthalene was the most abundant PAH, as also noted in other studies (Deb et al. 2000; DouAbul et al. 1997), followed by acenaphthylene and benzo[b]fluoranthene. Benzo[k]fluoranthene, benzo[a]pyrene, and indeno[1,2,3-cd]pyrene + benzo[ghi]perylene could not be detected in any of the sample, while benzo[a]anthracene + chrysene and dibenzo[ah]anthracene were present in one sample only. Concentration level of naphthalene (mean value: 13.50 ng g^{-1} wet wt) in the muscles of fish from Gomti river was relatively lower than that (mean value: 33.83 ng g^{-1} wet wt) reported by Liang et al. (2007), but higher than that (mean value: 5.00 ng g^{-1} wet wt) reported by DouAbul et al. (1997). Concentration levels of anthracene, fluoranthene, and pyrene were comparable with those reported by Liang et al. (2007) in the fish muscles. The absence or rather low detection of certain PAHs (benzo[a]anthracene + chrysene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[ah]anthracene, benzo[ghi]perylene, and indeno[1,2,3-cd]pyrene) in the fish muscles may be attributed to their rapid depuration or biotransformation (Deb et al. 2000). Accumulation and depuration of PAHs in fish can be influenced by various factors including route and duration of exposure, lipid content of tissues, environmental factors (e.g., salinity, temperature, etc.), differences in species, age, and sex and exposure to other xenobiotics (Varanasi et al. 1989). The fish efficiently metabolize PAH and during the metabolism

of petroleum derived aromatic hydrocarbons, the ability of an organism to process PAHs may be altered by the presence of polar components (Varanasi and Stein 1991), including the concentrations of produced PAH metabolites (Schmeltz et al. 1978).

Figure 2a and b presents spatial and temporal variations in the concentration levels of individual PAHs in the fish muscles. There could be observed neither spatial nor temporal trend in the concentration levels of PAHs. In general, naphthalene was observed with higher concentrations at all the sites in both the seasons, as compared to other PAHs. This may be due to its more water solubility and lower-particulate affinity than the larger molecular weight aromatic hydrocarbons (DouAbul et al. 1997). From Fig. 2a, it may also be noted that two- and three-ringed hydrocarbons, in general, had relatively higher concentrations at Site-2. This may be explained as, between Site-1 and Site-2, there are some 25 drains carrying about 400 million litres per day (MLD) of untreated sewage and industrial wastewater from different parts of the city, discharging directly into the river. Figure 3a presents the spatial and temporal variation of ∑PAHs concentrations as a sum of low-molecular weight PAHs (two- to three-ring PAHs) and high-molecular weight PAHs (>three-ring PAHs). There was no clear pattern in the spatial or temporal distribution of ∑PAHs. From Fig. 3a, it is clear that in the fish muscles low-molecular weight PAHs were prevalent than high-molecular weight PAHs. Deb et al. (2000) have also reported higher concentrations of low-molecular weight PAHs in the fish. Such patterns of accumulation are characteristic of PAHs generated by petrogenic processes (DouAbul et al. 1997). In general, the petroleum-derived residues contain relatively higher concentrations of two- and three-ringed PAH compounds (Tolosa et al. 1996). At low to moderate temperature, as in the wood stove (Lake et al. 1979), or as from the combustion of coal (Laflamme and Hites 1978), low-molecular weight parent PAH compounds are abundant. At high temperature, the high-molecular weight parent PAH compounds are dominant (Lee et al. 1977). Therefore, on account of the anthropogenic source, the low-molecular weight parent PAHs have both petrogenic and combustion (low-temperature pyrolysis) sources, whereas the high-molecular parent PAHs are predominantly pyrogenic. In terms of the number of fused rings present in the chemical structure of the polycyclic aromatic

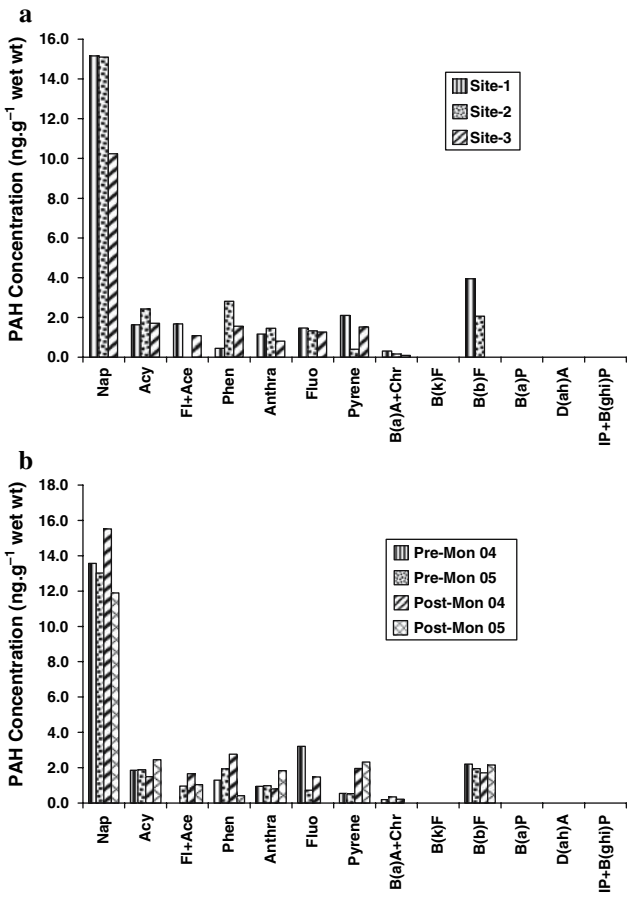

Fig. 2 (**a**) Spatial and (**b**) temporal variations in the concentration levels of PAHs in the fish muscles of the Gomti river

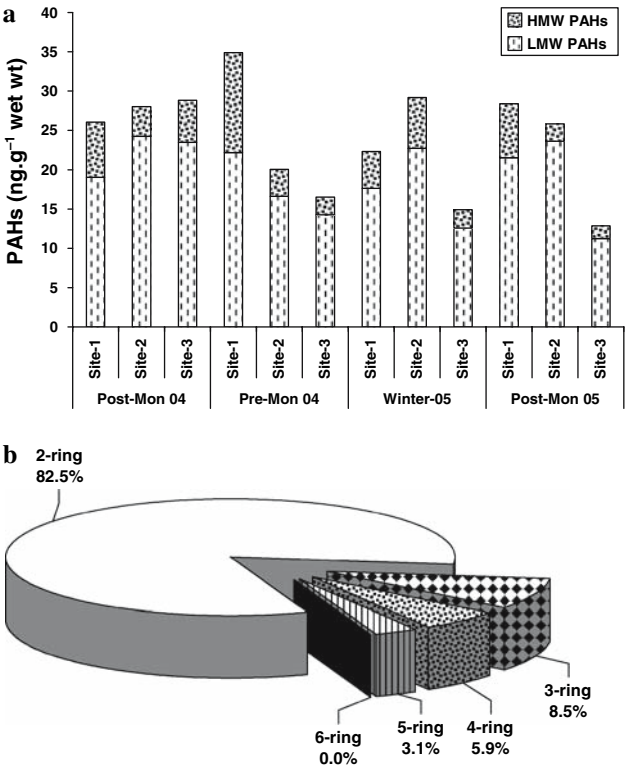

Fig. 3 (**a**) Spatial and temporal variations in the concentration levels of ΣPAHs and (**b**) ring-wise contribution of PAHs to the ΣPAHs burden in the fish muscles of the Gomti river

hydrocarbons, it was observed that on an average two- and three-ringed hydrocarbons contributed most (about 91%) to the ∑PAHs burden (Fig. 3b) in the fish muscles. These results are in agreement with Liang et al. (2007), who have also reported dominating accountancy (about 90%) of two- and three-ringed PAHs in the fish muscles. These results indicate dominance of PAHs from petrogenic/combustion origin. An earlier study has reported origin of hydrocarbons in the river catchments mainly from the combustion processes (Malik et al. 2004). Biomass based fuel combustion and open burning of biomass are the common activities in the region and these have been identified as among the major contributors to the PAHs release in the region (UNEP 2003).

The results of the present study indicate that fish of the Gomti river are contaminated mainly with low-molecular weight compounds, while the high-molecular weight PAHs (benzo[k]fluoranthene, benzo[a]pyrene, and indeno[1,2,3-cd]pyrene + benzo[ghi]perylene) could not be detected in any of the sample. Since very little work has been carried out previously, the present results could serve as baseline data. More detailed investigations, in terms of sampling network and sampling frequencies with a view to assess bioaccumulation potential of hazardous contaminants in different tissues of fish species are required to ensure the health of the river, its biota and human health in the river basin.

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References

Bouloubassi J, Saliot A (1991) Composition and sources of dissolved and particulate PAH in surface waters from the Rhone delta (NW Mediterranean). Mar Pollut Bull 22:588–594

Bouloubassi J, Saliot A (1993) Investigation of anthropogenic and natural organic inputs in estuarine sediments using hydrocarbon markers (NAH, LAB, PAH). Oceanol Acta 16:145–161

Deb SC, Araki T, Fukushima T (2000) Polycyclic aromatic hydrocarbons in fish organs. Mar Pollut Bull 40:882–885

DouAbul AAZ, Heba HMA, Fareed KH (1997) Polynuclear aromatic hydrocarbons (PAHs) in fish from the Red Sea coast of Yemen. Hydrobiologia 352:251–262

Kong KY, Cheung KC, Wong CKC, Wong MH (2005) The residual dynamic of polycyclic aromatic hydrocarbons and organochlorine pesticides in fishponds of the Pearl river delta, South China. Water Res 39:1831–1843

Laflamme RE, Hites RA (1978) The global distribution of PAH in recent sediments. Geochim Cosmochim Acta 42:289–303

Lake JL, Norwood C, Dimock C, Bowen R (1979) Origins of polycyclic aromatic hydrocarbons in estuarine sediments. Geochim Cosmochim Acta 43:1847–1854

Latimer JS, Zheng J (2003) Sources, transport, and fate of PAHs in the marine environment. In: Douben PET (ed) PAHs: an ecotoxicological perspective. Wiley, UK

Lee ML, Prado GP, Howard JB, Hates RA (1977) Source identification of urban airborne polycyclic aromatic hydrocarbons by gas chromatography-mass spectrometry and high resolution mass spectrometry. Biomed Mass Spectrom 4:182–186

Liang Y, Tse MF, Young L, Wong MH (2007) Distribution patterns of polycyclic aromatic hydrocarbons (PAHs) in the sediments and fish at Mai Po marshes nature reserve, Hong Kong. Water Res 41:1303–1311

Malik A, Singh KP, Mohan D, Patel DK (2004) Distribution of polycyclic aromatic hydrocarbons in Gomti river system, India. Bull Environ Contam Toxicol 72:1211–1218

Neff JM (1985) Polycyclic aromatic hydrocarbons. In: Rand GM, Petrocilli SR (eds) Fundamentals of aquatic toxicology. Hemisphere, New York

Payne JF, Mathieu A, Collier TK (2003) Ecotoxicological studies focusing on marine and freshwater fish. In: Douben PET (ed) PAHs: an ecotoxicological perspective. Wiley, UK

Schmeltz I, Tosk J, Hilfrich J, Hirota N, Hoffman D, Wynder EL (1978) Bioassay of naphthalene and alkylnephthalene for carcinogenic activity: relation to tobacco carcinogenesis. In: Jones PW, Freundenthal RI (eds) Carcinogenesis, polynuclear aromatic hydrocarbons, vol 3. Raven Press, New York

Tolosa J, Bayona JM, Albaigés J (1996) Aliphatic and polycyclic aromatic hydrocarbons and sulfur/oxygen derivatives in northwestern Mediterranean sediments: spatial and temporal variability, fluxes, and budgets. Environ Sci Technol 30:2495–2503

UNEP (2003) Global report on regionally based assessment of persistent toxic substances. UNEP Chemicals, Geneva

USEPA (1996a) Method 3540C: soxhlet extraction. US Environmental Protection Agency, Washington

USEPA (1996b) Method 3660B: sulfur cleanup. US Environmental Protection Agency, Washington

USEPA (1996c) Method 3620B: florisil cleanup. US Environmental Protection Agency, Washington

Varanasi U, Stein JE (1991) Disposition of xenobiotic chemicals and metabolites in marine organisms. Environ Health Perspect 90:93–100

Varanasi U, Stein JE, Nishimoto M (1989) Biotransformation and disposition of polycyclic aromatic hydrocarbons (PAH) in fish. In: Varanasi U (ed) Metabolism of polycyclic aromatic hydrocarbons in the aquatic environment. CRC Press, Boca Raton