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Abstract

Polycyclic aromatic hydrocarbons (PAHs) are a major concern in environmental studies as many of them have been labeled as probable carcinogens by the International Agency for Research on Cancer (IARC 1983). Due to their lipophilic properties and resistance to degradation, PAHs can accumulate in organic tissue. As a consequence, alarming concentrations of these compounds have been found in many aquatic species.

The European catfish (*Silurus glanis*) is a top food-chain predator that is considered to be a reliable bio-indicator of environmental pollution. From 2009 to 2011, 54 specimens of *S. glanis* were captured from four different sites covering the area of the Po River basin (Northern Italy). Fish muscles were analyzed in the laboratory to determine the levels of nine PAHs, namely: naphthalene, acenaphthene, fluorene, phenanthrene, anthracene, pyrene, benz[a]anthracene, chrysene, and benz[a]pyrene (BaP), which were detected by High-Performance Liquid Chromatography (HPLC). The total average concentration of PAHs was 26.90 ± 49.50 ng g^{-1} (min 0.60, max 275.75 ng g^{-1}). Analysis showed that 9.20% of the fish muscles exceeded the maximum levels of 2 ng g^{-1} set for BaP by European regulations (1881/2006 EC). Values measured for benz[a]pyrene ranged from 0.05 to 8.20 ng g^{-1} (mean 1.07 ± 1.58 ng g^{-1}). Chrysene and benz[a]anthracene, both considered potential human carcinogens (PAH2), were found at levels of 4.40 ng g^{-1} and 0.05 ng g^{-1} (mean values), respectively. The highest mean concentration was recorded for anthracene (12.92 ng g^{-1}), which has been recently included in the list of Substances of Very High Concern (SVHC) by the European Chemicals Agency (2009).

Keywords: PAHs, European catfish, Po river basin, HPLC

1. Introduction

Monitoring of PAH contaminants in food has received a growing interest in recent years. Dietary intake has been reported as the main cause of human exposure to PAHs (Scherer et al. 2000; Falcó et al. 2003). Indeed, for non–occupationally exposed subjects (and nonsmokers), 70% of PAH exposure arises from the diet. Moreover, several epidemiological studies have shown that a large proportion of human cancers are attributable, at least in part, to dietary factors (Doll et al. 1996). Consequently, one of the main reasons for concern about the exposure of humans to environmental contaminants is the evidence that a number of these contaminants are potentially carcinogenic.

Pollution by persistent chemicals significantly affect the organisms at higher trophic levels in the food chain. Marine fish can accumulate several-fold higher concentration of PAHs than the surrounding water (Johnson-Restrepo et al. 2008). Fish is a major source of protein and healthy lipids for people worldwide and long-chain omega-3 fatty acids in particular have been proven to have a variety of beneficial roles in human health and wellbeing (Ismail et al. 2005). Despite these proven benefits of a fish-based diet, the potential risk arising from exposure to toxic chemicals is an issue of grave concern (Domingo et al. 2007; Sion et al. 2008).

Polycyclic aromatic hydrocarbons (PAHs) mainly originate from incomplete combustion of fossil fuels and organic material as well as directly from petroleum contamination. Due to their hydrophobic nature, PAHs rapidly bind to suspended particles in an aquatic environment, and deposit in bottom sediments (Latimer and Zheng 2003). PAHs have been demonstrated to pose toxicity both in fish and birds (Payne et al. 2003; Albers 2003) by interfering with cellular membrane function and the associated enzyme systems (Neff 1985). Moreover, PAH metabolites can bind to proteins and DNA causing biochemical disruption and cell damage (Varanasi et al., 1987). In addition, carcinogenicity of PAHs can be increased by exposure to ultraviolet radiation, and 11 out of the 16 PAHs listed by the US Environmental Protection Agency as priority pollutants are considered to be photomutagenic (Yan and Wang 2004). Payne et al. (2003) suggested that elevated PAH levels, commonly found in many aquatic environments, are a significant risk factor for fish health. Moreover, Hart et al. (1998) reported an adverse histopathological and immunological response in tilapia exposed to PAHs.

Although benz[a]pyrene is considered a reliable marker for the occurrence and toxicity of total PAHs in food (Wenzl et al. 2006), this relationship is still not fully convincing. Therefore, the European Food Safety Agency (EFSA) has recently suggested that other PAHs, including benz[a]anthracene and chrysene, should also be taken into account when considering the occurrence

and associated carcinogenicity of contaminated foods (EFSA 2008a). Following a recommendation to further investigate PAH levels in certain foods (2005/108/EC), 18 Member States submitted almost 10,000 results for PAH levels in different food commodities. Evaluation of these data performed by the EFSA in June 2007, and updated in June 2008, demonstrated that benz[*a*]pyrene is present in about 50% of all samples. Furthermore, other carcinogenic and genotoxic PAHs were detected in about 30% of samples that were negative for benz[*a*]pyrene. Among the individual PAHs, chrysene was most commonly found in samples negative for benz[*a*]pyrene, and the highest level detected was 242 ng g^{-1}.

Moreover, it has been observed that the correlation between the sum of benz[*a*]pyrene and chrysene (PAH2) and the sum of the PAHs (PAH8) used by Culp et al. (1998) to assess carcinogenicity risk (benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*ghi*]perylene, chrysene, dibenz[*a,h*]anthracene and indeno[*1,2,3-cd*]pyrene: benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*ghi*]perylene, chrysene, dibenz[*a,h*]anthracene and indeno[*1,2,3-cd*]pyrene) is 0.92.

Considering other PAHs that can pose an environmental threat, anthracene has been recently included in the list of Substances of Very High Concern (SVHC) edited by the European Chemicals Agency (2009). Indeed, it is considered to be persistent, bio-accumulative and toxic for both freshwater and marine ecosystems. In particular, when released in freshwater environment, anthracene is rapidly adsorbed by the sediment and suspended particulate without being hydrolyzed. Moreover, Hall et al. (1989) reported that anthracene bio-concentrates in species which lack microsomal oxidase, an enzyme that allows rapid metabolization of poly-aromatic hydrocarbons.

Results about PAH contamination of fish species allow consumers to make decisions about food preferences in order to reduce risks from contaminant exposure and increase health benefits. However, there is limited information on PAH levels in fish muscle in the literature. In particular, to date no studies have been performed on *Silurus glanis*, an invasive species accidentally introduced about 30 years ago in the Po river and now widespread in Northern and Central Italy. This species was also introduced in several European Countries (i.e. Spain and France), where it is now successfully acclimatized. Being a top food-chain predator, *Silurus glanis* can be considered a reliable species for bio-monitoring studies, as it accurately reflects the state of the environmental organic and chemical pollution.

The aim of this study was to evaluate PAH contamination in European catfish muscles from Rivers within the area of the upper Po river basin (Northern Italy). In particular, compliance with the maximum levels established for BaP by the European Commission Regulation (1881/2006) in fish muscle was verified, and the distribution of other PAHs, known as being the most appropriate

105 indicators for the presence of carcinogenic and genotoxic PAHs in foodstuffs, with a particular
106 attention to PAH2 (EFSA 2008b), was discussed.
107 Results of the present study may provide reliable information about the environmental impact of
108 PAHs in North-West Italian Rivers, which is essential for the monitoring and management of the
109 study area.
110
111 **2. Materials and methods**
112 *2.1 Study species*
113 The European catfish (*Silurus glanis*), also known as Wels catfish, is one of the biggest European
114 freshwater fish. This species is native to Eastern Europe and Western Asia and is abundant in the
115 Danube and Volga basins. The European catfish inhabits the lower reaches of large rivers and
116 muddy lakes, and usually feeds on fish that are smaller than would be expected for its size and
117 mouth gape (Adàmek et al. 1999; Wysujack and Mehner 2005). *Silurus glanis* is a night predator,
118 foraging both on the river bottom and in the water column. Fry and juveniles are benthic, feeding on
119 a wide variety of invertebrates and fish, while adults prey on fish and other aquatic vertebrates.
120 Sexual maturity is reached at 2-3 years of age, and this species can survive for over 30 years in
121 natural conditions.
122 In recent years, the European catfish has received an increasing interest for consumption in
123 Northern Italy. Particularly, only the muscle of young specimens (weigh less than 15 kg or 33 lb) is
124 considered palatable. Larger specimens are highly fatty and not recommended for consumption.
125
126 *2.2 Field sampling*
127 Fifty-four specimens of *Silurus glanis* were collected in the following four sites from 2009 to 2011:
128 1. Po River (Lat. 45.138098, Long. 8.558135)
129 2. Tanaro River (Lat. 44.919446, Long. 8.6099719)
130 3. Bormida River (Lat. 44.906940, Long. 8.646197)
131 4. Parma River (Lat. 44.832150, Long. 10.314585)
132 Sampling sites belong to the hydrographical basin of the Po (the largest river in Italy) and were
133 selected according to accessibility and fish abundance.
134 Fish were captured using an electro fisher IG 600. Specimen sizes were 60 to 120 cm (age from < 1
135 to 12 years) with weights ranging from 1.5 to 10.5 Kg.
136 Captured fish were immediately euthanized and kept refrigerated while transported to the
137 laboratory. Fish were sexed and dissected to obtain muscle samples, which were immediately
138 frozen and stored at −20 °C.

Twenty-four samples were collected from the Po River (Alessandria district), ten from the Tanaro River (Alessandria district), nine from the Bormida River (Alessandria district), and eleven from the Parma River (Parma district).

2.3 Analytical Methods

5 g of freeze-dried sample was extracted for 15 minutes with *n*-hexane/acetone 1:1 (33 mL). The extraction procedure was performed using the Accelerated Solvent Extraction System (Dionex ASE® 200 Solvent Extractor – Dionex Corporation). This system increases the efficiency of the extraction process using conventional liquid solvents at elevated temperatures (100 °C) and pressures (102 atm). The solvent was gently removed by evaporation in a rotary evaporator, followed by addition of 50 mL of Ethanol 95% and 6 ml of Potassium Hydroxide 50% to the dried residue. Saponification was performed for 2 hours on a hot plate, connecting the flask with a water-cooled reflux condenser (Teledyne Isco, Lincoln NE, USA). When samples were returned to the 20 °C ambient temperature, a liquid-liquid extraction with *n*-hexane and distilled water was performed in a separatory funnel. PAHs were collected in the non-polar phase. After this procedure, the solvent was evaporated and the residue was loaded on top of a silica cartridge (Supelco Analytical S.p.A., Milano). Elutions were carried out with 5 mL of a mixture of *n*-hexane and diethyl ether 9:1 (v/v). Finally, dried extracts were suspended in isooctane for instrumental analysis, which was performed using an HPLC (1100 series, Agilent Technologies, Santa Clara, CAL, USA) equipped with auto sampler, quaternary pump and fluorescence detector. For the separation an Envirosep reversed phase for PAHs column 125 x 4.60 mm, 5 µm (Phenomenex Inc., CA, USA) was used. Acetonitrile and distilled water were used as eluents, at a flow rate of 1 mL min^{-1}. The gradient elution program was: 50% acetonitrile initially until 5 min, then increasing linearly to 100% acetonitrile in 20 min, holding in 100% for 15 min and finally back to initial conditions in 10 min. Column temperatures were maintained at 25 ± 0.1 °C. The fluorescence detector was programmed to optimize sensitivity for all peaks while minimizing interference. Fluorescence intensity was measured at the following excitation/emission wavelengths pairs: 275/325 nm for Na, Ac, F, Phe, 250/375 nm for Ant, Pyr, 285/475 for BaA, Chr and 270/380 for BaP.

2.4 Statistical analysis

Data were tested for normality by using the Kolmogorov-Smirnov test. Since the assumptions for parametric analyses were not met, a Kruskal–Wallis analysis of variance by ranks followed by Mann–Whitney U tests for pairwise comparisons were performed to assess differences in the concentration of BaP in the muscles of fish from different rivers. Differences were considered to be significant with a p value < 0.05.

174 Moreover, correlations between PAH2 and BaP as well as BaP and fish size/gender were examined
175 for each site using linear regression models.
176
177 **3. Results and Discussion**
178 Most of the PAHs detectable in the aquatic environment are localized to rivers, estuaries and coastal
179 waters (Hellou et al. 1994). Ascertaining contamination levels in rivers is instrumental to evaluate
180 the risks posed by human activity to the freshwater environment and consequently to human health.
181 In the present study, the levels of nine PAHs (naphthalene, acenaphthene, fluorene, phenanthrene,
182 anthracene, pyrene, benz[a]anthracene, chrysene, benz[a]pyrene) were measured in 54 samples of
183 *Silurus glanis* muscle from Northern Italy.
184 The values measured for BaP in fish muscle ranged from 0.05 to 8.2 ng g^{-1} (mean 1.07 ± 1.58 ng g^{-1}).
185 Five (9.2%) of the 54 analyzed samples exceeded the maximum levels (ML) of 2 ng g^{-1} set for
186 BaP by European regulations in fish muscle (1881/2006 EC). In particular, ML were exceeded in
187 three samples from the Tanaro River with values of 3.3, 4.1, 2.8 ng g^{-1} and in two samples from the
188 Po River (7.8 and 8.2 ng g^{-1}) (Fig. 1). Mean values registered for BaP (Table 1) in the sampling
189 sites were 1.07 (Po River), 2.15 (Tanaro River), 0.52 (Bormida River) and 0.57 ng g^{-1} (Parma
190 River), respectively. A significantly different concentration of BaP was found in fish muscles from
191 the four sampling rivers (Kruskal–Wallis, $p < 0.005$, df 3). However, multiple comparison Mann-
192 Whitney U tests showed that differences only exist between the Po and Tanaro ($U = 35$, $p < 0.001$),
193 the Po and Bormida ($U = 67$, $p < 0.05$), and the Tanaro and Parma ($U = 16$, $p < 0.005$) Rivers.
194 Results regarding the relationship between gender and total PAH concentrations (data not shown)
195 suggested that accumulation levels are similar for males and females in all sites. However, literature
196 concerning this subject is very limited. Moreover, we did not observe any relationship between
197 PAH concentration in fish muscle and size. Although some authors reported this correlation in fatty
198 fish (e.g. chub mackerel and sardine), suggesting that PAH bioaccumulation mostly occurs in lipid-
199 rich tissues (Meador et al. 1995), this topic still remains controversial, as fish are known to be
200 capable of rapidly metabolizing PAHs (Perugini et al. 2007; Akpambang et al. 2009).
201 The EFSA CONTAM Panel recently pointed out that benz[*a*]pyrene alone is not a suitable indicator
202 for the total PAHs in food. Indeed, benz[*a*]pyrene was detected in only 50% of the 10,000 samples
203 analyzed in the EFSA report (2008). Moreover, in approximately 30% of all the samples
204 considered, other carcinogenic and genotoxic PAHs were detected (most frequently chrysene),
205 despite the absence of benz[*a*]pyrene. Consequently, the CONTAM Panel concluded that the
206 toxicity and carcinogenicity risk characterization should be based upon the PAHs for which oral
207 carcinogenicity data are available. Therefore, PAH2 (the sum of chrysene and benz[a]anthracene),
208 PAH4 (the sum of benz[*a*]pyrene, chrysene, benz[*a*]anthracene and benz[*b*]fluoranthene) and PAH8

(the sum of benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*ghi*]perylene,chrysene, dibenz[*a,h*]anthracene and indeno[*1,2,3-cd*]pyrene: benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*ghi*]perylene, chrysene, dibenz[*a,h*]anthracene and indeno[*1,2,3-cd*]pyrene) can be considered suitable indicators for total PAH contamination in food.

Accordingly, in the present study, a linear regression model showed a strong relationship (R^2 = 0.98; $p < 0.001$) between PAH2 and BaP levels in the muscles of fish from the Tanaro River (Fig. 2).

Considering PAH2 values in all investigated sites, we can conclude that the major contribution was due to chrysene. In fact, the highest value detected for BaA was 0.3 ng g^{-1} in a sample from the Po River.

Chrysene contamination was negligible (mean value 0.05 ng g^{-1}) in the Po River, scarce in Bormida and Parma Rivers (mean values respectively 0.62 and 0.68 ng g^{-1}) while the Tanaro River revealed to be significantly more contaminated with the highest value of 119.1 ng g^{-1} and a mean value of 22.63 ng g^{-1} (Fig. 3).

The highest mean concentration recorded for anthracene was 28.0 ng g^{-1}, in the Tanaro River (min 0.05, max 119.1 ng g^{-1}) (Fig. 3). Considering the mean values of PAHs detected (Table 1), the Tanaro River was revealed to be the most polluted of the four rivers examined, with a decreasing concentration of the following PAHs: anthracene > chrysene > acenaphthene > phenanthrene > naphthalene > benz[a]pyrene > pyrene > fluorene > benz[a]anthracene. In Figure 4, the mean concentrations of ΣPAHs in the four locations are shown. The Tanaro River had the highest mean value of ΣPAH (80.92 ng g^{-1}), with a maximum value of 275.75 ng g^{-1}. The Bormida, Parma and Po rivers showed mean ΣPAH values of 24.56, 16.02 and 10.26 ng g^{-1}, respectively.

All PAHs are soluble in water and quickly become absorbed in organic and inorganic particulate matter, depositing in bottom sediments. Most PAHs remain relatively near to the point source and their concentrations decrease approximately logarithmically with the distance from the source. The basin of the Po river crosses the major industrial and populated area of Northern Italy. Therefore, it has a legacy of pollution which is reflected in the water quality of local rivers including the Tanaro, Parma and Bormida. In particular, the Tanaro was heavily affected by the presence of PAH contaminants. The Tanaro River is the most significant right-side tributary to the Po. The river flows close to an highway from the town of Alba to Alessandria. It is well-known that a large amount of PAHs are from incomplete combustion of fuels, especially in heavy-duty and diesel engines (Barakat, 2002). We hypothesize that vehicles could be a source of persistent, toxic pollutants that flows into the Tanaro and that seriously affect the water quality and river ecology.

Finally, the Council treatment plants cannot completely remove the entire load of pollutants and, as

a consequence, this river receives some quantities of incompletely treated wastewater and sewage from the urban areas.

4. Conclusions and recommendations

The European catfish is one of the world's largest (and the largest European) fish species (Stone 2007). Moreover, it is considered to be a suitable bio-indicator of environmental pollution. In this study, benz[a]pyrene (the most hazardous compound for human health) was detected in all the specimens collected, and the levels measured exceeded the limits set by the European Union in five out of 54 fish. As a consequence, we underline the importance of an adequate and more accurate treatment of industrial effluents prior to their drainage in the main water courses. Moreover, a continuous monitoring system of PAH residues in sentinel fish is necessary to prevent consumption of contaminated food. Finally, fishing areas must be maintained at a suitable distance from sources of industrial effluents.

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6. References

Adàmek, Z., Fasaic, K., Siddiqui, M.A. (1999). Prey selectivity in wels (*Silurus glanis*) and African catfish (*Clarias gariepinus*). *Ribarstvo*, 57, 47–60.

Akpambang, V.O.E., Purcaro, G., Lajide, L., Amoo, I.A., Conte, L.S., Moret, S. (2009). Determination of polycyclic aromatic hydrocarbons (PAHs) in commonly consumed Nigerian smoked/grilled fish and meat. *Food Additives & Contaminants*, 26, 1096–1103.

Albers, P.H. (2003). Petroleum and Individual Polycyclic Aromatic Hydrocarbons. In D.J. Hoffman, B.A. Rattner, G.A. Burton Jr. & J. Cairns Jr. (Eds), *Handbook of Ecotoxicology* (pp. 341-371). Boca Raton: Lewis Publishers.

Barakat, A.O. (2002). PAHs and petroleum biomarkers in the atmospheric environment of Alexandria City, Egypt. *Water Air Soil Pollution*, 139, 289-310.

COMMISSION REGULATION (EC). (2006). No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. *Official Journal of the European Union*, L 364/6-25.

Culp, S.J, Gaylor D., Sheldon, W.G., Goldstein, L.S., Beland, F.A. (1998). A comparison of the tumors induced by coal tar and benzo[a]pyrene in a 2-year bioassay. *Carcinogenesis,* 19,117-124.

279 Doll, R. (1996). Nature and nurture: possibilities for cancer control. *Carcinogenesis*, 17, 177-184.
280 Domingo, J.L., Bocio, A., Falcó, G., Llobet, J.M. (2007). Benefits and risks of fish consumption.
281 Part I. A quantitative analysis of the intake of omega-3 fatty acids and chemical contaminants.
282 *Toxicology*, 230(2-3), 219–226.
283 Falcó, G., Domingo, J.L., Llobet, J.M., Teixidó, A., Casas, C., Müller, L. (2003). Polycyclic
284 aromatic hydrocarbons in foods: human exposure through the diet in Catalonia, Spain. *Journal of*
285 *Food Protection*, 66(12), 2325–2331.
286 EFSA. (2008a). Scientific Opinion of the Panel on Contaminants in the Food Chain on a Request
287 from the European Commission on Polycyclic Aromatic Hydrocarbons in Food. Adopted 9 June
288 2008. http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902034842.htm.
289 Accessed 10 December 2012.
290 EFSA. (2008b). Monitoring polycyclic aromatic hydrocarbons presence across the EU.
291 http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178642214248.htm. Accessed 10
292 December 2012.
293 European Chemicals Agency. (2009). SVHC SUPPORT DOCUMENT, pp. 1-22.
294 http://echa.europa.eu/documents/10162/13608/mb_12_2010_general_report_2009_final_en.pdf.
295 Accessed 10 December 2012.
296 Hall, M., Forrester, L.M., Parker, D.K., Grover, P.L., Wolf, C.R. (1989). Relative contribution of
297 various forms of cytochrome P450 to the metabolism of benzo[a]pyrene by human liver
298 microsomes. *Carcinogenesis,* 10, 1815–1821.
299 Hart, L.J., Smith, S.A.S., Smith S.A., Robertson, J., Besteman, E.G., Holladay, S.D. (1998).
300 Subacute immunotoxic effects of the polycyclic aromatic hydrocarbon 7,12-
301 dimethylbenzanthracene (DMBA) on spleen and pronephros leukocytic cell counts and phagocytic
302 cell activity in tilapia (*Oreochromis niloticus*). *Aquatic Toxicology*, 41(1-2), 17-29.
303 Hellou, J., Upshall, C., Payne, J.F., Hodson, P.V. (1994). Polycyclic aromatic compounds in cod
304 (*Gadus morhua*) from the Northwest Atlantic and St. Lawrence Estuary. *Science of the Total*
305 *Environment*, 145, 71-79.
306 IARC. (1983). *Polynuclear aromatic compounds*. Part I: Chemical, Environmental and
307 Experimental Data. Lyon: International Agency for Research on Cancer.
308 Ismail, H.M. (2005). The role of omega-3 fatty acids in cardiac protection: an overview. *Frontiers*
309 *in Bioscience*, 10, 1079–1088.
310 Johnson-Restrepo, B., Olivero-Verbel, J., Lu S., Guette-Fernández, J., Baldiris-Avila, R., O'Byrne-
311 Hoyos , I., et al. (2008). Polycyclic aromatic hydrocarbons and their hydroxylated metabolites in
312 fish bile and sediments from coastal waters of Colombia. *Environmental Pollution*, 151(3), 452–
313 459.

Latimer, J.S., Zheng, J. (2003). The sources, transport, and fate of PAHs in the marine environment. In: P.E.T., Douben (Ed.), *PAHs: An Ecotoxicological Perspective* (pp. 9–33). West Sussex, England: John Wiley & Sons.

Yan, J., Wang, L. (2004). Photomutagenicity of 16 polycyclic aromatic hydrocarbons from the US EPA priority pollutant list. *Mutation Research*, 557(1), 99–108.

Meador, J.P., Stein, J.E., Reichert, W.L., Varanasi, U. (1995). Bioaccumulation of polycyclic aromatic hydrocarbons by marine organisms. *Reviews of Environmental Contamination & Toxicology*, 143, 79-165.

Neff, J.M. (1985). Polycyclic aromatic hydrocarbons. In G.M., Rand & S.R., Petrocelli (Eds.), *Fundamentals of aquatic toxicology* (pp. 416-454). New York: Hemisphere Publ. Corp.

Payne, J.F., Mathieu, A., Collier, T.K. (2003). Ecotoxicological studies focusing on marine and freshwater fish. In: P.E.T., Douben (Ed.), *PAHs: An Ecotoxicological Perspective* (pp. 191–224). Hoboken: Wiley.

Perugini, M., Visciano, P., Giammarino, A., Manera, M., Di Nardo, W., Amorena, M. (2007). Polycyclic aromatic hydrocarbons in marine organisms from the Adriatic Sea, Italy. *Chemosphere*, 66, 1904–1910.

Scherer, G., Frank, S., Riedel, K., Meger-Kossien, I., Renner, T. (2000). Biomonitoring of exposure to polycyclic aromatic hydrocarbons of non-occupationally exposed persons. *Cancer Epidemiology Biomarkers and Prevention*, 9(4), 373–380.

Sioen, I., Van Camp, J., Verdonck, J., Verbeke, W., Vanhonacker, F., Willems, J., De Henauw, S. (2008). Probabilistic intake assessment of multiple compounds as a tool to quantify the nutritional-toxicological conflict related to seafood consumption. *Chemosphere*, 71(6), 1056–1066.

Stone, R. (2007). Aquatic ecology. The last of the leviathans. *Science*, 316(5832), 1684-1688.

Varanasi, U., Stein, J., Nishimoto, M., Reichert, W., Collier, T. (1987). Chemical carcinogenesis in feral fish: uptake, activation, and detoxification of organic xenobiotics. *Environ Health Perspectives*, 71, 155–170.

Wysujack, K., & Mehner, T. (2005). Can feeding of European catfish prevent cyprinids from reaching a size refuge? *Ecology of Freshwater Fish*, 14, 87–95.

Wenzl, T., Simon, R., Kleiner, J., Anklam, E. (2006). Analytical methods for polycyclic aromatic hydrocarbons (PAHs) in food and the environment needed for new food legislation in the European Union. *Trends in Analytical Chemistry*, 25(7), 716-725.

Figure captions

Figure 1

Benz[a]pyrene distribution in *Silurus glanis* samples

Figure 2

Regression model: correlation between benz[*a*]pyrene and the sum of benzo[*a*]pyrene and chrysene (PAH2)

Figure 3

Chrysene and Anthracene levels in the four sampling sites

Figure 4

ΣPAH in Tanaro, Po, Bormida and Parma Rivers