

Evaluation of Heavy Metals and Polycyclic Aromatic Hydrocarbons (PAHs) in *Mullus barbatus* from Sicily Channel and Risk-Based Consumption Limits

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Abstract Our study is a preliminary step to evaluate water contamination of the Mediterranean Sea surrounding Lampedusa island and health risks for fish consumers. We analyzed muscle tissue of *Mullus barbatus* specimens to detect metals and polycyclic aromatic hydrocarbons (PAHs). Results show the following average concentrations of heavy metals: Pb 0.035, Cd 0.001, Hg 0.027, Cr 0.524, As 27.01, Cu 0.433, Co 0.013, Ni 0.042, Se 0.487, V 0.072, U 0.055 and Zn 3.360 ppm. PAHs ranged from 0.250 to 13.16 ppb. A possible risk to human health was determined based on consumption limits data and the content of total PAHs in fillets of this species.

Keywords Heavy metals · PAHs · Fish · Consumption limits · Health · Mediterranean Sea

During the last decades, many studies have been carried out to assess the status of chemical pollutants in marine ecosystems using fish as bioindicators. Manufacturing, ship traffic and other industrial activities cause the release of PAHs and trace metals in the marine environment. These chemicals can enter the food chain and be absorbed by marine organisms including fish. However, predicting rates

of bioaccumulation of chemicals in fish tissue is difficult as it depends on numerous factors such as fish trophic level and size (Burreau et al. 2006), species ecology (Szclinder-Richert et al. 2009), age of fish (Pandelova et al. 2008), lipid content (Kelly et al. 2007), ability to metabolize the contaminants (Ruangsomboon and Wongrat 2006), sex and phase of life cycle (Johnston et al. 2002). Thus biomonitoring is essential for the assessment of marine ecosystem health and for evaluating possible risks for human health due to fish consumption.

PAHs and metals released in the marine environment could express some toxicity, so several of them are classified as probable (2B) or possible (C) carcinogens by the International Agency for Research on Cancer (IARC 1973; Sciacca and Oliveri Conti 2009).

Until now, in foodstuffs from marine origin, only benzo(a)pyrene (BaP) has a limit set by European Regulation, which is 2 ppb wet weight (EC 2006). It would be opportune to also set a limit also for total PAHs. Among toxic metals, only Pb, Cd and Hg have a limit set by E.C. Reg. 1881/2006. However, essential metals may become toxic when elevate doses are ingested (Menzie et al. 2009).

The aim of our study was to estimate the Sicily Channel's pollution in an area that presents low industrial and municipal impacts, but does have an intensive ships traffic and a peculiar geochemical characterization of the bottom. (Di Leonardo et al. 2006). *Mullus barbatus*, recommended by FAO-UNEP as a bioindicator species (Fao-Unep 1993), was selected in this study. It is a sedentary benthic species with a large lipid content, which favors bioaccumulation of compounds such as PAHs (Kanalay and Harayama 2000; Liguori et al. 2006; Perugini et al. 2007). In particular we have estimated metals and a fingerprint of 16 PAHs in muscle tissue, and possible adverse health effect for consumers by evaluating the consumption rate and risk-based

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consumption limits proposed by the US EPA (2000) for carcinogenic health endpoints and chronic systemic health endpoints.

This topic has been explored by various authors in different areas (Bordajandi et al. 2004; Cizdziel et al. 2002; Di Leo et al. 2010; Domingo et al. 2007; Falco et al. 2006; Ginsberg and Toal 2009; Llobet et al. 2006, 2007; Mansilla-Rivera and Rodriguez-Sierra 2011; Marti-Cid et al. 2007, 2008; Spada et al. 2011; Storelli 2009, 2005; Turyk et al. 2012). This is the first report on these pollutants in fish from Sicily.

Materials and Methods

Specimens of *M. barbatus*, ranging in length from 14 to 16 cm, were sampled in March 2009, south of Lampedusa island, by a trawler net. Once 30 specimens were collected, fillets of muscle tissue with skin present, were stored in a sterile polyethylene tube and labelled with a code number. Samples were then transported in a styrofoam box containing wet ice and were kept in the laboratory at -20°C until analysis. Analytical methods for metals, including preparation of samples for analysis and instrumentation are detailed in recent publications (Copat et al. 2012; Tigano et al. 2009). Recoveries on standard reference material 1946 NIST was between 90 % and 110 %.

PAHs that were analyzed included naphthalene (NA), acenaphthylene (ACY), acenaphthene (AC), fluorene (FL), phenanthrene (PHE), anthracene (AN), fluoranthene (FA), pyrene (PY), benzo a anthracene (BaA), chrysene (CH), benzo b fluoranthene (BbF), benzo k fluoranthene (BkF), benzo a pyrene (BaP), dibenzo ah anthracene (DB a,h A), benzo g h i pyrene (B g,h,i P), indeno 123-cd pyrene (I 1,2,3-cd P). For the analysis, a sample of about 5 g of tissue was weighed with an analytical balance (Mettler Toledo AT 104, USA), homogenized, and sonicated with a solution of dichloromethane:acetone 1:1 (v:v) using a sonicator (ISCO, Italy). The extract obtained was concentrated with a stream of nitrogen and loaded into a Varian Bond Elut C18 cartridge (12 mL), previously conditioned with 5 mL of dichloromethane, 5 mL of isopropanol and 5 mL of distilled water. The collection vial was cleaned with 8 mL of 1:1 (v:v) dichloromethane:acetone and always loaded on the cartridge. The eluates were again concentrated in a stream of nitrogen, 1 mL of acetonitrile (ACN) was added, and analysis was performed by high performance liquid chromatography (HPLC) Prostar Varian, USA. The analytical method provided a mobile phase consisting of 1:1 (v:v) $\text{H}_2\text{O}/\text{CAN}$ for 5 min, which achieved 100 % ACN in 10 min with a flow of 1.5 mL/min. The ultraviolet determination was performed at 255 nm, while the fluorescent detection was conducted at 6

different wavelengths of excitation and emission. Recovery tests showed that between 85 and 120 % of the individual PAHs that were spiked into fish tissue were recovered.

The risk for human health as a result of eating these species was evaluated by estimation of the average daily exposure, on the basis of an average daily ingestion rate (IRd) of 2.5 g (Copat et al. 2012).

$$\text{EDI}_a = (C \times \text{IR}_d)/\text{BW}$$

C is the contaminant concentration in muscle fish analyzed ($\mu\text{g g}^{-1}$), and BW the body weight (kg), assuming it as 70 kg (EPA 2000).

Estimated daily intake was also calculated assuming a meal size of 227 g (EDIm) as suggested by US-EPA (2000).

Furthermore we calculated the Estimated Weekly Intake-average (EWIa) on the basis of a average weekly ingestion rate (IRw) of 17.5 g (Copat et al. 2012):

$$\text{EWI}_a = (C \times \text{IR}_w)/\text{BW}$$

All consumption rates thus calculated were compared with daily and weekly tolerable intake (TI) levels suggested by the World Health Organization (WHO 2011) for specific contaminants.

The allowable number of fish meals of a specific meal size that may be consumed over a given period of time was evaluated. For carcinogenic effects, we obtained the maximum allowable fish consumption rate (CRmmc) meals/month expected to generate a risk no greater than the maximum acceptable individual lifetime risk (ARL), considered to be 1 in 100.000 for this study. For non carcinogenic effects, we obtained the maximum allowable fish consumption rate (CRmmr) meals/month that would not be expected to cause adverse non-carcinogenic health effects.

As suggested by US-EPA (2000) we assumed the following: a meal size of 227 g; a body weight (BW) of 70 kg; a lifetime risk of 70 years; a time-averaging period of 30.44 day/month.

Results and Discussion

Data obtained from metals analysis (Table 1), show that As and Zn were present in the highest concentrations, with means of 27.01 and $3.476 (\text{mg kg}^{-1})$ respectively. Concentrations of toxic metals Pb, Cd and Hg, are within the limits of EC Regulation 1881/2006, that set acceptable levels for contaminants in foodstuffs.

Overall, most of metal concentrations found in muscle tissue of *M. barbatus* fall in the range of other studies done in the Mediterranean Sea, or are lower (Falco et al. 2006; Kucuksezgin et al. 2001; Perugini et al. 2009; Storelli et al. 2003; Storelli and Marcotrigiano 2005; Tepe et al. 2008;

Table 1 Mean (mg kg⁻¹ w.w.), standard deviation (SD) (mg kg⁻¹ w.w.) and consumption rates of metals analyzed

Metals	Mean ± SD	CSF ^a	RfD ^b	EDIa ^c	EDIm ^d	EWIa ^e	TI ^f	CRmmc ^g	CRmmr ^h
As	27.01 ± 7.548	NA ⁱ	NA ⁱ	0.964	87.56	6.75	15 µg/kg-w	NA ⁱ	NA ⁱ
Pb	0.035 ± 0.015	NA ⁱ	NA ⁱ	0.001	0.11	0.009	25 µg/kg-w	NA ⁱ	NA ⁱ
Cd	0.001 ± 0.001	NA ⁱ	1 × 10 ⁻³	0.000	0.003	0.000	7 µg/kg-w	NA ⁱ	>16
CH ₃ Hg ^j	0.027 ± 0.011	NA ⁱ	1 × 10 ⁻⁴	0.001	0.088	0.007	1.6 µg/kg-w	NA ⁱ	>16
Cr III ^j	0.524 ± 0.280	NA ⁱ	1.5	0.019	1.699	0.131	NA ⁱ	NA ⁱ	>16
Cr VI ^j	0.524 ± 0.280	NA ⁱ	3 × 10 ⁻³	0.019	1.699	0.131	NA ⁱ	NA ⁱ	>16
Cu	0.433 ± 0.210	NA ⁱ	NA ^a	0.015	1.404	0.108	NA ⁱ	NA ⁱ	NA ⁱ
Co	0.013 ± 0.007	NA ⁱ	NA ^a	0.000	0.042	0.003	500 µg/kg-day	NA ⁱ	NA ⁱ
Ni	0.042 ± 0.037	NA ⁱ	2 × 10 ⁻²	0.002	0.136	0.011	NA ⁱ	NA ⁱ	>16
Se	0.487 ± 0.111	NA ⁱ	5 × 10 ⁻³	0.017	1.579	0.122	NA ⁱ	NA ⁱ	>16
V	0.072 ± 0.056	NA ⁱ	9 × 10 ⁻³	0.003	0.233	0.018	NA ⁱ	NA ⁱ	>16
U	0.055 ± 0.015	NA ⁱ	3 × 10 ⁻³	0.002	0.178	0.014	NA ⁱ	NA ⁱ	>16
Zn	3.476 ± 1.885	NA ⁱ	0.3	0.124	11.27	0.869	1,000 µg/kg-day	NA ⁱ	>16

^a CSF cancer slope factor mg/kg-day, ^b RfD reference dose mg/kg-day, ^c EDIa estimated daily intake-average µg/kg-day, ^d EDIm estimated daily intake-meal µg/kg-day, ^e EWIa estimated weekly intake-average µg/kg-week, ^f TI tolerable intake suggested by FAO/WHO, ^g CRmmc maximum allowable fish consumption rate meals/month for carcinogenic effects; ^h CRmmr maximum allowable fish consumption rate meals/month for non-carcinogenic effects; ⁱ NA: CSF and RfD are not available in the EPA's Integrate Risk Information System (IRIS) for this pollutant and TI is not available in the Evaluations of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) online database; ^j We assume Hg as total CH₃Hg, Cr as total Cr III and as total Cr VI

Topcuoglu et al. 2002; Turan et al. 2009). Only concentrations of As and Se are not in agreement with those found in the literature. In fact, we found that As concentrations in muscle of various fish species from the Mediterranean Sea range between 0.44 and 0.49 mg kg⁻¹ for inorganic As, and between 5.31 and 14 mg kg⁻¹ for organic As (Storelli and Marcotrigiano 2005). In some works on *M. barbatus* caught in the coast of Catalonia, the total As concentrations in muscle reached 16.6 mg kg⁻¹ (Falco et al. 2006). As tends to settle in the benthos, whatever the source of release, and mainly benthic organisms tend to bioaccumulate it. So there is a strong correlation between the ecological niche occupied and concentration of total As in the tissues of analyzed species. On the basis of our data on As concentrations, which are considerably higher than those reported in the literature in the muscle of *M. barbatus*, we can indicate a contamination of the study area, even if it is a predominantly natural contamination due to the intense tectonic activity of the area (Di Leonardo et al. 2006; Ferrara et al. 2000). The consumption rate result of As EDIm is much higher than the TI suggested by the WHO (2011) (Table 1). However, we have analysed the total As, and it is known that the toxicity of As is caused by the inorganic form (Edmonds and Francesconi 1993), that in food typically accounts for no more than 1–3 % of the total arsenic present (FSA 2004).

Concerning the results of the 16 PAHs (Table 2), we found mean concentrations (µg kg⁻¹) ranging from 0.250 for BaP to 13.16 for NA. The finding of lower molecular weight PAHs arise from the fact that the PAHs with more

than 5 rings bind to sediment and are absorbed from the digestive tract and then metabolized. In contrast, PAHs from 2 to 4 rings show a greater solubility in water, and in fish are absorbed through the gills going directly into the interstitial liquid. Such evidence has also been confirmed in other work conducted in the Adriatic Sea (Perugini et al. 2007). This reinforces the hypothesis that the intense maritime traffic of the study area is the primary source of contamination with PAHs, that originate, not only from potentially incomplete combustion of fossil fuels, combustion of biomass, municipal waste, accidental spills and decomposition of organic matter (Guo et al. 2006, 2007; Liu et al. 2005), but also from vessel traffic (Hinkey et al. 2005).

Regarding consumption limits for PAHs, EPA's Integrated Risk Information System (IRIS) provides information on a cancer slope factor only for benzo (A) pyrene, which is considered as probable human carcinogen (group B2) based on sufficient evidence of carcinogenicity in animals. An EPA report (US-EPA 2000) considers that total PAHs have the same cancer slope factor as BaP.

Based on the CRmmc for total PAHs, the consumption of *M. barbatus* among Sicilian people could generate a risk greater than the maximum acceptable individual lifetime risk. Furthermore the EDIm is much higher than the TI suggested by WHO (2011).

In conclusion, our data provide information about health risks resulting from consumption of fish from the Sicily channel. Certainly, it would be appropriate in further studies expanding the study area as well as including other

Table 2 Mean ($\mu\text{g kg}^{-1}$ w.w.), standard deviation (SD) ($\mu\text{g kg}^{-1}$ w.w.) and consumption rates of PAHs analyzed

PAHs	Mean \pm SD	CSF ^a	RfD ^b	EDla ^c	EDIm ^d	EWla ^e	TI ^f	CRmmc ^g	CRmmr ^h
NA	3.526 \pm 6.130	NA ⁱ	0.02	0.126	11.43	0.882	NA ⁱ	NA ⁱ	>16
ACY	13.16 \pm 3.441	NA ⁱ	NA ⁱ	0.470	42.66	3.289	NA ⁱ	NA ⁱ	NA ⁱ
AC	0.974 \pm 1.129	NA ⁱ	0.06	0.035	3.159	0.244	NA ⁱ	NA ⁱ	>16
FL	1.614 \pm 1.889	NA ⁱ	0.04	0.058	5.234	0.404	NA ⁱ	NA ⁱ	>16
PHE	0.824 \pm 0.994	NA ⁱ	NA ⁱ	0.029	2.672	0.206	NA ⁱ	NA ⁱ	NA ⁱ
AN	0.503 \pm 0.132	NA ⁱ	0.3	0.018	1.631	0.126	NA ⁱ	NA ⁱ	>16
FA	0.961 \pm 2.003	NA ⁱ	0.04	0.034	3.116	0.240	NA ⁱ	NA ⁱ	>16
PY	0.584 \pm 0.447	NA ⁱ	0.03	0.021	1.894	0.146	NA ⁱ	NA ⁱ	>16
BaA	0.607 \pm 0.644	NA ⁱ	NA ⁱ	0.022	1.968	0.152	NA ⁱ	NA ⁱ	NA ⁱ
CH	0.726 \pm 0.795	NA ⁱ	NA ⁱ	0.026	2.354	0.182	NA ⁱ	NA ⁱ	NA ⁱ
BbF	0.529 \pm 0.287	NA ⁱ	NA ⁱ	0.019	1.715	0.132	NA ⁱ	NA ⁱ	NA ⁱ
BkF	0.615 \pm 0.421	NA ⁱ	NA ⁱ	0.022	1.994	0.154	NA ⁱ	NA ⁱ	NA ⁱ
BaP	0.250 \pm 0.068	7.3	NA ⁱ	0.009	0.811	0.063	10 ng/kg-d	>16	NA ⁱ
DahA	0.500 \pm 0.135	NA ⁱ	NA ⁱ	0.018	1.621	0.125	NA ⁱ	NA ⁱ	NA ⁱ
BghiP	0.591 \pm 0.335	NA ⁱ	NA ⁱ	0.021	1.917	0.148	NA ⁱ	NA ⁱ	NA ⁱ
IP	0.510 \pm 0.134	NA ⁱ	NA ⁱ	0.018	1.654	0.128	NA ⁱ	NA ⁱ	NA ⁱ
Σ PAHs	26.47 \pm 34.16	7.3	NA ⁱ	0.945	85.83	6.617	4 ng/kg-d-bw	<0.5	NA ⁱ

^a CSF cancer slope factor mg/kg-day, ^b RfD reference dose mg/kg-day, ^c EDla estimated daily intake-average ng/kg-day, ^d EDIm estimated daily intake-meal ng/kg-day, ^e EWla estimated weekly intake-average ng/kg-week, ^f TI tolerable intake suggested by FAO/WHO, ^g CRmmc maximum allowable fish consumption rate meals/month for carcinogenic effects, ^h CRmmr maximum allowable fish consumption rate meals/month for non-carcinogenic effects; ⁱ NA: CSF and RfD are not available in the EPA's Integrate Risk Information System (IRIS) for this pollutant and TI is not available in the Evaluations of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) online database

species having similar characteristics as bioindicators, in order to get a more comprehensive picture of the environmental contamination of the Sicilian sea, and of the risks for human health linked to fish consumption in the Sicilian region.

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