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Polychlorinated biphenyl and organochlorine pesticide contamination signatures in deep-sea fish from the Mediterranean Sea

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ABSTRACT

Polychlorinated biphenyl (PCB) and OCP concentrations were determined in the livers of two deep-sea fish species, roughsnout grenadier and hollowsnout grenadier, from the Adriatic Sea. In both species, contaminant concentrations were in the following order: PCBs > DDTs > > HCB. Contaminant load was higher in roughsnout grenadier (PCB: 12,327 ng g^{-1}; DDTs: 5357 ng g^{-1}; HCB: 13.1 ng g^{-1}) than in hollowsnout grenadier (PCB: 1234 ng g^{-1}; DDTs: 763 ng g^{-1}; HCB 6.3 ng g^{-1}). PCB patterns were dominated by higher chlorinated congeners (hexa-CBs: 50.3–52.1%, hepta-CBs: 29.6–35.5%, penta-CBs: 8.0–11.1% and octa-CBs :5.2–5.4%). PCBs 138, 153 180 and 187 were the most abundant. Regarding the DDT pattern, *p,p′*-DDE was prevalent in both species (roughsnout grenadier: 99.7%, hollowsnout grenadier: 90%), suggesting no recent DDT input. In both species, the total 2,3,7,8-tetrachlorodibenzo-*p*-dioxin toxic equivalent (TEQ) concentrations (roughsnout grenadier: mean 43.77 pg/g, hollowsnout grenadier: mean 20.49 pg/g), calculated from non- and mono-*ortho* PCBs, reached those encountered in marine organisms at higher levels in the trophic chain.

1. Introduction

Liphophilic persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs), organochlorine pesticides (DDTs) and hexachlorobenzene (HCB), are chemicals of the greatest concern owing to their persistence in environment and the fact that they are readily bioaccumulated and highly toxic for living organisms. Up to 70% of all organochlorinated compounds entering the environment are concentrated in the world's oceans (Tanabe, 1985). Transported by living biota or dead biota or absorbed to the detritus, these hydrophobic pollutants can reach the sea floor. From this final resting ground, they can be resuspended by physical mixing or by the activity of bottom-dwelling organisms and bioaccumulate as one moves up the food chain. Consequently, the deep environment not only will not be free from these pollutants but may even act as a sink for them, to the point that restrictive regulations for the use and disposal of these chemicals, which have resulted in a significant decrease of source inputs in recent decades, may still not be recognizable in these remote areas (Ramu et al., 2006). Different researches have continued to reveal that contamination in deep-sea biota is more elevated than surface living species, highlighting transport processes of POPs to the deep-sea environments. For example, Froescheis et al. (2000) and Looser et al. (2000) showed that the PCB levels in bottom dwellers at depths greater than 800 m were between 10 and 17 times higher than in the related surface species. Similarly, Kramer et al. (1984) find an enrichment of up to 600 times for DDT in Atlantic deepwater fish relative to surface living species. Nevertheless, there is a paucity of information regarding exclusively the feature of organochlorine contamination in deep-sea biota (Berg et al., 1997, 1998; Froescheis et al., 2000; de Brito et al., 2002a–c; Mormede and Davies, 2003), probably as a combination of the difficulty in obtaining samples and the low commercial interest of these fish species. The scarcity of data extends also to the Mediterranean basin (Solé et al. 2001; Storelli et al., 2004a, 2007), although it has been often reported that this marine area is heavily contaminated (Meadows, 1992; Kuetting, 1994; Borrell et al., 1997), as a consequence of its particular hydrographical characteristics and high anthropogenic pressure. However, many efforts have attempted to protect this delicate and complex ecosystem. The Barcelona Convention for the Protection of the Mediterranean Sea, including the Mediterranean Action Plan (MAP) and the Mediterranean Marine Pollution Monitoring and Research Program (MED POL), has encouraged the implementation of monitoring programs for evaluating the health status of this water body. Recently, United Nations Environment

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Program (UNEP) produced a regionally based assessment of sources, environmental levels, transport pathways and effects of persistent toxic substances in the environment, considering the Mediterranean Sea as one of the regions for the study (UNEP, 2002, 2003). In this renewed political context, the monitoring of PCBs, DDTs and HCB in the deep-sea environment of the Mediterranean basin remains of crucial importance not only to fill knowledge gaps on the state of its chemical contamination, but also to produce baseline data for taking decisions regarding future issues of environmental conservation.

Taking the above into account, this manuscript, which represents a component of a broader investigation including other environmental pollutants such as polybrominated diphenyl ethers (PBDEs; Covaci et al., 2008) and heavy metals (Storelli et al., in preparation), determines the occurrence and potential impact of PCBs, DDTs and HCB in two deep-sea fish species, *Trachyrincus trachyrincus* and *Coelorhinchus coelorhynchus*, collected in the Mediterranean Sea. Particular attention has been paid to the seven "target" PCBs (congeners 28, 52, 101, 118, 138, 153 and 180) recommended for PCB monitoring by international organizations (e.g. European Union, International Council for the Exploration of the Sea (ICES), World Health Organization) and to the "dioxin-like" PCBs (non- and mono-*ortho* PCBs) useful in the assessment of ecotoxicological impact.

2. Materials and methods

2.1. Sample collection

Specimens of *Trachyrinchus trachyrinchus* (roughsnout grenadier; specimen number: 307, length: 35.0–49.3, average: 40.60±4.70) and *C. coelorhynchus* (hollowsnout grenadier; specimen number: 203, length: 5.10–8.70 cm, average: 6.70±1.30) were caught along the Apulian coast from Trani to Castro (about 200 km) in the Southern Adriatic Sea (Mediterranean Sea) between June and September 2006. The sampling sites (Fig. 1) were selected based on the geographical features of the Adriatic Sea, which generally presents shallow waters except for a deep depression along the Apulian coast (300–1200 m). Species choice was based on their availability at the sampling location. The analyses were conducted in composite samples. From the total number of specimens, pools were formed (hollowsnout grenadier: no. 6; roughsnout grenadier: no. 9) within which individual fish were gathered as a function of their similar size. From fish of each pool, liver was taken, homogenized and kept in deep freeze at −20 °C until chemical analysis.

2.2. Organochlorine compound

The following PCB congeners (no. 18, 28, 31, 44, 47, 49, 52, 74, 77, 87, 95, 99, 101, 105, 110, 118, 126, 128, 132, 138, 146, 149, 151, 153, 156, 158, 169, 170, 171, 172, 174, 177, 180, 183, 187, 194, 195, 196/203, 199, 205, 206 and 209), together with the DDT compounds (*p,p′*-DDT, *p,p′*-DDE, *p,p′*-DDD, *o,p′*-DDT, *o,p′*-DDE and *o,p′*-DDD) and HCB were investigated using previous methods (Covaci et al., 2002; Voorspoels et al., 2004).

Briefly, approximately 0.6 g of pooled fish liver was grinded with Na_2SO_4 and spiked with 20 ng of PCB 143 used as internal standard. Samples were extracted for 2 h by hot Soxhlet with a mixture of acetone/hexane (1/3, v/v). The extract was evaporated and cleaned by passing through 8 g of acid silica (H_2SO_4, 44% w.w.), using 50 mL of a mixture of hexane/dichloromethane (1/1, v/v) for elution of the analytes. The eluate was evaporated to dryness and redissolved in 100 μL of iso-octane. For the separation of non-*ortho* PCB congeners (no. 77, 126 and 169) from other PCBs, the method reported by Tanabe et al., (1987), involving fractionation on 125 mg of activated carbon (434455 C. Erba, Milano, Italy), was used.

2.3. High-resolution gas chromatography–mass spectrometry (GC–MS)

For the analysis of PCBs, an Agilent 6890 GC connected with an Agilent 5973 MS operated in electron impact ionization (EI) mode was equipped with a 25 m × 0.22 mm × 0.25 μm HT-8 capillary column (SGE, Zulte, Belgium). The ion source, quadrupole and interface temperatures were set at 230, 150 and 300 °C, respectively. The MS was used in the SIM mode with two ions monitored for each PCB homolog group or pesticide in specific windows. One microliter of the cleaned extract was injected in cold pulsed splitless mode (injector temperature 90 °C (0.03 min) then to 300 °C with 700 °C/min) for splitless time 1.50 min, pulse pressure time 1.50 min and pressure pulse 25 psi. Helium was used as the carrier gas at constant flow (1.0 ml/min). The temperature of the HT-8 column was held at 90 °C for 1.50 min, then increased to 180 °C at a rate of 15 °C/min, further increased to 280 °C at a rate of 5 °C/min, further increased to 300 °C at a rate of 40 °C/min and held for 7 min.

2.4. Quality assurance and quality control

QA/QC was performed through the analysis of procedural blanks, a duplicate sample and a standard reference material (SRM 1945, PCBs and OCPs in whale blubber) for each set of 10 samples. For the replicate and SRM 1945, the relative standard deviations (RSDs) were <10% for all the detected compounds. Additionally, the method performance was assessed through successful participation to interlaboratory studies organized by NIST (NIST/NOAA Interlaboratory Comparison Exercise Program for Organic Contaminants in Marine Mammal Tissues). Obtained values were deviating with less than 20% from the consensus values. Multi-level calibration curves ($r^2 > 0.999$) in the linear response interval of the detector were created for the quantification. The calibration curves were prepared to result in a range of 2–10,000 ng g^{-1} lipid weight for *p,p*-DDE, PCB 153, PCB 138 and PCB 180 and in a range of 2–2000 ng g^{-1} lipid weight for the other PCB congeners. The identification of target analytes was based on the relative retention times (RRTs) to the internal standard used for quantification, ion chromatograms and intensity ratios of the monitored ions. A deviation of the ion intensity ratios within 20% of the mean values obtained for calibration standards was considered acceptable. For analytes detected in the procedural blanks (PCBs 101, 138, 153 and 180), the mean value of each analyte in the procedural blanks was used for subtraction. After blank subtraction, the limit of quantification (LOQ) was set at 3*SD of the value obtained in the procedural blanks. For analytes that were not detected in procedural blanks, LOQs were calculated for a signal-to-noise ratio equal to 10. LOQs for individual PCBs and OCPs ranged between 1 and 2 ng g^{-1} lipid weight. Concentrations of individual compounds below LOQ were substituted with a value equal to zero. The RSD value below 3.6% for all pesticides and PCB congeners shows the good repeatability of the method used. Concentrations of PCBs, DDTs and HCB means of 2 replicate measurements are presented as ng g^{-1} on a lipid weight basis.

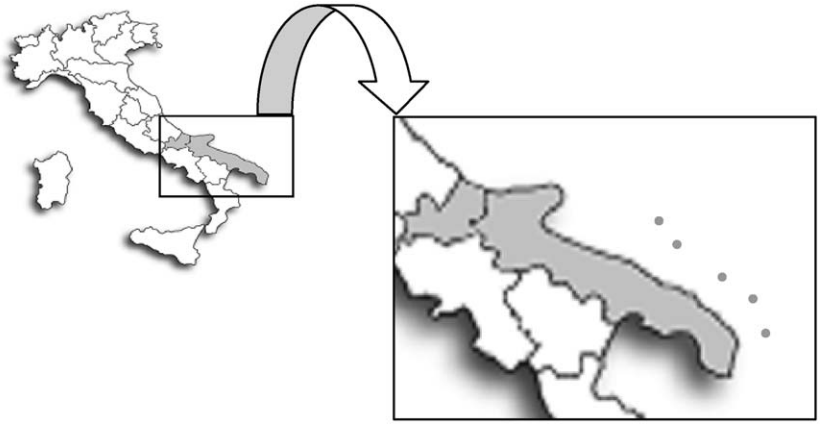

Fig. 1. Location of sampling stations along the southeast coast of Italy.

2.5. Statistical analyses

Mann–Whitney's *U* test was conducted to verify the difference in the levels of PCB, DDT and HCB accumulation, to determine whether there were differences as a function of species and to test the profile differences. Simple linear regression coefficient was used to examine the correlations between PCBs and DDTs and between the total organochlorine concentration and length of fish samples. The level of significance was set at $P<0.05$.

3. Results and discussion

3.1. Organochlorine load and bio-accumulation patterns

Concentrations of PCBs, DDTs and HCB in the liver of the species of deep-sea fish are summarized in Tables 1 and 2. Of the 41 PCB congeners investigated in this study, PCBs 52, 95, 99, 101, 105, 110, 118, 126, 128, 132, 138, 146, 149, 151, 153, 156, 170, 171, 172, 174, 177, 180, 183, 187, 199, 196/203, 194, 206 and 209 were detected in all samples, whereas congener PCBs 18, 31, 28, 44 and 169 were not detected in any of the examined samples. For the remaining congener PCBs 47, 49, 74, 77, 195 and 205 the frequency of occurrence was species dependent. As revealed by statistical analysis, in both species the contaminants were in the following ranking order: PCBs > DDTs > > HCB. Furthermore, total contaminant concentration was higher in roughsnout grenadier (PCBs: 12,327 ng g^{-1}; DDTs: 5357 ng g^{-1}; HCB 13.1 ng g^{-1}) than in hollowsnout grenadier (PCBs: 1234 ng g^{-1}; DDTs: 763 ng g^{-1}; HCB: 6.3 ng g^{-1}). Bioaccumulation of contaminants in marine biota can be viewed as a result of the interaction of numerous factors. Besides the level of contaminant input to the marine system, also biological (species, sex, age or size class, trophic level, reproductive status and metabolism) and environmental parameters (temperature, depth and habitat location) influence the pollutant body burden. Trophic level appears to be a determinant factor due the high biomagnification potential of POPs. Diet composition data of Mediterranean fish assign the species in question to different trophic levels, with the highest to roughsnout grenadier (Stergiou and Karpouzi, 2002). Consequently, the higher levels in this latter species liver can be imputed to distinct feeding strategies leading to a major uptake of contaminants in comparison with the hollowsnout grenadier. However, it should be stressed that for both species, feeding habits change depending on age classes. Smaller roughsnout grenadiers eat mainly decapods, while the diet of older animals consists of decapods and fish. In a similar way, young hollowsnout grenadiers feed on polichaetes, amphipods and copepods, those of medium size prefer polichaetes, decapods and fish, while larger specimens prey mainly on decapods (Stergiou and Karpouzi, 2002). Such distinct food preferences dependent on the animal's growth stage can also be responsible for the absence of significant correlation between concentrations of these contaminants and length of fish examined (roughsnout grenadier: $r = 0.37$, $P>0.05$; hollowsnout grenadier: $r = 0.06$, $P>0.05$). Another important factor that can play a key role in determining the different contaminant burdens between the species is the depth at which organisms live. In general, higher concentrations of POPs have been detected in fish from deeper waters (Mormede and Davies, 2003; Serrano et al., 2000; de Brito et al., 2002a). This agrees well with our results, roughsnout grenadier being an organism inhabiting deeper marine areas with respect to hollowsnout grenadier (FAO, 1987). To visualize the PCB profiles in the different species, congeners were separated into homolog groups depending on the number of chlorine atoms in the molecule (Table 3). The contribution of each PCB group to the total load was almost the same for both species—the highest homologs were hexa-CBs (50.3–52.1%), followed by hepta-CBs (29.6–35.5%), penta-CBs (8.0–11.1%) and octa-CBs (5.2–5.4%), while tetra-CBs, nona-CB and deca-CB PCB 209 (0.9–1.8%) made up only a small percentage. PCBs 138, 153 and 180 were the most abundant, collectively accounting for 52.7% in hollowsnout grenadier and

Table 1
Minimum, maximum and mean concentrations (ng g^{-1} lipid weight basis) of individual PCB congeners in hollowsnout grenadier and roughsnout grenadier liver.

	Hollowsnout grenadier				Roughsnout grenadier			
	Min.	Max.	Mean	SD	Min.	Max.	Mean	SD
Lipids (%)	30.6	45.8	41.2	5.7	20.6	33.0	26.7	4.5
PCB 47	ND	3	1.9	1.6	ND	5	2.4	1.7
PCB 49	–	–	–	–	ND	9	3.1	2.5
PCB 52	4	10	6.7	2.0	3	10	6.9	2.5
PCB 74	–	–	–	–	ND	6	1.6	2.3
PCB 77	ND	2	0.5	0.8	ND	2	1.0	0.9
PCB 87	ND	2	0.6	1.0	9	35	15.8	9.3
PCB 95	6	13	8.3	2.2	3	5	3.7	0.6
PCB 99	15	41	23.1	9.7	74	214	118.7	53.5
PCB 101	15	31	20.4	5.7	92	296	145.3	73.7
PCB 105	15	29	18.4	5.2	85	286	149.8	73.9
PCB 110	2	6	3.1	1.7	6	28	15.0	6.5
PCB 118	43	93	56.5	18.6	284	1048	528.1	270.7
PCB 126	1	10	4.0	3.3	ND	18	7.9	6.0
PCB 128	16	43	24.5	9.7	48	115	71.9	24.8
PCB 132	2	35	27.2	12.8	9	26	15.8	6.5
PCB 138	110	302	165.9	70.1	980	3190	1669	829.2
PCB 146	28	73	39.1	16.7	288	978	483.0	253.3
PCB 149	19	59	31.1	14.3	128	344	205.1	82.4
PCB 151	11	27	14.8	5.8	65	200	110.1	50.1
PCB 153	217	579	319.3	132.6	2206	6496	3456	1608
PCB 156	10	22	13.8	4.5	83	300	156.6	74.1
PCB 158	5	14	7.9	3.1	26	62	39.2	14.8
PCB 170	33	82	44.8	18.8	326	1115	580.7	287.9
PCB 171	6	15	8.5	3.2	28	86	47.3	21.3
PCB 172	6	14	8.0	3.0	56	200	100.7	51.3
PCB 174	3	12	6.0	3.4	52	179	89.7	47.6
PCB 177	4	17	8.6	4.4	97	328	176.4	83.1
PCB 180	116	304	165.6	69.4	1145	3792	2009	945.6
PCB 183	24	60	33.0	13.5	191	618	328.9	153.4
PCB 187	71	167	90.9	37.5	52	179	89.7	47.6
PCB 194	11	27	18.1	5.4	ND	382	176.8	127.4
PCB 195	3	6	4.6	1.3	ND	ND	ND	ND
PCB 196/203	13	31	21.4	6.4	125	438	226.1	111.9
PCB 199	16	35	22.8	6.6	ND	520	249.0	173.0
PCB 205	ND	1	0.5	0.6	ND	8	1.7	3.0
PCB 206	2	10	5.3	2.5	23	95	44.1	25.3
PCB 209	3	18	8.9	5.3	20	88	42.1	22.5
ΣPCBs	**937**	**2127**	**1234**	**449**	**7455**	**23,500**	**12,327**	**5932**

ND = not detected.
Number of replicates = 2.

Table 2
Minimum, maximum and mean concentrations (ng g^{-1} lipid weight basis) of DDT and HCB in hollowsnout grenadier and roughsnout grenadier liver.

	Hollowsnout grenadier				Roughsnout grenadier			
	Min.	Max.	Mean	SD	Min.	Max.	Mean	SD
p,p′-DDT	4	40	24.5	12.9	ND	16	7.6	6.9
p,p′-DDE	377	1254	686	341	2869	9945	5339	2712
p,p′-DDD	28	51	42.7	8.0	3	18	9.0	5.4
o,p′-DDT	ND	10	4.2	3.4	ND	3	0.9	0.9
o,p′-DDE	–	–	–	–	–	–	–	–
o,p′-DDD	4	6	5.0	1.1	ND	3	0.8	1.2
ΣDDT	**444**	**1361**	**763**	**353**	**2887**	**9959**	**5357**	**2711**
HCB	**4**	**9**	**6.3**	**1.7**	**9**	**16**	**13.1**	**2.2**

Number of replicates = 2.

Table 3
PCB homolog profile (%±SD) in hollowsnout grenadier and roughsnout grenadier liver.

Homologue	PCB congeners	Hollowsnout grenadier	Roughsnout grenadier
Tetra-CBs	44, 47, 49, 52, 74, 77	0.7±0.2	0.2±0.02
Penta-CBs	87, 95, 99, 101, 105, 110, 118, 126	11.1±1.5	8.0±1.4
Hexa-CBs	128, 132, 138, 146, 149, 151, 153, 156, 158	52.1±8.5	50.3±9.4
Hepta-CBs	170, 171, 172, 174, 177, 180, 183, 187	29.6±4.6	35.5±5.5
Octa-CBs	194, 195, 196/203, 199, 205	5.4±0.8	5.2±0.9
Nona-CBs	206	0.4	0.4
Deca-CBs	209	0.7	0.3

57.8% in roughsnout grenadier, followed by PCB 187 (roughsnout grenadier: 8.5%; hollowsnout grenadier: 7.4%). The abundance of these congeners is consistent with their high prevalence in technical mixtures, high lipophilicity, stability and persistence, which facilitate adsorption to sediments and accumulation in the aquatic ecosystem, and to their molecular structure. PCBs 138, 153 and 180, being refractory to metabolic attack by monooxygenases, tend to be more slowly eliminated because of their high degree of chlorination and the lack of adjacent unsubstituted H-atoms in *ortho–meta* and/or *meta–para* position on the aromatic ring. The comparison of homolog groups between the two species indicated that there were no significant profile differences, except for hexa-CBs, which were slightly more abundant in roughsnout grenadier ($P<0.03$). It is difficult to indicate the reasons for such a difference; one possibility is to assume that the species in question have a selective metabolism of individual congeners and/or some congeners have a higher biomagnification potential leading to a selective enrichment in organisms higher in trophic web. In this regard, Bodin et al. (2008) found the highest trophic magnification factors for congeners with 2,4,5-substitution, characteristic structure of many hexa-CBs (i.e. PCBs 138, 146, 149, 153 and 156). For DDTs, the pattern of metabolite composition was DDE>DDD>DDT in both species. *p,p′*-DDE accounted always for the largest percentage, reaching 90–99.7% of ∑DDTs, while *o,p′*-DDE was below LOQ in all samples. For the remaining compounds, DDD isomers accounted for 0.21% and 6.3%, while the compositional percentages of DDT isomers were 0.12% and 3.7% in roughsnout grenadier and hollowsnout grenadier, respectively. Differences in the relative concentrations of DDT-related compounds in these two species might reflect specie-specific capacity for DDT biotransformation. For HCB, the observed levels were always modest, probably reflecting the traditionally small inputs of this compound in the environment and a lower persistence compared to DDTs and PCBs, particularly in warm environments, where its decomposition rate is higher (Calamari et al., 1991).

The organochlorine pollution of the Adriatic Sea ecosystem is attributable to many sources: atmospheric fallout, wastewater discharge from the intensively cultivated areas, the densely populated urban centers and the large industrial complexes. In addition, agrochemical, industrial and domestic effluents drained by major Italian rivers, Po and Adige, into the sea can also play a key role in organochlorine contamination of this aquatic environment. The ratio of ∑PCBs/∑DDTs, proposed as an indicator of the provenience of the chemicals (Tanabe et al., 1997) has evidenced values higher than 1 (roughsnout grenadier: 2.3; hollowsnout grenadier: 1.6), suggesting a proportionally higher contribution of pollutant releases of industrial origin to agricultural origin. In addition, the high values of DDE/DDT ratio (0.89–0.99), commonly used to assess the chronology of DDT input into the ecosystem, indicate that the contamination found is due not to a recent input but to remote use of DDT in agricultural activity. It is also interesting to highlight that PCB 153 and *p,p′*-DDE, representative compounds of the two contaminant classes because they are the most relevant in terms of abundance, were significantly correlated in both species (roughsnout grenadier: $r=0.90$, $P<0.02$; hollowsnout grenadier: $r=0.91$, $P<0.0001$). Such high relationship suggests that these organic micropollutants may come from similar sources and reflects an old contamination that may represent the background pollution in this area.

3.2. Comparison with other studies

Despite the difficulties encountered when comparing published data on marine organisms because of differences in analytical methods, number of congeners, species analyzed or sampled tissue it appears that the organochlorine levels observed in hollowsnout grenadier are generally comparable to those found in similar species liver from Mediterranean and non-Mediterranean regions. In contrast, roughsnout grenadier shows a very different picture of pollution with levels higher than most of the corresponding published data from deep-sea fish (Table 4). Concerning the PCB isomer pattern, similarities with literature data have been observed, especially with those reported for deep-sea fish showing an enrichment in the heavier homologs (particularly in hexa-CBs and hepta-CBs) and a poor presence of less chlorinated homologs (Solé et al., 2001; Mormede and Davies, 2003; Storelli et al., 2004b, 2007). This agrees well with their octanol–water partition coefficient (K_{ow}). The less chlorinated homologs have a lower $\log K_{ow}$ and, as a consequence, they have a lower propensity to leave the aqueous environment for organic compartments, while the higher chlorinated homologs having a high $\log K_{ow}$ are readily adsorbed to sediments, and only a small fraction remains in the bioavailable dissolved form (Connell, 1990). Moreover, because of their high degree of chlorination and the particular structural feature, these high-chlorinated biphenyls are refractory to metabolic attack, tending, thus, to be more strongly bioaccumulated than the low-chlorinated congeners (Walker, 2001). For DDTs, the contamination image is similar to those of PCBs, with hollowsnout grenadier having levels of the same order of magnitude as those in literature and roughsnout grenadier having much higher concentrations.

3.3. "Dioxin-like" PCBs and "target" PCB congeners

Among the 209 congeners of PCBs, the scientific community has spotted two particular congeners groups: the "dioxin-like" PCBs (non-*ortho* DL-PCBs: 77, 126 and 169; mono-*ortho* DL-PCBs:105, 118 and 156) and the "target" PCBs (PCBs 28, 52, 101, 118, 138, 153 and 180). Dioxin-like PCBs share with polychlorinated dibenzo-*p*-dioxins and dibenzofurans the bind ability to the aryl hydrocarbon (Ah) receptor. On this bases the World Health Organization has elaborated the Toxic Equivalent Factor (TEF) methodology, which is the most appropriate approach for toxicological risk assessment studies (Van den Berg et al., 1998).

Table 4
Mean concentrations of PCBs, DDTs, HCB and sum of seven congeners (PCBs 28, 52, 101, 118, 138, 153 and 180; ng g^{-1} lipid weight) in liver of deep-sea fishes from different regions of the world.

Species	Location	PCBs	DDTs	HCB	ΣPCB7	References
Hollowsnout grenadier	Mediterranean Sea (Adriatic Sea)	1234	763	6.3	734	This study
Roughsnout grenadier	Mediterranean Sea (Adriatic Sea)	12,327	5357	13.1	7814	This study
Hollowsnout grenadier	Mediterranean Sea (Adriatic Sea)	427	344	–	415	Storelli et al. (2007)
Roughtip grenadier	Mediterranean Sea (Adriatic Sea)	313	239	–	300	Storelli et al. (2007)
Roughhead grenadier	Atlantic Ocean (Davis Strait)	1058	390	59	602	Berg et al. (1997)
Round-nose grenadier	Atlantic Ocean (North Sea)	2400	5230	15	–	Berg et al. (1998)
Giant grenadier	Pacific Ocean (Tohoku Region)	2200	460	15	–	de Brito et al. (2002a)
Round-nose grenadier	Atlantic Ocean (West of Ireland)	664–876	1010–1500	26–31	460–601	Mormede and Davies (2003)
Deep-sea fishes	Pacific Ocean (Suruga Bay)	1000	280	19	–	Lee et al. (1997)
Deep-sea fishes	Pacific Ocean (Tosa Bay)	340	290	11	–	Takahashi et al. (2001)
Deep-sea fishes	Pacific Ocean (East China Sea)	230	700	18	–	Tanabe et al. (2005)
Deep-sea fishes	Pacific Ocean (Sulu Sea)	58	150	6.8	–	Ramu et al. (2006)
Greenland halibut	Atlantic Ocean (Davis Strait)	559	473	84	268	Berg et al. (1997)
Redfish	Atlantic Ocean (Davis Strait)	947	652	29	509	Berg et al. (1997)
Black dogfish	Atlantic Ocean (Davis Strait)	545	955	37	294	Berg et al. (1997)
Blue hake	Atlantic Ocean (Davis Strait)	1156	1446	68	672	Berg et al. (1997)
Tusk	Atlantic Ocean (Davis Strait)	939	992	73	528	Berg et al. (1997)
Velvet belly	Atlantic Ocean (North Sea)	2390	6030	64	–	Berg et al. (1998)
Ling	Atlantic Ocean (North Sea)	2960	4640	31	–	Berg et al. (1998)
Tusk	Atlantic Ocean (North Sea)	11,700	27,000	22	–	Berg et al. (1998)
Monkfish	Atlantic Ocean (Rockall Trough)	275–492	206–376	17.4–18.2	–	Mormede and Davies (2001)
Black scabbard	Atlantic Ocean (Rockall Trough)	140–364	385–410	23–24	–	Mormede and Davies (2001)
Walleye pollock	Pacific Ocean (Bering Sea)	780	340	74	–	de Brito et al. (2002b, c)
Walleye pollock	Pacific Ocean (Gulf of Alaska)	1000	420	30	–	de Brito et al. (2002b, c)
Walleye pollock	Pacific Ocean (Japan Sea)	1800	1400	87	–	de Brito et al. (2002b, c)
Ratfish	Pacific Ocean (Tohoku Region)	130	91	13	–	de Brito et al. (2002a)
Ghostsharks	Mediterranean Sea (Adriatic Sea)	387	–	–	274	Storelli et al. (2004a)
Skates	Mediterranean Sea (Adriatic Sea)	889	–	–	662	Storelli et al. (2004a)
Angler fish	Mediterranean Sea (Adriatic Sea)	4372	4423	–	3334	Storelli et al. (2004b)

Each concentration of an individual congener in a mixture is multiplied with its TEF, and the resulting TCDD equivalents are added up and expressed in terms of concentrations (toxic equivalents—TEQs). This methodology applied to risk analysis of chloroaromatic compounds is important in the consideration of numerous toxic effects caused by these pollutants, including hormonal, immunological, neurological and reproductive system damage. Total concentrations of DL-PCBs in hollowsnout grenadier and roughsnout grenadier were 93.2 and 843.4 ng g^{-1}, respectively. Congener-specific analysis showed that these DL-PCBs were always present, except PCB 169, which was not detected in any fish samples. In both species, mono-*ortho* PCBs were predominant with respect to non-*ortho* PCBs, contributing more than 95% of the total DL-PCBs. Among the mono-*ortho* congeners, the residue level of PCB 118 was the highest, constituting about 63%, while the proportion of PCBs 105 and 156 was lower and collectively made up about 36%. In the case of non-*ortho* DL-PCBs, the contribution by congener PCB 126 was higher than that of PCB 77, constituting 88.8% in both species. Calculations of TEQs (roughsnout grenadier: 43.77 pg-TEQ g^{-1}; hollowsnout grenadier: 20.49 pg-TEQ g^{-1}) showed that pentachlorinated non-*ortho* PCB 126 contributed the most to total toxicity, ranging from 90% in roughsnout grenadier samples and reaching 98% in hollowsnout grenadier. It is evident that, while mono-*ortho* PCBs mainly contribute to the concentration levels, the non-*ortho* congeners, and particularly PCB 126, accounted mostly for the toxicity, although this consistent contribution is as a result of the high TEF assigned to this congener. A comparison with literature data shows that our TEQ concentrations are sensibly higher than those in fish liver from the Mediterranean Sea (Storelli et al., 2003, 2004a, b, 2006; Oliveira Ribeiro et al., 2008), but comparable with those encountered in marine organisms located in the highest position of trophic web, such as swordfish, tunas and sharks (Serrano et al., 2000; Kannan et al., 2002; Storelli and Marcotrigiano, 2006; Storelli et al., 2008). The similarity between TEQ concentrations in these species and those encountered in long-lived organisms with the highest trophic positions underlines the high degree of PCB contamination of these species and, consequently, the contamination of their biotope. The International Council for the Exploration of the Sea has recommended the systematic consideration of some congeners for monitoring purposes. The existence of these "target" PCBs is relevant for the prediction of the degree of lipophilic contamination, although their toxicity is less than that of "dioxin-like" PCBs. In our study, among the seven target congeners only PCB 28 was below the limit of detection, while the others were omnipresent in all fish samples. The sum of these congeners was 7814 ng g^{-1} in roughsnout grenadier and 734 ng g^{-1} in hollowsnout grenadier. As shown in Table 4, the results of the seven target PCBs indicate that PCB contamination of hollowsnout grenadier is generally in the same order as that in fish liver from different geographical regions, where concentrations vary between 268 and 672 ng g^{-1}. In contrast, roughsnout grenadier samples were contaminated to a much higher degree, showing values comparable to those encountered in deep-fish from ecosystems considered severely polluted (Berg et al., 1998; de Boer et al., 2001).

4. Conclusions

Notwithstanding the complete ban of DDT and HCB and all the legal restriction about production and use of PCBs, widespread pollution is still present in the Mediterranean ecosystem. Long-range transport of these compounds from eastern countries, where they are still used, or some unknown contamination point sources due to improper disposal, accidental spills and discharge of industrial, agricultural and municipal effluents can be responsible for contamination of this semi-enclosed basin. The Adriatic

Sea, in particular, is an area that for the reduced hydrodynamics, high riverine inputs and intensive agricultural and industrial activities is subject to high anthropogenic impact (Viganò et al., 2001; Frignani et al., 2001, 2004; Solis-Weiss et al., 2004). The data of the seven target congeners confirm the presence of an extensive contamination and highlight especially that deep-sea environment is a hot spot of PCB contamination. In addition, the detection of so consistent concentrations in these deep-sea organisms undoubtedly renders a negative effect on the health of hydrobionts themselves. The high TEQ values, combined with consistent toxic load due to other PCB congeners and OCPs, constitute a warning signal that cannot be ignored. Assessments of organochlorine contaminant levels, their evolution and patterns are, thus, needed in the management and long-term conservation of this delicate marine ecosystem.

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