SLOW DECLINE OF PCBs IN EDIBLE MARINE FISH SPECIES FROM THE MEDITERRANEAN SEA

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Introduction

Polychlorinated biphenyls (PCBs) are toxic and persistent chemicals able to biomagnify along the food web, posing potential health risks for humans and wildlife[1]. In recent years many studies have focused on the assessment of these pollutants' concentrations in different environmental compartments and wildlife[2]. Most of these investigations depict a common scenario, i.e. a slow but steady decrease in PCB environmental concentrations resulting from the wide ban occurred in many countries in the mid 80's and the enforcement of the Stockholm Convention in 2004 [3,4,5]. Despite this apparent decrease, in some areas, high levels of PCBs are still reported today, especially in species at the top of the food web[6,7].

The Mediterranean Sea is an area where a slow temporal decrease in PCB levels has been described in different species while it remains, by some authors, a PCB hot spot for marine mammals[8,9]. On the other hand, the slight reduction reported for some fish species appears to be less noticeable in last years, which suggests potential new inputs and/or remobilization of these contaminants[10]. This, in turn, may be explained in terms of inefficient mitigation measures and ineffectiveness of global efforts towards PCB contamination, which is in concert with the inability of some countries to achieve the 2025 and 2028 targets of the Stockholm Convention at the current rate of mitigation and reduction of PCB environmental levels, as suggested by others authors[9].

The aim of this study was to assess the degree of PCB contamination in three different important commercial fish species of the Mediterranean Sea: sardine (*Sardina pilchardus*), anchovy (*Engraulis encrasicolus*) and bogue (*Boops boops*) in order to further provide new data on concentrations, and to assess potential risks for fish consumers. Nonetheless, this study is part of a largest project aimed to investigate the impact of micro-plastics on these species and the potential relationship between micro-plastics' content and associated organic contaminants.

Materials and methods

Sampling

Forty-eight fish specimens (16 sardines, 16 anchovies and 16 bogues) were obtained from Italian local fisheries in spring 2017. Samples corresponded to three species commonly fished and consumed in the Mediterranean diet.

Sample processing

PCBs were extracted from freeze-dried fish muscle samples using Soxhlet apparatous (24h) with a n-hexane:dichloromethane (9:1) mixture. Samples were previously homogenized with anhydrous sodium sulfate (Na_2SO_4) and spiked with a suite of ^{13}C-labeled PCBs. Obtained extracts were purified by low pressure chromatography on multilayer columns packed with neutral and acid silica gel modified with sulfuric acid (H_2SO_4). Final extract was evaporated using a TurboVap® (Zymarck Inc., Hopkinton, MA, USA) system until ~1 mL, transferred to vials, and dried under a gentle nitrogen stream. A few microliters of ^{13}C-labeled injection standards of PCBs were used for reconstitution of each sample prior to instrumental analysis.

Instrumental determination

Eighteen PCBs (#28,52,77,81,101,105,114,118,123,126,138,153,156,157,167,169,180,189) were targeted by gas chromatography high resolution mass spectrometry (GC-HRMS) using a Trace GC Ultra gas chromatograph (Thermo Fisher Scientific, Milan, Italy) coupled to a high-resolution mass spectrometer (DFS, Thermo Fisher Scientific, Bremen, Germany). Quantitation was carried out by the isotopic dilution technique. A full description of the instrumental parameters can be found elsewhere[6].

Data handling

All concentrations are given in ng/g (dl-PCBs) on wet weight (w.w.) basis. Toxic equivalent quantities (TEQ) for dl-PCBs were determined using the World Health Organization (WHO)-2005 toxic equivalency factors (TEF) for fish[11]. Data dl-PCBs and TEQs are reported in upper bound (i.e. substitution of non-detected compounds for detection limit values).

Results and discussion:

Target PCBs were found in all samples at concentrations ranging between 1.01 and 17.9 ng/g w.w. A detailed description of PCB content per species along WHO-TEQ values is compiled in Table 1.

Table 1. Mean, median and range of PCB concentrations (in ng/g w.w. or pg/g w.w.) and TEQ values (pg/g w.w.) in muscle from three Mediterranean Sea fish species.

	Sardine (*Sardina pilchardus*)				**Bogue (*Boops boops*)**				**Anchovy (*Engraulis encrasicolous*)**			
	concentration (ng/g w.w.)											
	Mean	**Median**	**Range**		**Mean**	**Median**	**Range**		**Mean**	**Median**	**Range**	
∑ICES7[a]	9.32	8.56	3.88	16.9	3.27	2.77	1.39	6.90	3.34	2.78	0.991	6.80
∑non-*ortho*-dl-PCBs[b] (pg/g w.w.)	17.6	14.4	9.10	40.5	4.12	3.10	0.854	15.3	3.30	3.36	7.76	9.27
∑mono-*ortho*-dl-PCBs[c]	1.35	1.36	0.577	2.62	0.384	0.296	0.174	1.058	0.322	0.272	0.0417	0.757
∑dl-PCBs	1.37	1.38	0.587	2.66	0.388	0.299	0.176	1.07	0.325	0.276	0.0424	0.776
	TEQ (pg/g w.w.)											
∑non-*ortho*-dl-PCBs	0.715	0.689	0.372	1.15	0.123	0.117	0.0637	0.236	0.194	0.201	0.0755	0.377
∑mono-*ortho*-dl-PCBs	0.202	0.205	0.0915	0.358	0.0565	0.0452	0.0246	0.152	0.0491	0.0471	0.00508	0.102
∑TEQs	0.917	0.905	0.473	1.44	0.180	0.164	0.0967	0.388	0.243	0.255	0.0817	0.435

[a]ICES7: PCB-28, 52, 101, 118, 138, 153, 180 [b]non-*ortho*-PCBs: 77, 81, 126, 169 [c]mono-*ortho*-PCBs: 105, 114, 118, 123, 156, 157, 167, 189

Sardine -the species with the highest fat content- showed the higher concentrations of contaminants; instead, anchovy and bogue showed similar average levels, about three times lower than those of sardine, in line with previous studies[12-15]. Strictly from a temporal perspective, values found in this survey depict a level of contamination significantly lower when comparing with PCB concentrations reported in the late 90's or early 2000's. However, and taking in consideration the wide range of values reported for sardine and anchovy, values found in this study are not significantly lower –while in some occasions higher- than those measured in the last years.

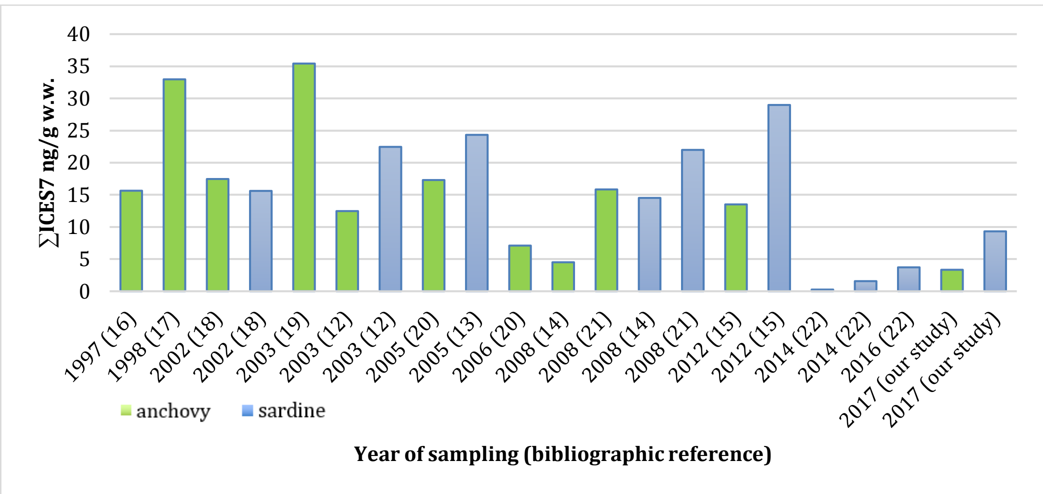

Figure 1. Reported data on PCB concentrations (ng/g w.w.) in Mediterranean sardine and anchovy

Much less abundant are data about PCB levels in bogue. The ICES7 values reported in 2011 for caught fish in the Adriatic Sea[23] are in the same order of magnitude that those reported in this study; conversely, studied specimens in 2017 from the Atlantic Ocean (Canary Islands) showed levels of PCBs about one order of magnitude higher[24].

In terms of congener abundance, the same predominance of PCB-153>138 >180 was observed in the three species (Figure 2A) in line with what is commonly found in marine fish[17,25]. Regarding dl-PCBs, the three species showed the same mono-*ortho* congener profile (118>105>156); however, distinct abundances of non-*ortho* congeners were observed depending on the species. Sardine and bogue exhibited the profile PCB-77>126>169>81, which is commonly found in other studies in the same and different fish species from the Mediterranean Sea and other parts of the world [26,27,28]. Instead, the pattern of non-*ortho* PCBs was quite different in anchovy (126>77>169) with a contribution of congener PCB-126 about 71% and no detection of PCB-81. This profile is different from that in anchovies caught in the Adriatic Sea[17], but the same as that of anchovies caught in the Black Sea[20] and in samples collected in Catalonia[29]. These differences in non-*ortho* PCB accumulation patterns could be attributed to different feeding habits, but also to important dissimilarities in toxicokinetics between species, involving metabolism and selective distribution across tissues.

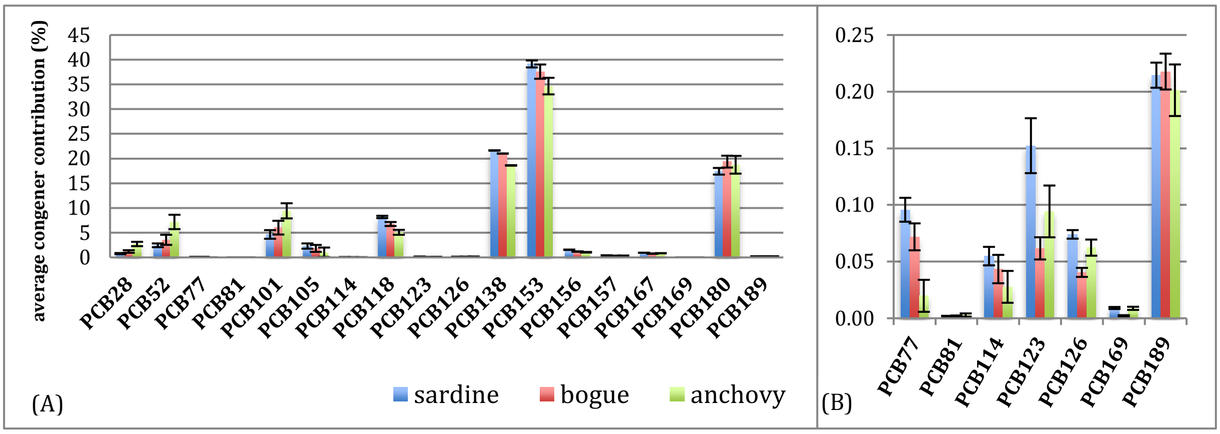

Figure 2. Average PCB congener profile of (A) all congeners and (B) non-*ortho* and the least abundant mono-*ortho* congeners

dl-PCB concentrations in pg WHO-TEQ/g is given in Table 1 for all species investigated in this study. As with total PCB concentrations, in comparison to the few reported TEQ data on the same species (Table 2) our results are in concert with a steady slow decline of TEQs in the Mediterranean, being in fact not significantly different from most values previously reported, especially in sardines.

Table 2. Mean WHO-TEQ concentration of dl-PCBs in sardine and anchovy from Mediterranean Sea. Data are expressed in pg WHO-TEQ/g w.w

Reference (year)	Geographic area	Sampling year	Species	pg WHO-TEQ/g w.w.
Llobet et al.(2003)[29]	Spanish markets	2000	sardine	2.5
Gómara et al.(2005)[26]	Spanish markets	1995-2003	sardine	1.91
Bocio et al.(2007)[30]	Spanish markets	2005	sardine-anchovy	1.19-1.24
Miniero et al. (2014)[14]	Southern Adriatic Sea Southern Tyrrhenian Sea Ionian Sea	2007-2008	sardine-anchovy	1.76-0.51

Mean levels of total TEQs reported by our study for sardine, bogue and anchovy, did not exceed the applicable Maximum Levels (ML) of 6.5 pg/g w.w. for muscle meat of fish and fishery products (Regulation (EU) 1259/2011[31]). However, it should be highlighted that our results did not include PCDD/F concentrations while official MLs relate to sum of both PCDD/Fs and dl-PCBs. Also the concentration of the 6 indicator PCB congeners (PCB 28, 52, 101, 138, 153 and 180) did not exceed the applicable ML of 75 ng/g w.w. in any species analyzed in this study.

An estimated weekly intake (EWI) of dl-PCBs was calculated by multiplying the weekly pelagic and demersal fish consumption data for Italy, respectively 85 g/week and 135 g/week[32], by the mean dl-PCB concentrations in muscle of analyzed fish (sardine, anchovy, bogue), and dividing by 70 Kg as average body weight. EWIs (g/week) deriving from the estimated weekly fish consumption in Italy were 1.12, 0.29 and 0.35 for sardine, anchovy and bogue, respectively. These values are far below the tolerable weekly intake (TWI) of 14 pg

TEQ/Kg established by the Scientific Committee on Food (SCF) of the EU FAO/WHO (2003)[33]. Therefore, our results show that the consumption of these important commercial species might not pose a significant risk for human health derived from their dl-PCB content. Alike MLs, in the comparison of this study's results with the regulated TWI it should be taken into account that EWIs were calculated without the contribution from PCDD/F contents (even if PCDD/F contribution to TEQs tend to be generally lower than that of dl-PCBs).

It is important to underline that comparability in concentrations among other studies' values may be highly influenced by variables such as different capture sites, year and season of sampling related to the spawning activity, as well as different lab procedures and quantification methods. Nevertheless, our results seem to indicate that current PCB burdens in the three Mediterranean fish species studied are in full compliance with the regulated levels in diet. At the same time, our results are often not different from values reported for the same species in the last years, which underpins the idea of a virtual halt or very slow decline in PCB concentrations in the Mediterranean, suggested by other authors. All these reasons justify ongoing concern and research on these pollutants, and make it essential continuous and effective PCB monitoring activities in the Mediterranean Sea area.

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