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Indicator PCBs in farmed and wild fish in Greece - Risk assessment for the Greek population

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ABSTRACT

Health benefits of fish consumption could be counterbalanced by the intake of contaminants after long term fish consumption, burdened even in trace levels. The presence of the indicator PCBs (NDL-PCBs and PCB 118) in farmed and wild seabream and seabass was evaluated. For the determination of PCB, a GC-MS method was developed and evaluated. The association of PCB accumulation in fish with seasonality, locality, production mode and species was also investigated. A new approach for the risk characterisation after exposure to NDL-PCB through fish consumption in Greece was developed, based on the real exposure and the permitted maximum levels of both aggregated dietary exposure and exposure through fish consumption. PCB levels determined in fish were below established permitted limits (6.24 ng/g 95th percentile), while PCB levels and congener distribution varied significantly between farmed and wild fish ($p = 0.001$). Seasonality was highlighted as an important factor affecting NDL-PCBs accumulation, with high levels coinciding with the reproduction period of each species. Differences were also depicted for sampling sites, with PCB 118 presenting significantly higher values in open seas while NDL-PCB congeners in closed seas. Risk assessment of NDL-PCB intake through fish consumption corrected for the aggregated exposure revealed no risk for the consumers.

1. Introduction

Fish consumption has been well established as part of a healthy diet, mainly due to the rich content in essential nutrients and ω-3 polyunsaturated fatty acids (PUFA) of fish meat. Numerous health benefits have been linked to the intake of PUFA and high quality protein found in fish, with protection against cardiovascular diseases being the most important. However, diet exposure to various toxicants and xenobiotics bioaccumulated in fish, could counterbalance these beneficial effects (Storelli, 2008; Domingo, 2016).

Wild, as well as farmed fish, are exposed to a set of various organic contaminants, such as Polychlorinated biphenyls (PCBs), Polychlorinated dibenzo-p-dioxins (PCDDs), Poly-chlorinated dibenzofurans (PCDFs) and Polycyclic aromatic hydrocarbons (PAHs) however, PCBs exhibit sharper biomagnification in the food chain (Çakiroĝullari et al., 2010; Paiano et al., 2013; Costopoulou et al., 2016). Moreover, PCB levels in fish tissue increase in relation to the fish fat content. PCBs are a synthetic group of persistent organic contaminants (POPs), which were manufactured to be used as plasticizers, heat exchanging fluids, additives in pesticides and were adopted in electrical equipment and pigment industry as well. Their release in the environment was of anthropogenic origin and although their use and production has been banned, their occurrence in the environment and food chain is ubiquitous due to their low elimination rate and high resistance to metabolic degradation (Schrenk and Chopra, 2017).

PCBs constitute a class of 209 congeners which are categorized under 2 major groups based on their toxic potential: the dioxin-like PCBs (DL-PCBs), which share a common toxicity mechanisms with dioxins, and the non-dioxin-like PCBs (NDL-PCBs) (Arnich et al., 2009; Schrenk and Chopra, 2017). Both groups are described as potential food contaminants, yet the sum of seven congeners (ΣPCB-7) (PCBs 28, 52, 101, 118, 138, 153, and 180) is commonly used as a gauge to determine

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Abbreviations

DL-PCBs	dioxin-like PCBs
NDL-PCBs	non- dioxin-like PCBs
ΣPCB-6	sum of the 6 NDL-PCBs
ΣPCB-7	sum of seven congeners
EDI	estimated daily intake
EDI_f	EDI from fish consumption
HI	hazard index
F_C	correction factor
HI_f	corrected fish specific hazard index
$MPDI_f$	maximum permitted daily intake through fish consumption
$MPDI_A$	maximum permitted daily intake through the whole diet

the total PCB burden in environmental as well as tissue and food samples. Six of these seven are NDL- PCBs (PCB 28, PCB 52, PCB 101, PCB 138, PCB 153 and PCB 180), and one is a DL-PCB (PCB 118). These seven PCBs, often called "indicator PCBs" were identified by international bodies such as the European Commission (European Commission, 2011) and the International Commission for the Exploration of the Seas (ICES). The indicator PCBs have been since, routinely monitored by researchers and are also included in the Water Framework Directive (European Commission, 2011) for their monitoring (Schrenk and Chopra, 2017; Squadrone et al., 2015; St-Gelais et al., 2017; Storelli and Perrone, 2010).

According to EFSA (2012), for a large portion of food samples, the sum of six indicator PCBs represents about 50% of the total NDL PCB load. Moreover, EFSA reported in 2012 that the highest levels of NDL-PCBs were observed in products derived from aquatic animals and specifically for fish muscle cited a mean of 23.3 μg/kg w/w, while average exposure to NDL-PCB indicators ranged from 4.3 to 25.7 ng/kg bw per day and the 95th percentile between 7.8 and 53.7 ng/kg bw per day. Consumption of fish, was one of the highest contributing food groups to dietary exposure. Additionally, the EU has set maximum tolerable levels (MLs) for the sum of the six indicators NDL-PCBs (ΣPCB-6) to 75 ng/g wet weight (European Commission, 2011). However, there is no valid established value for tolerable intake (TI) or guidance value for ΣPCB-6 or the ΣPCB-7 by international legislating authorities, since their toxicities for humans have not yet been fully characterized (EFSA, 2012b; WHO, 2016). An ADI value of 10 ng/kg bw/day has been proposed by the National Institute for Public Health and the Environment for the sum of six NDL-PCB indicators (Baars et al., 2001) and has been employed in certain risk assessment studies (Arnich et al., 2009; Giandomenico et al., 2016).

PCB burden in wild and farmed fish strongly depends on fish species, as well as area of sampling, demonstrating wide differentiations among farmed and wild-caught (Hayward et al., 2007). Lundebye et al. (2017) reported higher PCB levels in wild fish than farmed while Carubelli et al. (2007) showed that farmed sea bass was 2 times more burdened than wild and similar results were published by Ferreira et al. (2010) who reported higher levels in farmed than wild.

According to FAOSTAT total fishery aquaculture and capture in Greece, in 2015, was 105.969 and 65.188 tons respectively, while per capita supply was 19.3 kg in 2013, 8.8 kg of which was demersal fish and 3.2 kg pelagic fish. Greek consumers demonstrate different preferences as to farmed or wild-caught fish, depending on various criteria such socioeconomic status and area of residence; however, there are no official published data.

Human health implications associated with PCBs include, endocrine disruption, reproductive defects, neurological disorders and potentially cancer (Schantz et al., 2003; Buck et al., 2005; Meeker and Hauser, 2010; Boas et al., 2012; IARC, 2015; Paliwoda et al., 2016; Petrakis et al., 2017) There is also evidence of PCBs synergistic effects during co-exposure with other substances, such as the ones with Cd on thyroid function (Buha et al., 2013). NDL-PCBs in particular, seem to adversely affect dopamine neurotransmitter levels, calcium homeostasis and induce CYP enzymes in the liver (Fattore et al., 2008; Schrenk and Chopra, 2017). It has also been suggested that CYP polymorphisms could be responsible for adversities caused by exposure to PCBs (Docea et al., 2017). Regarding the presence of PCBs in marine fish from the Greek market, to the best of our knowledge, there are 2 studies assessing PCB levels in fish tissue, one of which focuses on DL-PBCs alone (Papadopoulos et al., 2004) while the second reports levels of both DL-PBCs and NDL-PBCs in farmed fish and wild caught fish (Costopoulou et al., 2016).

The aim of this study is to investigate the burden of the indicator PCBs (ΣPCB-7) in farmed and wild-caught fish from the Greek market as well as to assess the risk of human exposure through fish consumption for the Greek population.

As far as we know, there is not a previously described methodology for the risk characterisation of exposure from a specific food item. All the current methodologies refer to risk characterisation due to aggregated dietary intake. In our study we aimed in the estimation of risk due to exposure to NDL-PCB but only through fish consumption. For this purpose, we used a newly developed approach which is based on the classic hazard index of unit but corrected for the intake due to a specific food.

2. Materials and methods

2.1. Chemicals – reagents

Standard solution PCB-Mix 3, containing all the investigated congeners (PCBs 28, 52, 101, 118, 138, 153, 180) at 10 ng/μL, was purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Diethyl ether (for analysis) and *n*-hexane (95%) were supplied from PanReac AppliChem ITW Reagents. SiliaFlash® Irregular Silica Gels, 70-230 mesh, 60 Å (R10140B) was supplied from SiliCycle. Hexachlorobenzene (HCB) was used as an internal standard (Dr. Ehrenstorfer GmbH). Aluminium oxide 15 μm was supplied from Agilent.

2.2. Sample collection

Fish samples of both species (gilthead seabream and sea bass) were collected from aquaculture sites as well as the fish market of Heraklion, Crete during the period August 2017–March 2018. All collection sites are located in the Aegean Sea and the Sea of Crete (FAO fishing area 37, subarea 37.3, division 37.3.1). A total of 101 fish of both species namely gilthead seabream (*Sparus aurata*) (n = 47) and seabass (*Dicentrarchus labrax*)(n = 54) were collected. More specifically 81 samples (gilthead seabream n = 37, sea bass n = 44) were collected from aquaculture sites and 20 samples from the fish market (10 fish from each species) which were caught in Cyclades and Dodecanese, Greece.

There were three distinct periods of collection (months) from fish farms: 21 samples (25.9%) on August (summer), 30 samples (37.0%) on November (autumn) and 30 samples (37.0%) on February–March (winter-early spring).

In the laboratory, length and weight of fish were measured; samples were labelled and stored at −20 °C until dissection. Upon dissection of the fish, dorsal muscle tissue was collected in glass vials and stored at −20 °C until further analysis. An amount of 4 gr of fish muscle tissue wet weight (ww) was collected from each sample and all fish samples were freeze-dried.

2.3. Sample extraction and clean-up

Each freeze-dried fish sample was placed in a glass vial with a screw cap and crushed with a spatula. In each vial the internal standard HCB (20 ng) and 9 ml of n-hexane were added. Vials were hermetically sealed and placed in an ultrasonic bath for 1 h at 50 °C. Silica gel (4.5 g) and aluminium oxide (3.6 g) were placed in a Schott filter used as a short glass column. Each column was washed with 25 ml of a mixture of hexane-diethyl ether (95:5). Subsequently, the extract obtained after the ultrasonic bath was passed through the sorbents and washed twice with 9 ml of hexane-diethyl ether (95:5). The purified extract was collected in a 100 ml glass flask and was evaporated to a volume of approximately 1 ml before being transferred to a 2 ml vial. The flask was washed with 600 µl of a mixture of hexane-diethyl ether (95:5) and combined with the previously evaporated extract. The obtained extract was evaporated to dryness under a gentle stream and reconstructed in 100 µl of hexane.

2.4. GC-MS equipment and analysis

Instrumental analysis was performed with a gas chromatograph coupled to a mass spectrometer (GC-MS Shimadzu QP2010 Ultra) equipped with an AOC-20i/s autosampler. An HP-5 MS column (30 m ×0.25 mm, film thickness: 0.25 µm) was used for separation. The initial oven temperature was set at 100 °C, then raised to 110 °C with a heating rate of 4 °C/min. The temperature was finally raised to 280 °C at 15 °C/min and held for 15 min. Injection volume was 2 µl in splitless mode and helium was employed as carrier gas (purity ≥ 99,999%) with a constant flow of 1.0 ml/min. The temperature of the ion source was 230 °C. The MS was operated in single ion monitoring (SIM) mode and the mass traces acquired (m/z) for each congener and IS are reported in Table 1.

2.5. Method evaluation

All blanks and spiked samples were preparedas described. Method validation was carried out and the examined analytical parameters were linearity, limits of quantification (LOQ), % recovery, inter-day precision (%RSD) and % accuracy. The quality standards were set to meeting ISO/IEC 17025 standards (ISO/IEC 17025:2005).

The standard and spiked curves were constructed using the ratio of each compound area to IS area and used of the study of instrument response as well for the quantification of the target compounds in fish muscle tissue. The LOQ (S/N > 10) was determined from the lowest spiked blank sample. The % recovery, the % inter-day precision (%RSD) and the % accuracy were determined at 4 spiked levels: 0.125 (lower limit of quantification, LLOQ), 0.625 (quality control low level, QCL), 1.25(quality control medium level, QCM), 2.5(quality control high level, QCH) ng/gr) using 3 repetitions (n = 3) and the obtained data are presented in Table 1. For the determination of % recovery, PCB values obtained for a QCL sample spiked before extraction were compared to values obtained for a standard low level (STD-L) sample spiked after extraction. For the investigation of the carryover effect, the blank sample was injected immediately after the spiked sample at the upper limit of quantitation. The chromatogram of the blank sample was evaluated by comparing the peak area of the analyte in LLOQ samples and the analyte area in the blank sample.

2.6. Statistical methods

Levels of each PCB congener as well as of the sum of the 6 NL-PCBs (ΣPCB-6) and the sum of the 7 indicator PCBs (ΣPCB-7) were expressed in the form of mean and standard deviation (SD) and median. Levels and % of detection of individual PCB congeners, ΣPCB-6 and ΣPCB-7 were estimated using only samples with levels > LOQ values. Statistical analysis and exposure assessment were conducted using the following approach: Detected values < LOQ were replaced with LOQ/2 and not detected with LOQ/6.

Median, 3rd quartile and 90th percentile of PCBs were also calculated as indicators of exposure, for the dietary exposure assessment. Analysis of two or more groups were performed using non-parametric Mann-Whitney and Kruskal Wallis, respectively. Multiple linear regression using log scale values of PCB congeners as well as for the sums of PCBs, namely ΣPCB-6 and ΣPCB-7 as dependent variable and area (closed vs. open seas), species (Seabass vs. Gilthead seabream), collection period (August, September, February–March) as explanatory variables were applied. Stacked bar charts of mean concentrations as % contribution of each PCB congener to ΣPCB-7 were used for the graphical representation of PCB levels. Statistical analysis was carried out using IBM SPSS Statistics 24.0 and a level of acceptance of null hypotheses was set at 0.05.

2.7. Exposure assessment

For the exposure assessment, we considered only the sum of the 6 NDL-PCBs (ΣPCB-6) since they share a common mode of action (MoA) and we regarded it as an assessment group. This corresponded to a whole-mixture approach and it is consistent with the current globally agreed practice for the NDL-PCBs (EFSA, 2012; WHO, 2016). We conducted the exposure assessment by calculating the estimated daily

Table 1
Analytical and validation parameters of the applied method.

			PCB 28	PCB 52	PCB 101	PCB 118	PCB 138	PCB 153	PCB 180
m/z			256, 186	292, 220	326, 256	326, 256	360, 290	360, 290	396, 324
Rt *(min)*			16.20	16.69	17.88	18.69	18.97	19.36	20.38
		C(ng/g)							
Inter-day precision (%RSD)	*LLOQ*	*0.125*	14.9	7.2	9.7	1.7	10.3	6.8	10.7
	QCL	*0.625*	3.2	5.9	4.2	6.9	1.6	0.9	0.6
	QCM	*1.25*	6.8	2.2	8.3	9.3	6.7	6.1	5.2
	QCH	*2.5*	1.4	1.3	1.8	1.7	0.6	0.3	1.7
		Mean (± SD)	6.6 ± 6.0	4.1 ± 2.8	6.0 ± 3.6	4.9 ± 3.8	4.8 ± 4.5	3.5 ± 3.4	4.5 ± 4.5
% Accuracy	*LLOQ*	*0.125*	90.0	86	116	116	116	112	112
	QCL	*0.625*	97.6	102.4	95.2	95.6	96.4	96	98.8
	QCM	*1.25*	98.2	101.0	102	100.4	97.8	99.4	96.2
	QCH	*2.5*	101.3	100.8	102.4	103	102.1	102.4	104.6
		Mean (± SD)	96.8 ± 4.8	97.5 ± 7.7	103.9 ± 8.7	103.7 ± 8.7	103.1 ± 8.9	102.4 ± 6.9	102.9 ± 7.0
% Recovery	*QCL*		99.4	96.3	89.2	82.6	84.9	84.1	87.4

*LLOQ: lower limit of quantification. QCL: quality control low level. QCM: quality control medium level. QCH: quality control high level.

intake (EDI) (ng contam/kg bw/day) of the ΣPCB-6 from the measured occurrence (contamination) in the studied fish and the daily fish consumption in Greece.

The EDI from fish consumption (EDI_f) was calculated as follows:

$$EDI_f = \frac{Cf \times Occ}{BW}$$

Where:

Cf is the daily fish consumption for the Greek population (g/person),
Occ is the ΣPCB-6 occurrence (contamination) in fish tissue expressed as the 95th percentile (ng contam/g fish) of ΣPCB6 determined in this study and
BW is the mean body weight for an adult consumer (70 kg).

The daily fish consumption value for the Greek population was considered from two consumption databases: FAOSTAT (http://www.fao.org/faostat/en/) and DAFNE-ANEMOS software (http://www.hhf-greece.gr/DafnesoftWebV2/). According to FAOSTAT Cf is 24,1 g/person/day (value for demersal fish), whereas DAFNE-ANEMOS reports 38 g/person/day (value for all fish).

2.8. Risk characterisation

For the risk characterisation, a newly developed approach was used. According to classic approach of HI (U.S. EPA, 2007) the ratio of aggregated exposure to the ADI (or the sum of EDI/ADI for mixtures in the component-based approach) should be less than the HI of one. However, in our study we aimed to evaluate the risk through fish consumption and not all food. For this purpose, we proceeded to the refinement of the HI based on an appropriate correction factor (F_C), determining the corrected fish specific hazard index HI_f. The Fc expresses the contribution of fish to the total ΣPCB-6 daily intake and it is equal with the ratio of the maximum permitted daily intake through fish consumption, $MPDI_f$ (fish consumption * permitted occurrence in fish) to the maximum permitted daily intake through the whole diet, $MPDI_A$ (SUM of MPDIi = SUM (food$_i$ consumption * permitted occurrence in the food$_i$),where *i* represents each food group considered for the dietary intake).

$$MPDI_A = \sum MPDIi$$

$$F_C = \frac{MPDIf}{MPDI_A}$$

For the calculation of PMDI*i* in ng/kg bw per day for each relevant food group (*i*) we used the current EU Maximum levels (European Commission, 2011) and the consumption data from the aforementioned databases. The food groups we considered were: cheese, eggs, demersal fish (FAOSTAT), pelagic fish(FAOSTAT), fish (DAFNE-ANEMOS), meat and products, milk and products and total added lipids since they represent the food sources responsible for almost the total intake of ΣPCB-6 (WHO, 2016).

Since for the moment there is no ADI value for the ΣPCB-6 officially set by the regulatory bodies, we used as the existed proposed guidance value (ADIg) of 10 ng/kw bw per day reported in the literature (Baars et al., 2001).

For considering no-risk it should be:

$$HIf > \frac{EDI_f}{ADI_g}$$

where $HI_f = Fc \; x \; HI$.

The results were also normalised to produce a different way of expression.

3. Results

3.1. Method validation

The developed method was validated in terms of linearity, accuracy, quantification limits, precision, recovery and carry over effect. The results are presented in Table 1. For quality assurance and quality control of the PCB quantification method, contamination was evaluated by blank controls and results were always below the detection limit. The accuracy was evaluated by analysis of spiked samples and recoveries for the analysed congeners ranged between 82.64 and 99.41%. The precision was calculated on replicate analysis giving an overall variability of 0.3–14.9%. LLOQ for individual PCBs is 0.125 (ng/g). Spiked curves were linear with $r^2 > 0.99$ (Table 1). The detector's response to the analyte retention time in a blank sample was less than the response in the LLOQ sample and the effect on the retention time of the internal standard was lower than 5% of IS mean. Thus, no carry-over effect was observed.

3.2. PCB levels in fish muscle tissue

PCB levels in fish muscle tissue were far below the maximum permissible limits set by the EU for both approaches used as it is presented in Table 2.

The % of detection for each PCB congener in farmed and wild fish samples is presented in Fig. 1 for gilthead seabream (a) and seabass (b). Although the two species present a similar pattern of detection for farmed samples, they exhibit distinct differences for wild fish samples. More specifically, PCB 52 and PCB 180 are detected in wild gilthead seabream in 60% and 40% of the samples respectively and are absent in all of the wild seabass samples. On the other hand, PCB 138 and PCB 153 were more often detected in wild fish for both species. PCB 138 was present in 100% of wild seabream and 90% of wild seabass samples, while PCB 153 in 90% of wild seabream and 80% of wild seabass samples.

For all farmed fish in total, PCB 138 was the predominant congener, followed by PCB 118 and PCB 153. PCB 138 was the most abundant for wild fish as well followed by PCB 153 and then PCB 118.

Regarding gilthead seabream, when considering imputed values, statistical analysis revealed significant differences for PCB 101 ($p < 0.001$), PCB153 ($p = 0.015$) and PCB180 ($p = 0.015$) between farmed and wild fish, with wild fish presenting higher mean values. Wild seabass showed significantly higher values for PCB 153 than farmed seabass ($p = 0.014$). Moreover, mean detected values of PCB congeners for farmed seabream decreased in the following order: 118 > 138 > 153 > 52 > 101 > 180 > 28 and for wild seabream in a different order: 138 > 153 > 118 > 101 > 52 > 180 > 28. Farmed seabass showed a similar pattern to farmed seabream: 118 > 138 > 153 > 52 > 101 > 28 > 180 and wild seabass one resembling more to wild seabream: 138 > 118 > 153 > 101 > 52 > 180 > 28.

Table 2
Mean ± SD, median and range (ng/g) of ΣPCB-6 and ΣPCB-7 for farmed and wild fish for imputed and not imputed values.

			Mean ± SD	Median	Range
Imputed	**ΣPCB-6**	Farmed	1.96 ± 1.55	1.68	0.11–7.17
		Wild	2.43 ± 1.92	1.82	0.16–6.85
	ΣPCB-7	Farmed	4.68 ± 6.94	2.97	0.18–57.7
		Wild	3.14 ± 2.29	2.84	0.41–7.93
Not imputed	**ΣPCB-6**	Farmed	1.95 ± 1.54	1.68	0.13–7.09
		Wild	2.47 ± 1.92	1.70	0.21–6.83
	ΣPCB-7	Farmed	4.73 ± 7.02	2.96	0.13–57.6
		Wild	3.04 ± 2.33	2.74	0.21–7.92

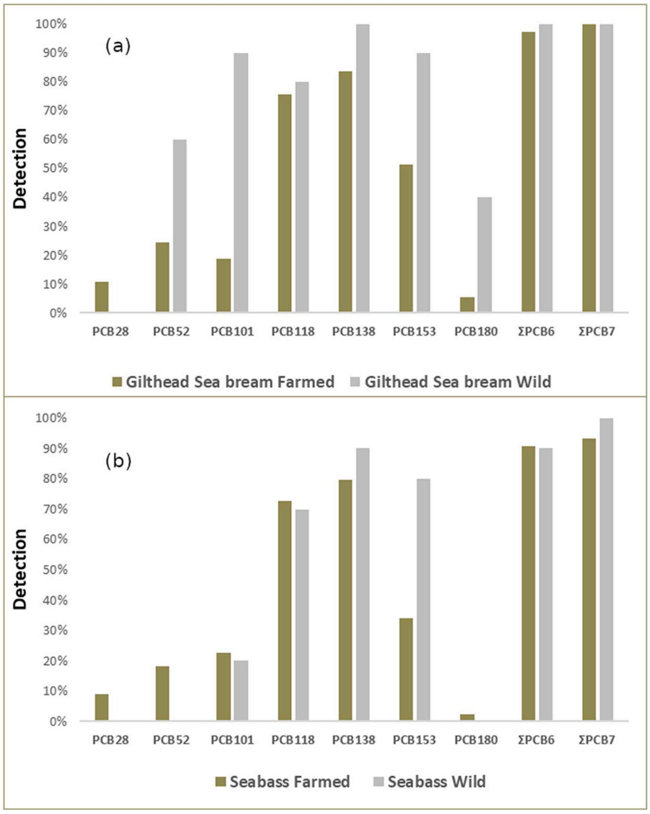

Fig. 1. % Detection of each PCB congener for gilthead seabream (a) and seabass (b).

3.3. Distribution of PCB levels in fish muscle tissue depending on seasonality

PCBs distribution was studied in relation to the sampling season for farmed gilthead seabream and seabass and the results are presented in Fig. 2. In all cases PCB 118 seams to contribute the most to the ΣPCB-7 and the distribution of congeners seams to differentiate according to the sampling season. Further statistical analysis revealed that for gilthead seabream median PCB 28 is significantly higher during the summer season: 0.06 ng/g (August) ($p = 0.036$) compared to the other periods. PCB 138 is significantly higher in November with a median value of 2.14 ng/g ($p = 0.003$) while median PCB 153 in late winter-early spring: 0.65 ng/g ($p = 0.002$). For seabass samples, PCB52, PCB 101 and PCB153 showed significantly higher median values in late winter-early spring: 0.06 ng/g ($p = 0.016$), 0.33 ng/g ($p < 0.001$) and 0.44 ng/g ($p < 0.001$), respectively (see Fig. 2).

3.4. Distribution of PCB levels in fish muscle tissue depending on collection site

PCB distribution was additionally studied in relation to the collection site for farmed gilthead seabream and seabass and more specifically for fish collected from open seas (NE Aegean and Dodecanese) compared to fish obtained from closed seas (Crete, Mainland and Saronic Gulf). There is a clear variation between farmed seabass collected from open seas and seabass collected from closed seas and more specifically median PCB 118 is significantly higher in fish obtained from open seas ($p = 0.023$), whereas PCB28 and PCB52 present significantly higher medians in fish collected from closed seas ($p = 0.021$ and $p = 0.004$ respectively) (see Fig. 3).

3.5. Analysis of main effects of PCB levels in fish tissues

Multiple linear regression models using log-scaled PCB levels as dependent variables were applied using species, origin (farmed or wild) seasonality (from "warmer" periods to "colder" periods) and farming areas (closed/open seas). Species appears to be the main factor affecting both ΣPCB-6 and ΣPCB-7 levels ($p = 0.037$ and $p = 0.001$ respectively) as can be seen in Table 3. With respectto factors affecting specific PCB congeners, statistical significance was demonstrated for PCB 153, PCB 28 and PCB 101. Specifically, for PCB 153 and PCB 101 primary factor proven to affect their levels was sampling season ($p < 0.001$), while for PCB 28 farming site and sampling season as well ($p < 0.05$).

3.6. Exposure assessment

Exposure assessment, through the EDI calculation, was based on the occurrence (contamination) of NDL-PCB in the Greek fish as determined in the current study, and fish consumption data in Greece from the FAOSTAT and DAFNE-ANEMOS databases (Table 4). The 95th percentile of occurrence in our samples, determined in fish tissue, was 6.24 ng PCB/g fish. Exposure assessment in the 95th percentile of occurrence revealed that the NDL-PCBs intake values for the Greek population through the consumption of demersal fish (EDI_f) range

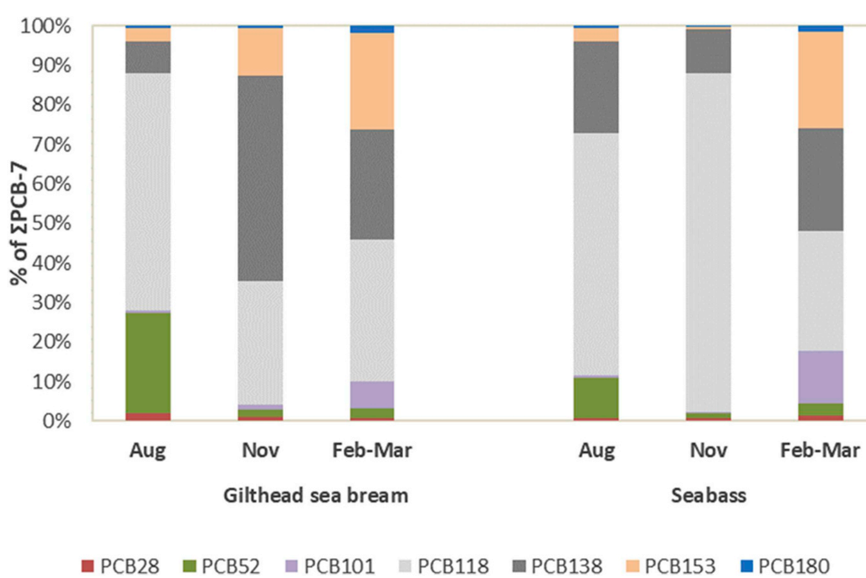

Fig. 2. % Contribution of each PCB congener to ΣPCB-7 in relation to sampling season for farmed gilthead seabream and farmed seabass.

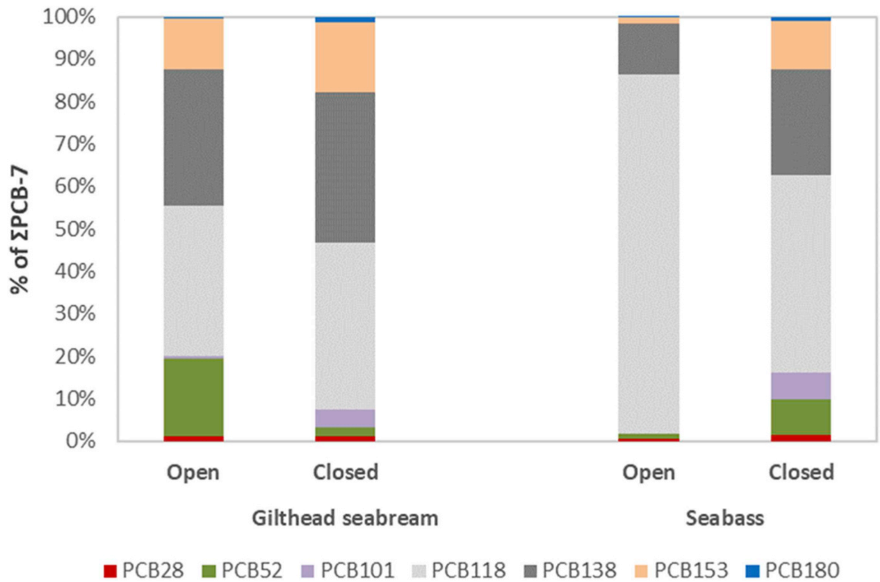

Fig. 3. % Contribution of each PCB congener to ΣPCB-7 in relation to sampling site (open or closed seas) for farmed gilthead seabream and farmed seabass.

Table 3
Adjusted Beta coefficient and 95 CIs using ΣPCB-6 and ΣPCB-7 as dependant variables.

		B	95%LB	95%UB	p	R^2
Log (ΣPCB-7)	Species	−0.18	−0.35	−0.01	**0.037**	0.09
	Farming site	−0.19	−0.41	0.03	0.097	
	Origin	−0.17	−0.48	0.14	0.278	
	Sampling Season	−0.06	−0.19	0.07	0.335	
Log (ΣPCB-6)	Species	−0.251	−0.402	−0.100	**0.001**	0.124
	Farming site	−0.081	−0.282	0.119	0.422	
	Origin	0.051	−0.225	0.328	0.714	
	Sampling Season	−0.023	−0.140	0.094	0.700	

Table 4
Consumption data for the NDL-PCBs related food and their respective Maximum Permitted Daily Intakes.

	CONSUMPTION (g food/person/day)		MPDI (ng contam/g bw/d)	
DATABASE	FAOSTAT	DAFNE-ANEMOS	FAOSTAT	DAFNE-ANEMOS
Food group				
Cheese	92.0	63	9.65	6.61
Eggs	24.8	17	1.57	1.08
Demersal Fish	**24.1**	**-**	**25.8***	
Pelagic fish	9.00	-	9.36	
Fish	**-**	**38**		**40.7***
Meat and products	371	164	7.42	3.28
Milk	219	162	5.01	3.70
Milk Products	-	37	-	1.40
Total Added lipids	2.42	0.88	1.38	0.50
SUM	**742**	**468**	**60.2****	**57.29****
% contribution of fish	3%	8%		

* MPDEf. ** $MPDE_A$.

between 2.15 and 3.38 ng/kg bw/day.

Our estimations regarding fish consumption for the Greek population indicate that demersal fish, such as gilthead seabream and seabass, contribute to about 3–8% to the whole diet, while the exposure to NDL-PCB through fish consumption climbs to 43-72% of the total dietary intake, when considering as occurrence in these fish and the various dietary products (contamination) the maximum levels set by EFSA (2012).

3.7. Risk characterisation

According to the used approach the EDI for fish, the $MPDE_A$, MPDEf, Fc and HIf were calculated (Table 5). The ratio EDIf to ADI with data consumption from FAOSTAT and DAFNE-ANEMOS (0.22 and 0.34 respectively) are well below the respective HIf (0.43 and 0.72 respectively) indicating no-risk for the Greek population after exposure to NDL-PCB through fish consumption. Normalizing our results to HIf equal to 1 we ended up to the normalised EDIf/ADI ratios being 0.50 for FAOSTAT and 0.48 for DAFNE-ANEMOS (50% and 48% risk respectively).

4. Discussion

4.1. PCB levels in fish muscle tissue of farmed and wild fish

With regard to PCB congener distribution in fish, results obtained from this study, demonstrate that for farmed fish in total, PCB 138 was the predominant congener followed by PCB 118 and PCB 153, whereas PCB 138 was the most abundant in wild fish as well followed by PCB 153 and then PCB 118. This is in accordance with results reported in the relevant literature where PCB 138 and PCB 153 are described as the most common and at higher levels detected congeners(Antunes and Gil, 2004; Baptista et al., 2013) In several cases, PCB 153 has been reported

Table 5
Hazard characterisation parameters.

	FAOSTAT	DAFNE-ANEMOS
EDI_f (ng/kg bw/day)	2.15	3.38
ADI (ng/kg bw/day)	10.0	10.0
EDIf/ADI	**0.22**	**0.34**
MPDEf (ng/kg bw/day)	25.8	40.7
$MPDE_A$ (ng/kg bw/day)	60.2	57.3
F_C = HIf	**0.43**	**0.72**
Risk %	**50%**	**48%**

$MPDE_A$: Aggregated Maximum Permitted exposure, MPDEf: Maximum Permitted Exposure from fish, HIf: Hazard Index for fish.

as the predominant congener, followed by PCB 138 (Giandomenico et al., 2016; Trocino et al., 2009; Vuković et al., 2018). Nevertheless, both congeners (PCB 138 and PCB 153) appear to be the most frequently detected due to their high persistence and stability which are attributed to their chemical structure as di-ortho substituted and highly chlorinated congeners. Additionally, these congeners are amongst the major components of commercial PCB mixtures (Rodríguez-Hernández et al., 2017). Their resistance to degradation and lipophilicity leads to high levels of accumulation in fish tissue.

In our study, although the two species present a similar pattern of detection for farmed samples, they exhibit distinct differences for wild fish samples. Besides the fact that wild fish present higher mean values for the sum of NDL-PCBs (ΣPCB-6) (2.43 ng/g) than farmed (1.96 ng/g), differences have been depicted in congener distribution as well. More specifically, for gilthead seabream PCB 153, 101 and 180 differed significantly between farmed and wild samples, while for seabass a difference was disclosed for PCB 153. On the other hand, for the sum of all PCBs analysed (ΣPCB-7) values were higher for farmed fish, suggesting that PCB 118 presence is higher in farmed fish (2.73 ng/g) compared to wild ones (0.71 ng/g). Similar differences between farmed and wild fish have been illustrated by several authors. Serrano et al.(2008a, b) reported higher PCB levels in wild gilthead seabream in relation to its farmed counterpart and such variations were published in other studies as well (Ferreira et al., 2010). Conversely, seabass collected from natural environment presented lower PCB levels than cultivated seabass according to Antunes and Gil (2004). Several other authors determined higher values in farmed fish (Carubelli et al., 2007; Henríquez-Hernández et al., 2017; Lundebye et al., 2017) and it has generally evolved into a debate on account of conflicting results. Most authors ascribe differences in PCB levels between farmed and wild fish to variations in lipid content, suggesting that higher lipid content in farmed fish justifies for higher PCB levels (Antunes and Gil, 2004; Trocino et al., 2009). Moreover, many authors associate feed composition administered to farmed fish with differences in PCB levels and PCB distribution between farmed and wild fish (Carubelli et al., 2007; Serrano et al., 2008b; Çakiroğullari et al., 2010; Cirillo et al., 2009; Henríquez-Hernández et al., 2017; Ginés et al., 2018). It has also been suggested that variations can be attributed to biological effects as fish growth and metabolic activities of fish, especially for various stages of fish farming (Ferreira et al., 2008). However, we tried to eliminate this factor in our study by selecting fish samples of commercial size in all cases. Variations between farmed and wild fish in our study could be explained by diversified types of diet exposure due to aquafeeds.

Our results on PCB levels determined in fish muscle tissue, for both species and mode of production were far below the maximum permissible limits set by the European Commission (2011). This is in accordance with levels reported for these species in the Mediterranean, as it is presented in Table 6.

4.2. Factors affecting PCB levels and congener distribution in fish tissues

Our results highlight seasonality as an important factor affecting

Table 6

Sum of concentrations of indicator PCBs concentrations (ΣPCB-6, ΣPCB-7) in the muscle tissues (expressed as means and range ng/g ww) of gilthead seabream and seabass in the Mediterranean Sea, reported in the recent literature.

Species	Season	ΣPCB-6	ΣPCB-7	Origin	REGION	REFERENCE
Gilthead seabream		8.02		farmed	Greece	Costopoulou et al. (2016)[b]
	May	22.1		farmed (1)	Turkey	Çakiroğullari et al. (2010)
	September	3.94		farmed (2)		
	March	14.9		farmed (4)		
	winter		3.7	farmed red muscle	Spain	Serrano et al., 2008a
	automn		34	farmed red muscle		
	automn		2.4	farmed white muscle	Spain	Serrano et al., 2008a
	winter		< LoQ	farmed white muscle	Spain	Serrano et al., 2008a
	automn		2.2	farmed white muscle		
	summer	**1.71**	**4.3**	farmed	Greece	This study
	autumn	**2.54**	**3.71**	farmed		
	winter	**2.31**	**3.6**	farmed		
	winter	**3.35**	**3.14**	wild		
	winter		11	wild red muscle	Spain	Serrano et al., 2008a
	winter		< LOQ	wild white muscle		
	automn		23	wild red muscle		
	automn		< LOQ	wild white muscle		
	automn		0.15	wild white muscle		
Seabass		5.24		farmed	Greece	Costopoulou et al. (2016)[b]
	October to January		2.2	farmed (extensive)	Italy	Trocino et al. (2009)
	May	8.01		farmed (1)	Turkey	Çakiroğullari et al. (2010)
	September	8.29		farmed (2)		
	October	3.05		farmed (3)		
	March	9		farmed (4)		
		7.02 ± 2.79		farmed	Italy	Carubelli et al. (2007)
	October to January		12.4	farmed (intensive concrete tanks)	Italy	Trocino et al. (2009)
	October to January		8.8	farmed (Sea-cages)		
	October to January	10.6		farmed (Semi-intensive ponds)		
		3.69 ± 2.38		farmed (1)	Italy	Paiano et al. (2013)
		10.73 ± 8.27		farmed (2)		
	summer	**1.67**	**4.3**	farmed	Greece	This study
	automn	**1.36**	**9.5**	farmed		
	winter	**1.94**	**2.78**	farmed		
	winter	**1.31**	**1.96**	wild		
	spring and autumn		192 ± 159	wild	France	Bodin et al. (2014)[c]
	automn		242 ± 169	wild		
	spring	122 ± 136		wild		
	June-Jyuly	3.85 ± 2.35		wild	Italy	Carubelli et al. (2007)

[a] mean, [b] upperbound,[c] dry weight.

PCB accumulation and distribution in fish muscle tissue. ΣPCB-6 for farmed seabream is higher in autumn, while for farmed seabass higher mean ΣPCB-6 value is recorded in winter-early spring (Table 5). In both cases, seasons presenting the highest values coincide with the beginning of the reproduction period of each species. This association could be explained by higher lipid content before reproduction season, due to increased food intake, as it is discussed by other authors as well (Serrano et al., 2008b; Blanes et al., 2009). Seasonal alterations in PCBs levels on that context are also species dependent, as PCB accumulation is dictated by each species ecology and biological cycle (Baptista et al., 2013; Henríquez-Hernández et al., 2017). Moreover, different levels between these species were presented by a recent study in Greece, where higher values were observed in gilthead seabream in comparison to seabass, a fact which was attributed to gilthead seabream's higher lipid content(Costopoulou et al., 2016). In our study, the species effect was further underlined by the multiple linear regression models, which revealed that both ΣPCB-6 and ΣPCB-7 levels are primarily affected by the fish species.

Moreover, our results regarding ΣPCB-7 reveal a differentiated distribution of PCB congeners in relation to sampling season for each species, which is significant for PCB 28, 138 and 153 for seabream, counter to PCB 52, 101 and 153 for seabass. The predominant congener detected in our study (PCB 138) follows the concentration fluctuation dictated by the reproduction hypothesis, whereas PCB 153, one the most persistent congeners is significantly higher for both species in winter-early spring. This is hard to interpret, yet a possible explanation could be the influx of contaminant loads into the marine environment in early spring, due to the water cycle. Nevertheless, regression analysis revealed the sampling season to be the main factor affecting PCB 153 levels, in accordance with results reported by Serrano et al., 2008a.

As regards to the collection site effect, interestingly, PCB 118 presented significantly higher values in fish collected from open seas, while PCB 28 and PCB 52 present significantly higher values in fish collected from closed seas. Furthermore, PCB 28 appeared to be mainly influenced by farming site, besides sampling season, in the regression analysis models carried out in our study. PCB congener distribution and levels are area dependent according to other authors as well, who report that location of fish growth and collection is of great importance to PCBs accumulated in fish tissues (Baptista et al., 2013; Paiano et al., 2013; Henríquez-Hernández et al., 2017). Additionally, PCB 28, PCB 118, PCB 52 and 101 are considered as indicators of recent contamination (Arnich et al., 2009; Giandomenico et al., 2016). Therefore, distinctions in PCB congeners between open and closed seas could be attributed to different types and sources of contamination.

4.3. Exposure assessment

Exposure assessment for the Greek population showed that NDL-PCB intake through fish consumption (2.15–3.38 ng/kg bw/day) is comparable to other European countries, reaching about 50% of the total dietary intake (EFSA, 2012; WHO, 2016). A WHO report recently published (WHO, 2016) reviewing the relevant literature, describes a range of whole dietary exposure between 8 and 45 ng/kg bw/day.

In relation to the aggregated dietary exposure to NDL-PCBs for the general population or subgroups a number of studies have been published in the literature (Arnich et al., 2009; Cirillo et al., 2009; Cimenci et al., 2013; Mihats et al., 2015; Perelló et al., 2015; Costopoulou et al., 2016; Giandomenico et al., 2016; Rodríguez-Hernández et al., 2016).

As previously mentioned the fish consumption data obtained from the two different databases led to slightly divergent exposure results (2.15 and 3.38 ng/kw bw/day), underlying the importance of dietary habits to exposure. Indicative to the importance of consumption data use, is additionally the fact that although the contamination levels in fish determined in our study represents approximately the 8% of the maximum established NDL-PCB maximum permitted limits, and thus they may considered safe, this was translated in approximately 50% of the fish contribution in the overall intake from the NDL-PCBs in the Greek population. Moreover, a recent biomonitoring study in Greece, assessing PCB occurrence and levels in the hair of two Greek population groups (residents of different agricultural regions) revealed significant differences in PCB levels between the two groups, as well as differences in congener distribution which could be a reflection of divergent dietary habits among other reasons (Barbounis et al., 2012). Similar differences regarding PCB prevalence and congener distribution and were found between hair of children from rural and urban regions (Tzatzarakis et al., 2014).

Comparison with other studies on exposure assessment and risk characterisation becomes complicated for reasons which include the different approaches used. However, based on our results NDL-PCB intake for the Greek population through consumption of fish is estimated at 150-237 ng/person/day, higher the intake of the Spanish population (87 ng/person/day) recently reported (Rodríguez-Hernández et al., 2016) for the same group of fish.

4.4. Risk characterisation

Risk characterisation for NDL-PCB is challenging on account of the fact that there are no established values for acceptable daily intake due to lack of determined NOAELs. Although, recent studies on PCB effects on hepatotoxicity through oxidative stress induction, support the use of benchmark dose (BMD) concept in the prediction of health risks associated with PCBs exposure, instead of the NOAEL approach (Buha et al., 2015). Moreover, a comparative approach using the minimum effect doses from available studies was developed in order to estimate margin of exposure MOEs and to provide guidance on human health risk (WHO, 2016), however, this approach has not been conclusive or finalized.

In our study we estimated the NDL-PCBs intake of the Greek population from fish consumption only, aiming to assess the weighted risk arising from fish consumption. Up to date, the classical HI approach (HI = 1) has been used extensively in risk assessment studies dealing with exposure from single food items (Tsakiris et al., 2013, 2015; Renieri et al., 2014, 2019). With a view to refine current risk assessment methods, we chose instead of using the classical HI to use a newly developed food specific HI approach. Based on this approach fish contribution to the maximum permitted aggregated dietary exposure was considered, arriving to a lower value for the HI.

It must be noted that however realistic we aspire to be in risk assessment, true risk is difficult to identify for numerous reasons and existing methods assessing the risk from PCB intake contain a rather ample amount of uncertainty. Besides the lack of ADI values, dietary exposure falls in the context of long term-low dose exposure, whose adverse effects have not been yet characterized. Moreover, human behaviour including dietary habits varies greatly based on various criteria such as locality and socioeconomic status. Furthermore when considering specific single chemicals or assessment groups, such as NDL-PCBs in our case, we can determine just a part of the overall risk and cannot provide an integrated assessment of the multiple risks triggered by exposure to different toxic stimuli (Hernández et al., 2013; Tsatsakis et al., 2016; Hernández et al., 2017; Tsatsakis et al., 2017, 2018; Docea et al., 2018; Kostoff et al., 2018).

Finally, we have to accept certain limitations to our study such as the fact that we considered only adult consumers, not taking into account children or sensitive groups due to lack of available data. Additionally, we estimated the NDL-PCB contamination in two fish species. Regardless, these are the most frequently consumed fish by the Greek population and the ones more likely to contribute to the PCB intake. Although we applied a new risk characterisation approach, further refinement is possible, with more targeted and updated consumption data which are currently lacking and contamination data in more fish species as well. Even though we covered an appreciable range of areas of the Greek seas that we collected fish from, more sites could be represented in the future. Our study provides data on the contamination of frequently consumed fish in Greece and further elucidates the risks involved in fish consumption, regarding the NDL-PCB intake which could contribute to the efforts of researchers, health advisories and regulative authorities to balancing health benefits and risks of fish consumption.

5. Conclusions

The levels of the sum of indicator PCBs and congener distribution determined in fish muscle tissue were found to vary depending on fish species, seasonality, sampling area and mode of production. In most cases differences were attributed to variations in lipid content, fish biology and PCB occurrence in aquafeeds. Results obtained from this study show that PCB levels in seabream and seabass were well below the established maximum permitted limits in fish tissue and risk assessment for the NDL-PCBs revealed no risk for Greek consumers. It is important that in the future we work with a view of improving the dissemination of consumption and contamination data in order to build readily accessible databases which could assist the advancement of more integrated risk assessment methods for aggregated and cumulative exposures.

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