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Influence of sex and age on PCBs accumulation in the commercial fish C*helon labrosus*

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Abstract

Thicklip grey mullet, *Chelon labrosus*, is an important commercial fish species and has been studied worldwide. However, no recent studies have been made regarding polychlorinated biphenyls (PCBs) in wild *C. labrosus*. Due to that, the concentration of 13 PCBs congeners were measured in muscles and livers, of males and females, of *C. labrosus* of different ages, allowing the estimation of PCB bioaccumulation throughout the species lifespan, in the Mondego estuary, a southern European temperate estuary. Male muscle sample concentrations ranged from 32 to 96 ng g^{-1} (lipid wt.) and in females from 32 to 62 ng g^{-1} (lipid wt.). In male liver samples concentration ranged from 106 to 158 ng g^{-1} (lipid wt.), while female concentrations ranged from 88 to 129 ng g^{-1} (lipid wt.). The most abundant congeners presenting higher percentages in all samples were CB 138, 153 and 180. No significant differences were found between the concentrations in both sexes, but muscle and liver PCB concentrations in males tended to increase with age whereas in females concentrations remained stable throughout the species lifespan. Significant differences were found between concentrations in muscle and liver.

1. Introduction

Polychlorinated biphenyls (PCBs) are environmental pollutants that tend to persist in the environment for long periods of time. Due to their lipophilic character they tend to accumulate in fishes (Howell et al., 2008; Nfon and Cousis, 2006). Fishes can accumulate PCBs from the surrounding environment (bioconcentration) (Antunes and Gil, 2004) or by prey intake (biomagnification) (Mackay and Fraser, 2000). The differences in the PCB congeners' patterns can demonstrate their distribution in the aquatic environment. High chlorinated congeners are the most persistent ones and have higher accumulative properties (Bazzanti et al., 1997; Bodin et al., 2008).

The Mondego estuary has been much studied for many years, especially its estuarine fish (Martinho et al., 2007), benthic (Cardoso et al., 2010) and plankton community (Primo et al., 2011). In the case of contaminants, some studies have been made on mercury (Tavares et al., 2011), and only a few studies have been made on PCBs in fish and sediments (Nunes et al., 2011; Pereira et al., 2005). The main contamination sources of the Mondego estuary are wastewaters (mostly resulting from high population density and hence domestic sewage), industrial activities (industrial sewage), agriculture runoff (which requires great amounts of fertilizers and pesticides) and Figueira da Foz harbour (responsible for industrial pressure in the estuarine area) (Nunes et al., 2011).

Thicklip grey mullet (*Chelon labrosus*) is a marine-estuarine opportunist species, since it enters the estuaries mainly as juveniles, and as an alternative habitat to other coastal areas (Baptista et al., 2010). This species is omnivorous (feeds mainly on benthic diatoms, epiphytic algae, small invertebrates and detritus) (Zouiten et al., 2008). More recently, the research in *C. labrosus* has increased due to its economic relevance and to its potential to be cultured in fish farms (Boglione et al., 2006; Khemis et al., 2006; Zouiten et al., 2008). Moreover *C. labrosus* usually supports artisanal fisheries (Cardona et al., 2008). Since they are consumers of low trophic level species, they can be used in an economic and efficient way in extensive culture (Khemis et al., 2006). PCBs studies on this species are scarce, and only laboratory studies have been made (Narbonne, 1979). So far, only the accumulation of a mixture of PCBs was considered and no field studies regarding bioaccumulation of specific congeners throughout the species lifespan have been developed. For that reason, the aim of this work was to study bioaccumulation of PCBs according to age and sex in *C. labrosus*, in two different tissues (muscle and liver). Furthermore, it was expected to find higher concentration in liver than in muscle, differences in male and female bioaccumulation, and concentrations below the European Union tolerance limit for human intake. Accordingly, several hypothesis were raised: A) Do these contaminants bioaccumulate during *C. labrosus* life cycle?; B) Are there any differences between sexes concerning bioaccumulation?; C) Are there any differences between the sexes in the effects of PCB congeners bioaccumulation?; D) Is this species safe for human intake in estuaries with low contamination?

2. Material and Methods

2.1. Study site and laboratory work

The Mondego estuary is a small estuary (area 8.6 km^2), located in the western coast of Portugal (40º08' N, 8º50' W) (Fig.1). The estuary comprises two arms, the north and the south, separated at a distance of 7 km from the coast and joining again near the mouth of the estuary. The two arms present different hydrologic characteristics. The north arm is deeper with 5-10 m depth at high tide and 2-3 m tidal range, and it is frequently dredged to maintain its depth for harbour activities. The south arm is shallower with 2-4 m depth at high tide and 1-3 m tidal range; 75% of the area comprises intertidal mudflats. The south arm is largely silted up in the upstream areas causing the freshwater to flow mainly through the north arm, while the water circulation in the south arm mainly depends on the tides and on a small amount of freshwater input from a tributary, the Pranto River, which is controlled by a sluice, according to the water needs of the rice fields from the Mondego agricultural valley.

Due to the fishes' life-cycle, juveniles are preferentially located in the inner areas of the estuarine system, and adults are located close to the mouth of the estuary. Fishes were collected from September 2007 to April 2008, in the south arm (mainly juveniles, age 2+) and at the Mouth of the Mondego estuary (adults, age ≥3+) (Fig.1). Three individuals from each age group and sex were analysed for PCBs, giving a total of 35 individuals analysed. Fishing took place during low tide, using a traditional beach-seine net, to catch the younger fishes, and a trammel net to catch the older fishes. All fishes were weighed (g) and measured (cm) before collecting samples of muscles and livers. Sex was determined by examination of the gonads. Fishes were aged using scales analysis. All individuals <3+ were considered immature (Table 1). For both sexes, ages were estimated at between 3+ and 8+. Muscle samples from immature fish (2+) were also included in the analysis. From each age and sex, three individuals were stored and analysed for PCBs individually. Due to the lack of mass for the PCBs analysis, female livers for fish age 5+ and 6+

were not determined. After laboratory processing, fish samples were freeze-dried, homogenised and stored in the freezer (-20ºC), until analyzed.
Sediments (0-5 cm depth) are composed mainly of mud and silt. They were collected in August of 2008, in the south arm and at the mouth of the estuary. After sampling, sediments were taken into the laboratory, where they were freeze-dried, homogenised and stored at -20ºC until analyzed. For comparison with the European Union limits, *C. labrosus* wet weight concentration was calculated using the formula: $\sum_6$ PCB concentration (dry wt.)]*(100 – water (%)).

2.2. PCBs analysis

The procedures for extraction and cleanup of fish tissues were adapted from United States Environmental Protection Agency methods. A detailed description can be found as Electronic Supplementary Material. Briefly, 3 grams of muscle and 1g of liver of each individual were extracted by sonication with *n*-hexane:acetone (1:1) followed by a cleanup with sulphuric acid and solid phase extraction with florisil. Liver extracts were further cleaned with acid silica gel. Sediment (10 g) samples were Soxhlet-extracted, submitted to an alumina cleanup as described by Cachada et al. (2009), and an additional cleanup was performed with acid silica gel (as performed for liver samples). Instrumental analyses of the extracts were performed by GC-MS with selected ion monitoring (details can be found in Supplementary Material). Thirteen congeners (IUPAC nos. 18, 28, 31, 44, 52, 101, 118, 138, 149, 153, 170, 180 and 194) according to EN 12766/CEN and EN 61619 were analysed in muscle and liver tissues and in sediment samples. Total PCB ($\sum_{13}$PCB) content (ng g^{-1}, lipid wt.) was based on the sum of the concentrations of the detected congeners.
For quality assurance and quality control of PCB quantification methods, contamination was evaluated by blank controls and the recovery checked by analysis of spiked samples. The levels for blanks were below the detection limit and for the analyzed congeners mean recoveries in spiked samples ranged between 71-106%. Reproducibility was calculated on replicate analysis giving an overall error of 4-20%.

2.4. Statistical analysis

A linear regression (SigmaPlot 11.0 software) analysis was performed to determine the trends in concentrations in males and females PCBs concentration for $\sum_{13PCB}$. To evaluate differences within males and females, for both muscle and liver, a one-way ANOVA was performed. No transformation was applied to the data, since all values had a normal distribution. A 5% significance level was used for all the analyses.

3. Results and Discussion

Sediment samples from the Mouth station (M station) presented higher $\sum_{13}$ PCB concentration, with 2.0 ng g^{-1} (dw), than the south arm, with 0.28 ng g^{-1} (dw). Moreover, at M station nine congeners were detected, whereas in the south arm only two congeners were detected (Fig. 2A). This pattern results from the higher urban pressure at this station, including the proximity of a tourist boat marina only 100m away. Studies performed in the Mondego estuary by Pereira et al. (2005) and Vale et al. (2002) are in accordance with the present results. Sediment concentration from the Mondego estuary is low when compared with others estuaries, eg. Sado Estuary (Portugal), with 87 to 1100 ng g^{-1}, dw (Costa et al., 2008), or Er-Jen Estuary (Taiwan), with 5 to 65 ng g^{-1}, dw (Fu and Wu, 2006).
In the Mondego estuary *C. labrosus* size varied between 19 and 53 cm (Table 1). Male muscle $\sum_{13}$ PCB concentrations varied between 32–96 ng g^{-1} (lipid wt.), while in females muscle concentrations varied between 32–62 ng g^{-1} (lipid wt.) (Fig. 2B). According to the one-way ANOVA, male and female muscle concentration did not present significant differences (p=0.268). Nevertheless, male muscle increased its concentration with age, and significant differences were found across the species lifespan (r^2=0.634; p=0.032). On the other hand, female muscle concentration remained constant (r^2=0.094; p=0.617). Differences in female and male accumulation can be explained by differences in their gross growth efficiency. If one sex requires more energy (food intake) than the other sex to attain the same size, then the sex requiring more energy would have higher PCB concentrations (Madenjian, 2011). Another explanation, and the most common, is that females can lose a portion of their PCB body burdens by releasing their eggs. Depuration systems can be observed in other species, such as in largemouth bass (*Micropterus salmoides*) (Rypel et al. 2007), hake (*Merluccius merluccius*) (Bodiguel et al., 2009) or sea bass (*Dicentrarchus labrax*) (Loizeau et al., 2001).
Regarding liver samples, male $\sum_{13}$ PCB concentration varied between 106–158 ng g^{-1} (lipid wt.), while female $\sum_{13}$ PCB concentration varied between 88–129 ng g^{-1} (lipid wt.) (Fig. 2C). According to the one-way ANOVA, no differences were found between both sexes (p=0.707). Both male and female concentration remained constant throughout the species lifespan (r^2=0.646, p=0.054; r^2=0.300, p=0.452, respectively), although males' concentration had a tendency to increase with age (Fig 2C).

Muscle and liver concentrations presented significant differences (p=0.011, for males; p=0.021 for females). Livers presented higher concentrations since the liver is the main organ for PCB storage and metabolism (Bodiguel et al., 2009; Fernandes et al., 2008). Also this organ presented higher lipid content than the muscle (Table 1). In males, concentrations in muscle lipids varied between 2–17%, while in livers they varied between 21–35% (Table 1). In females, muscle lipid concentrations varied between 2–10%, while those in livers varied between 17–31% (Table 1).

For all samples the most abundant congeners were CB 138, 153 and 180 (Table 1). High chlorinated congeners are the most prevalent and significant in environmental samples (Bazzanti et al., 1997; Fernandes et al., 2008). Similar results were found in sea bass (Antunes and Gil, 2004), sardines (Antunes et al., 2007; Coelhan et al., 2006) and hake (Bodiguel et al., 2009).

Human exposure occurs mostly through contaminated food. *C. labrosus* is a suitable species for aquaculture, and therefore may be part of the human diet (Boglione et al., 2006; Zouiten et al., 2008). Recently the European Union has recommended a tolerance limit of 75 ng g^{-1} (wet wt.) for fish muscle and fishery products, and 200 ng g^{-1} (wet wt.) for fish liver, for the sum of the 6 ecological indicators ($\sum_6$ PCB 28, 52, 101, 138, 153 and 180) (Commission Regulation (EU) No 1259/2011). In the Mondego estuary, *C. labrosus* muscle and liver $\sum_{6PCB}$ concentration are far below the respective tolerance limit. Male muscle concentration varied between 1.7–3.3 ng g^{-1} (wet wt.) and female muscle was around 1.1–2.4 ng g^{-1} (wet wt.). Male liver values varied between 7.2–13.4 ng g^{-1} (wet wt.), while values in female livers varied between 5.8–9.0 ng g^{-1}(wet wt.). Therefore, the consumption of *C. labrosus* from the Mondego estuary can be considered safe for humans, with respect to PCBs. The Mondego estuary is a lightly contaminated system, with few contamination sources.

Contrary to expectations, no statistical differences were found between PCBs concentration of each sex for each tissue. Nevertheless, the PCB concentrations of male *C. labrosus* increased with age, whereas female concentrations tended to remain stable. Despite the fact that male livers did not present significant differences throughout the species lifespan, PCB concentrations tended to increase with age. As expected, livers presented higher concentrations than muscles due to their high lipid content.

High chlorinated congeners (CB 138, 153 and 180) presented higher concentration than other PCBs.

The Mondego estuary can be a useful site for the study and calibration of PCBs uptake. Moreover, *C. labrosus* concentration is below the tolerance limit, therefore safe for human intake.

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Figure 1. The Mondego estuary and sampling stations location

Figure 2. PCB congeners concentration (ng g^{-1}, dw) in sediments (A); and mean $\sum_{13}$PCB (ng g^{-1}, lipid wt.) concentration according to age and sex in muscle (B) and liver (C). Error bars represent the standard error.

Table 1. *C. labrosus* congeners (%), $\sum_{13}$ PCB concentration (ng g^{-1}, lipid wt.), lipid content (%) and mean size (cm) (deviation pattern between brackets), for male and female, muscle and liver according to age.

Age		18	28	31	44	52	101	118	138	149	153	170	180	194	$\sum_{13PCB}$ (ng g^{-1}, lipid wt.)	Lipid (%)	Size (cm)
									Muscle								
2+	**Male**								40.3	29.2	30.5				32.3	3.25	19.30
	Female								40.3	29.2	30.5				32.3	3.25	
3+	**Male**					4.5	5.1	7.7	20.6	9.4	28.5	11.9	12.3		55.2	4.15	29.68 (±1.47)
	Female						6.4	8.0	31.4	13.3	29.1	3.7	8.1		51.1	7.83	
4+	**Male**	3.0	4.5	3.8	2.7	7.3	6.8	7.4	18.4	7.6	21.7	8.7	8.1		88.8	2.44	34.52 (±1.98)
	Female	1.2			1.6	5.7	18.4	4.2	18.3	10.4	21.9		14.6	3.7	62.0	2.49	
5+	**Male**			1.2	1.4	3.6	8.2	6.7	22.8	10.4	26.8	8.3	10.6		86.7	4.28	38.92 (±1.35)
	Female																
6+	**Male**	3.6	1.6	1.8	0.8	2.4	5.3	1.8	25.2	10.4	28.4	4.4	13.7	0.6	86.7	9.95	44.28 (±1.38)
	Female																
7+	**Male**	0.3		0.8	2.6	7.1	8.5	7.0	19.9	9.4	26.0	7.1	10.9	0.4	79.1	6.80	47.36 (±0.57)
	Female	3.5	3.5		1.2	5.0	6.6	5.6	20.9	11.7	26.8	2.7	10.6	1.9	45.6	6.86	
8+	**Male**		0.8		1.6	4.5	11.0	9.5	22.2	10.7	25.4	4.4	9.9		95.5	5.59	53.13 (±2.02)
	Female	1.4			5.3	9.2	8.0	11.2	17.5	6.8	23.7	6.3	10.6		51.9	10.26	
									Liver								
3+	**Male**	1.0	4.9	3.4	0.2	4.5	7.5	4.7	20.0	12.8	24.9	2.8	13.1	0.2	106.3	28.33	
	Female	3.7	1.2	4.2	9.2	6.9	13.7	2.7	7.9	5.5	34.8		10.2		88.4	20.89	
4+	**Male**	4.6	5.5	4.4	1.0	5.5	8.4	7.7	12.3	10.9	17.3	12.3	8.7	1.4	122.2	33.45	
	Female	1.8	6.5	4.3	6.5	8.2	2.3	4.1	16.7	14.0	27.0	5.9	2.7		112.2	16.52	
5+	**Male**		4.7	3.2	2.1	3.5	7.7	5.5	16.7	10.3	27.2	8.4	9.2	1.3	106.1	24.75	
	Female																
6+	**Male**	2.1	3.5	2.9	3.7	3.2	2.8	3.2	19.3	11.0	30.5	3.3	10.7	3.8	110.4	35.36	
	Female																
7+	**Male**	2.4	5.5	3.7	3.5	5.3	8.2	3.5	11.5	9.3	26.9	9.2	8.8	2.2	142.6	24.19	
	Female	1.1	3.1	1.1	1.7	5.6	8.7	9.3	18.8	15.1	25.4	3.6	6.5		93.4	34.95	
8+	**Male**		0.6			1.0	5.9	7.0	28.1	12.1	35.7	2.7	6.9		157.9	20.90	
	Female	0.1	9.3	12.0	1.3	5.1	5.4	8.0	11.0	9.9	22.6	3.0	8.5	3.8	128.5	29.84	


Sampling Stations
M Station
South arm
Image © 2012 DigitalGlobe
Data SIO, NOAA, U.S. Navy, NGA, GEBCO
Image © 2012 GeoEye
Google earth
Data das imagens: 20/3/2011
2003
40°07'44.59"N 8°49'21.95"O elev 7 m
Altitude de visualização 8.60 km

Figure 1

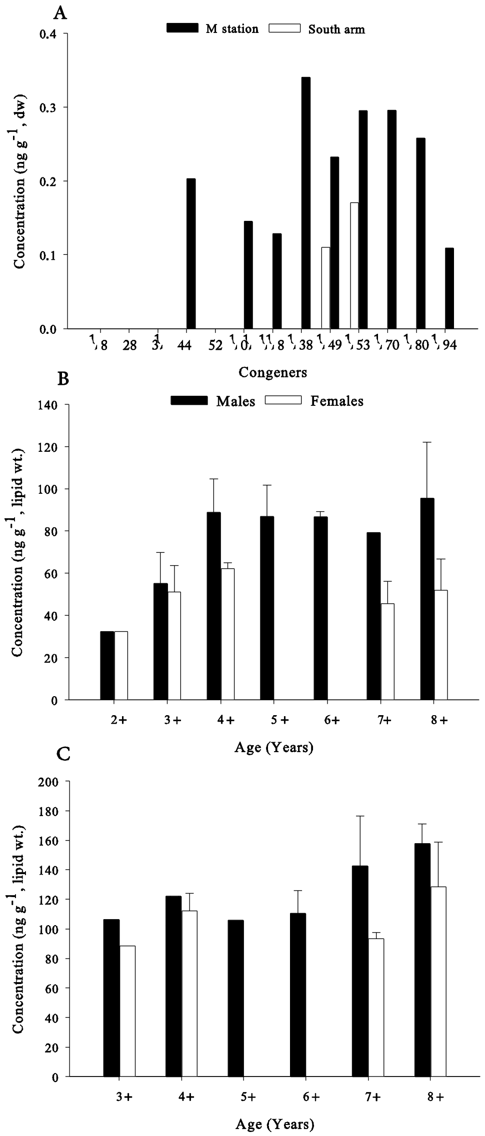
A
M station
South arm
Concentration (ng g-1, dw)
0.0
0.1
0.2
0.3
0.4
28
44
52
Congeners
B
Males
Females
Concentration (ng g-1, lipid wt.)
0
20
40
60
80
100
120
140
2+
3+
4+
5+
6+
7+
8+
Age (Years)
C
Concentration (ng g-1, lipid wt.)
0
20
40
60
80
100
120
140
160
180
200
3+
4+
5+
6+
7+
8+
Age (Years)

Figure 2

Research highlights

- The Mondego estuary is poorly contaminated, with low sediment contamination.
- *Chelon labrosus* was studied for PCBs.
- PCBs concentration in male muscle and liver tended to increase with age.
- PCBs concentration in female muscle and liver tended to remain stable with age.
- The most abundant congeners in liver and muscle were 138, 153 and 180.