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PCB bioaccumulation in three mullet species—A comparison study

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ABSTRACT

Polychlorinated biphenyls (PCBs) are lipophilic contaminants that tend to accumulate in organisms. PCBs were detected in *Chelon labrosus*, *Liza aurata* and *Liza ramada*, along different age groups. *L. ramada* presented the highest concentration, and it increased with age, whereas *C. labrosus* and *L. aurata* concentration remained constant. *L. ramada* high concentration can be attributed to its ecological niche, since this species is able to accumulate PCBs along its different age groups even in low environmental contamination conditions. PCBs 101, 118, 138, 149, 153, 170 and 180 were the congeners that more contributed to these species contamination, being PCB 138 and 153 the congeners with higher concentration. Mullets are edible in many countries, being important in fisheries and aquaculture. *L. ramada* is the most common mullet for capture and human consumption. All species presented concentrations below the regulation limit establish by the European Union, and therefore safe for human consumption.

1. Introduction

Polychlorinated biphenyls (PCBs) have been an environmental concern for many years, due to their high toxicity and bioaccumulative properties (Bodiguel et al., 2009). PCBs are widely spread in the environment, and can accumulate in organisms (Masmoudi et al., 2007). PCBs are considered dangerous pollutants, given a broad spectrum of toxicological responses, including immunotoxicity, endocrine disruption and tumor and carcinoma development (Bodiguel et al., 2009). Therefore, it is important to measure PCBs, in order to determine the quality status of the environment.

Fishes are often used as indicators of contamination in coastal systems, because they are relatively large and easy to identify (Coelhan et al., 2006; Tavares et al., 2011). Many authors pointed out that the consumption of fish contaminated by PCBs increases the risk for human contamination (Fu and Wu, 2005; Matthiessen and Law, 2002; Ulbrich and Stahlmann, 2004). So, obtaining information on organochlorine concentration and bioaccumulation in fish is essential given the importance for public health.

The Mugilidae family can be found in estuaries, coastal waters and rivers (Almeida, 1996), due to its food plasticity. They are omnivorous and detritivorous consuming a wide selection of food items (Boglione et al., 2006; Zouiten et al., 2008). Mugilidae have the advantage of using food resources provided by the primary producers, and therefore contribute decisively to the energy and organic matter flux in the estuarine ecosystem (Almeida, 2003). Mullet species play an important role in the world fisheries and aquaculture (Masmoudi et al., 2007; El-Halfawy et al., 2007; Mousa, 2010).

The presence and distribution of PCBs in edible fish is important not only for public health, but also from an ecological perspective. Many studies regarding contaminants have been made in mullet species (Baker et al., 1998; Bilbao et al., 2010; Tavares et al., 2011), but only a few studies have been made for PCBs, for *Liza aurata* (Masmoudi et al., 2007; Licata et al., 2003), *Liza ramada* (Bocquené and Abarnou, 2013) or *Chelon labrosus* (Narbonne, 1979). Moreover there are no studies regarding PCBs along different age groups in the three species. So, the purpose of this work was to assess PCB contamination in three Mugilidae species: thinlip gray mullet (*L. ramada*, Risso 1810), golden gray mullet (*L. aurata*, Risso 1810) and thicklip gray mullet (*C. labrosus*, Risso 1827). Accordingly, some questions were addressed: (a) Do these species bioaccumulate PCBs along their different age groups?; (b) Are there differences between species in the accumulation patterns?; (c) Which congeners have higher contribution for PCB contamination?; (d) Is the size/age of the fish important, concerning public health?

2. Methods

2.1. Sampling location

The Mondego estuary is a small estuary located in the western coast of Portugal (40°08′N, 8°50′W), with an area of 8.6 km^2 (Fig. 1). The estuary comprises two arms,

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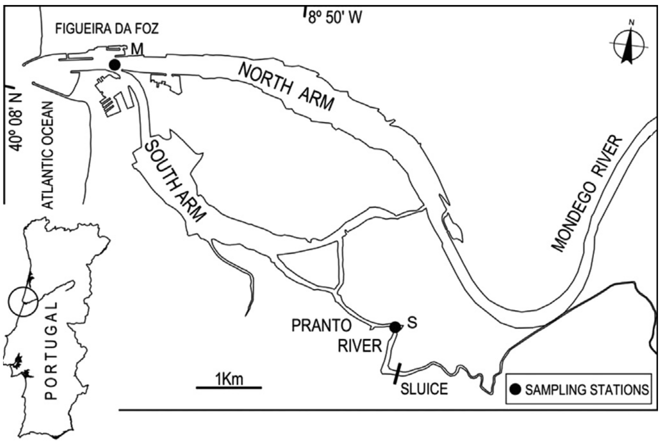

Fig. 1. Locations of the sampling sites in Mondego estuary.

the north and the south arm, separated at about 7 km from the shore joining again near the mouth of the estuary. The north and the south arm have distinct hydrologic characteristics. The north arm is deeper with 5–10 m of water column in high tide and 2–3 m tidal range. It is dredged frequently to maintain its depth, due to harbor activities. The south arm is shallower with 2–4 m deep in high tide and 1–3 m tidal range. The water circulating in the south arm is mainly depending on the tides and on small fresh water input from the Pranto River. The Mondego estuary is a well described system and poorly contaminated, regarding PCBs (Vale et al., 2002). In this scenario of low environmental contamination it is important to understand how fish species accumulate PCBs along their lifespan and specifically the ones that are consumed by humans reaching considerable prices in some markets.

2.2. Sampling and laboratory work

Three species of mullets were caught in the Mondego estuary: *C. labrosus*, *L. aurata and L. ramada*. Fishes and surface sediments (0–5 cm depth) were collected from September 2007 to April 2008 at the south arm (mainly juveniles, and adults from *L. aurata* and *L. ramada*) and at the mouth (mainly *C. labrosus* adults) (Fig. 1). In each location a sediment composite sample (from four sub-samples) was collected to increase the representativeness of the sampling locations. Surface sediments were composed mainly of mud and silt. After sampling 10 g of sediment were taken into the laboratory, where they were freeze-dried, homogenized and stored at −20 °C until analytical procedure. Regarding fish, a traditional beach-seine net was used to catch the younger fish, and a trammel net was used to catch the older fishes. Fishes were taken into the laboratory where they were weighted (g) and measured (cm) before collecting muscles samples. Fishes were aged using scales analysis. For each species and age three individuals were stored and analyzed for PCBs. For PCB analysis 3 g (dw) of muscle was used. Due to the lack of material some samples were analyzed as composite samples with three individuals. For *C. labrosus* were used individuals from 2+ to 8+ years, for *L. aurata* from 0+ to 4+ years and for *L. ramada* from 3+ to 11+ years. Due to the lack of material for PCB analysis, age 10+ of *L. ramada* and age 6+ of *C. labrosus* were not determined. After laboratory processing, samples were freeze-dried (Snijders scientific), homogenized and stored in freezer (−20 °C), until the analytical procedure.

2.3. PCBs analysis

The method was described in detail by Baptista et al. (2013). Briefly, sediments were Soxhlet-extracted with a hexane/acetone mixture, the extract was treated with activated copper and further cleaned through solid phase extraction (SPE) in two steps: first with alumina and neutral silica and secondly with acid silica gel. Fish muscles were extracted by sonication with hexane/acetone and the extract was submitted to a cleanup with sulfuric acid, followed by SPE with Florisil. PCBs quantification was carried out on a gas chromatograph equipped with a MDN-12 silica capillary column (30 m × 0.25 mm × 0.25 μm) coupled to a mass spectrometry detector (GCMS-QP5050A, Shimadzu). Total PCB content dry weight (ng g^{-1}, dw) was based on the summed concentrations of the twelve congeners analyzed (Σ_{12PCBs}) (IUPAC nos. 18, 28 and 31-tri; 44 and 52-tetra; 101 and 118-penta; 138, 149 and 153-hexa; 170 and 180-hepta). Within these congeners, six of them are considered as biological indicators to assess marine pollution, by the European Union (Σ_{6ICES} 28, 52, 101, 138, 153, and 180) (Comission of the European Communities, 1999).

For quality assurance and quality control of the PCBs quantification method, contamination was evaluated by blank controls and results were always below detection limit. The recoveries of the analytical method for the analyzed congeners were tested by analysis of spike samples and the recoveries mean was 98 percent (standard deviation of eleven percent). Reproducibility was calculated on replicate analysis giving an overall variability lower than twenty percent. The detection limits for individual PCBs ranged between 0.1 and 1.0 ng g^{-1} (dw).

2.4. Statistical analysis

To detect differences in PCB concentration and in the lipid content of the different age classes a linear regression was performed (SigmaPlot 11.0). To detect differences in the concentration of all species a Kruskal–Wallis one-way analysis of variance on ranks was performed. Afterward, an All Pairwise multiple comparison, Dunn's test, was applied in order to detect differences between each species. To detect differences between lipids of all species a one-way ANOVA was developed using the average lipid percentage for the three species (SigmaPlot 11.0). A data square-root transformation was applied to the data. A multidimensional scaling (MDS) analysis (Primer 6β) was performed to detect differences between the mullet species concentration. The MDS analysis was performed using the congeners' concentration of each species. A SIMPER analysis (Primer 6β) was used to detect differences in the congeners' distribution of each species. This analysis was performed using the Bray–Curtis similarity, with a cut-off of 90 percent, and the congeners' concentration of each species' age. A five percent significance level was used for all the analyses.

3. Results

3.1. PCB analysis in the abiotic and biotic compartment

Sediments from the Mondego estuary presented low PCB concentration, with M station presenting a concentration (Σ_{12PCBs}) of 2.6 ng g^{-1} (dw) and the south arm a concentration of 1.4 ng g^{-1} (dw).

C. labrosus PCB concentration varied between 1.6 and 8.4 ng g^{-1} (dw), *L. aurata* concentration varied between 3.3 and 6.4 ng g^{-1} (dw) and *L. ramada* concentration varied between 8.8 and 52.3 ng g^{-1} (dw) (Fig. 2A). *L. aurata* and *L. ramada* concentration presented significant differences (R^2=0.976, p=0.012 and R^2=0.869, p=0.002, respectively), along their different age groups. On the other hand *C. labrosus* PCB concentration did not present significant differences (R^2=0.0093, p=0.856). *L. aurata* age 0+ PCB concentration were below the detection limit, and therefore not able to be quantified.

L. ramada had the highest concentrations among the three mullet species and greatly increased along different age groups. According to the Kruskal–Wallis test each species concentration presented significant differences (p=0.002). An All Pairwise test reveled differences between *L. ramada* vs. *L. aurata* and *C. labrosus* (post hoc test, $p < 0.05$). On the other hand, *L. aurata* vs. *C. labrosus* did not present significant differences (post hoc test, $p > 0.05$).

Regarding lipids, no relation was found between lipid and age (R^2=0.456, p=0.066; R^2=0.018, p=0.867; R^2=0.456, p=0.141, for *L. ramada*, *L. aurata* and *C. labrosus*, respectively). According to the one-way ANOVA no significant differences were found between lipid content of the three species (p=0.523) (Fig. 2B).

In the MDS analysis the three species were positioned according to their concentration. *L. ramada* older individuals presented higher concentration, therefore were separated from the other species (Fig. 3). Until age 6+ *L. ramada* was included in the same group as *L. aurata* and *C. labrosus*, since they presented similar concentrations (Fig. 3). Due to the low concentration of *C. labrosus* at age 2+, this age was separated from the other groups (Fig. 3). According to the SIMPER analysis higher chlorinated congeners were the congeners that more contributed for the mullet species contamination (Table 1).

CB 118 was the only congener analyzed considered as a dioxin-like PCB. Both *L. aurata* and *C. labrosus* presented values lower than 0.5 ng g^{-1} (dw), while *L. ramada* presented higher concentration varying between 0.5 and 6.5 ng g^{-1} (dw) (Fig. 4A). *L. ramada* PCB

138 concentration varied between 2.0 and 10 ng g^{-1} (dw), on the other hand, *L. aurata* and *C. labrosus* concentrations were similar, varying between 1.0–2.0 ng g^{-1} (dw) (Fig. 4B). PCB 153 presented the higher concentration within the three mullet species. *L. ramada* concentration ranged from 2.0 to 15 ng g^{-1} (dw) and *L. aurata* and *C. labrosus* concentration varied between 0.3 and 2.6 ng g^{-1} (dw) (Fig. 4C). Only *L. ramada* concentration increased along the different age groups for the three congeners.

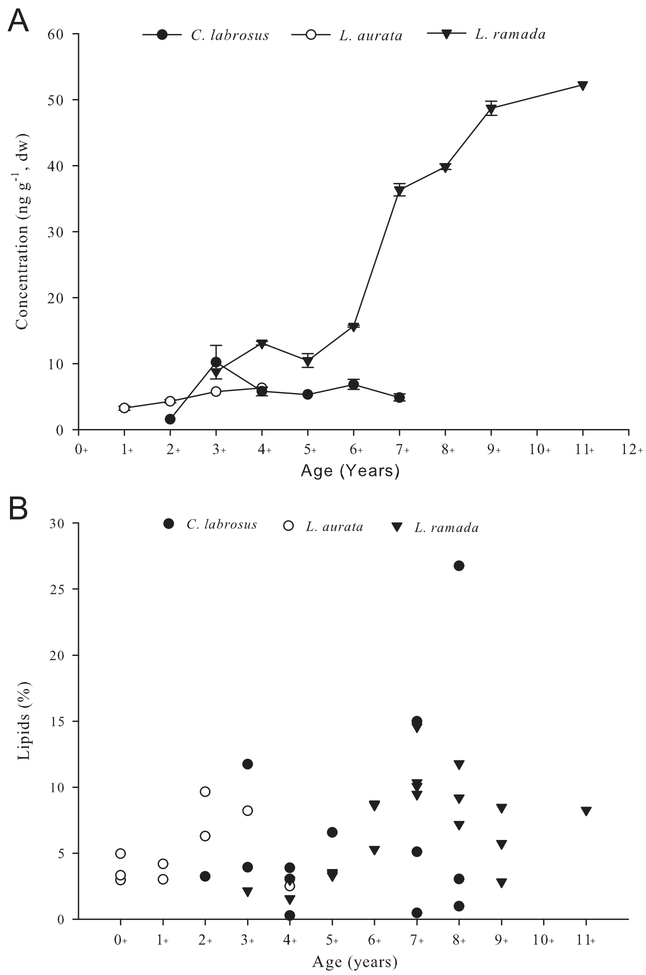

Fig. 2. PCB concentrations (A) and lipid proportion (B) in different age groups of the three mullet species (standard deviation between brackets).

4. Discussion

4.1. Mugilidae PCBs contamination

The Mondego estuary is a small estuary with few and diffuses contamination sources. Domestic and industrial sewage, agricultural runoff (contaminated by the use of fertilizers and pesticides, from the Mondego agricultural valley) and Figueira da Foz harbor (Nunes et al., 2011) are the main sources for the Mondego estuary PCB contamination. Therefore, the estuary presented low PCB concentration, also observed by Pereira et al. (2005). Moreover, PCB concentration present in sediments from the Mondego estuary was lower than those found by Lána et al. (2008), in Czech Republic, and Howell et al. (2008), in the Houston Ship channel, USA.

PCBs are very lipophilic and in many studies higher lipid content is associated with higher PCB concentration (eg. Coelhan et al., 2006; Trocino et al., 2009). However, in the current study lipid levels were not associated with PCB concentration, since all species presented similar lipid content and different PCB concentration. *L. ramada* PCB concentration increased with age but its lipid content remained stable. So, other factors must also play a

Table 1
SIMPER analysis with the percentage of individual PCB congeners in the three mullet species.

	Contribution (percent)						
	PCB 101	PCB 118	PCB 138	PCB 149	PCB 153	PCB170	PCB180
Chelon labrosus	6.3	5.7	25.0	15.4	29.0	–	10.1
Liza aurata	–	–	27.9	15.9	37.5	–	15.7
Liza ramada	10.4	7.7	21.4	9.7	26.8	5.8	11.9

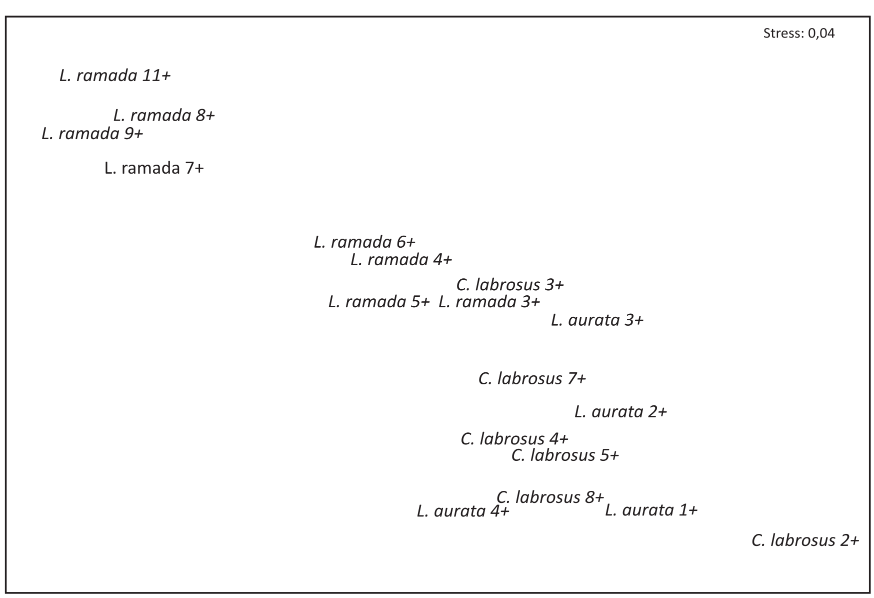

Fig. 3. Multidimensional scaling (MDS) analysis of the concentration in the three mullet species.

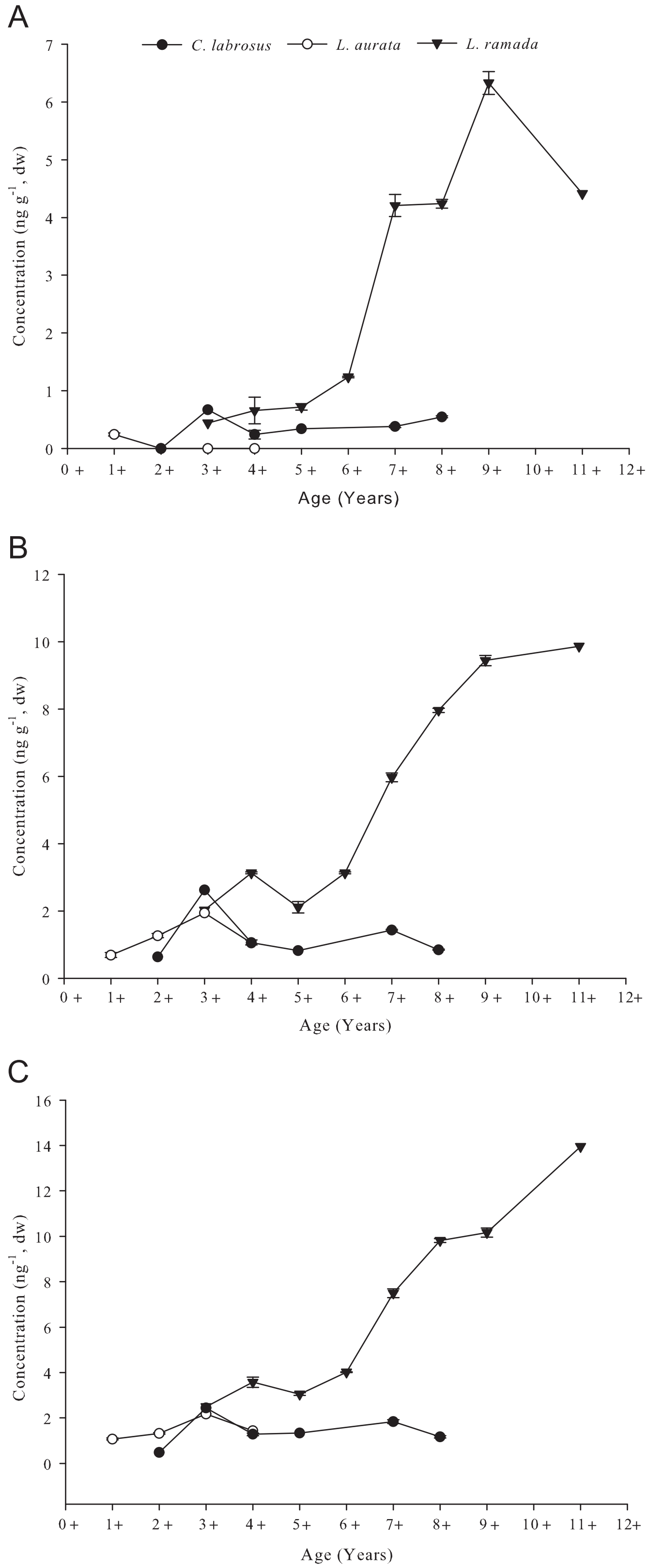

Fig. 4. Concentrations of congeners 118 (A), 138 (B) and 153 (C) in different age groups of the three mullet species (standard deviation between brackets).

key role in the metabolic pathways involved in the bioaccumulation of PCBs by the fish species.

Mugilidae species can live for a long period of time, *L. ramada* reported age is around 10 years, *C. labrosus* reported age is estimated to be 25 years. No information regarding *L. aurata* is available (http://www.fishbase.org, 2013). Mullets can run long distances in short periods of time eventually moving out of the estuarine systems, or remain inside particularly around urban sewages discharge (Antunes et al., 2007), leading to a high uptake of contaminants (Masmoudi et al., 2007).

Only juveniles' mullet species presented similar PCB concentration, which can be explained by the fact that the three species juveniles spent a part of their life in the same environment, inside the Mondego estuary (Fig. 5). *L. aurata* and *C. labrosus* presented lower PCB concentration than *L. ramada*. These two species are marine estuarine opportunists, entering the estuary regularly in substantial numbers, particularly as juveniles, but use near shore marine waters as a preferential habitat (Fig. 5). On the other hand, *L. ramada* concentration increased with age, since this species spends its entire lifespan inside or near the estuarine system, only migrating to the sea to spawn (Fig. 5). According to Baptista et al. (2013) species that spend more time in the Mondego estuary present higher PCB concentration than species that spend less time in the estuary. According to Tavares et al. (2011), *L. aurata* mercury contamination, in the Mondego estuary, decreased with fish age. The decrease of this bioaccumulative pollutant can be explained by the ecology of the species that tend to spend most of the time in the coastal area and not inside the estuarine system, which can lead to a low uptake of contaminants. This can also explain the fact that PCBs increased very slowly in *L. aurata* individuals. As a catadromous species, *L. ramada* has the capacity to osmoregulate even in fresh water (Cardona et al., 2008). Furthermore, this species is often found in more contaminated areas (Kottelat and Freyhof, 2007), and is more tolerant to coastal organic pollution and eutrophication (Boglione et al., 2006).

The relation between species concentration and age is not well established in literature. This relation can occur due to extended exposure to contaminants in older fish. Nevertheless, a wide range of variables can affect bioaccumulation, such as growth rate, diet and lifespan location. The relation between concentration/age is only observed in *L. ramada* corresponding to the only species that spent the entire life cycle inside the estuarine system.

In the Mondego estuary, the three mullet species have similar feeding behavior, being detritivorous and omnivorous. Mugilidae are characterized by a wide range of feeding adaptations in the estuarine environment according to the trophic availability (Zouiten et al., 2008). Each species is able to utilize the food distributed from the thin water surface film to the bottom mud, either by direct grazing or using plant-detritus food chain (Boglione et al., 2006). Dietary intake is recognized as the main source of PCB contamination, and increases in high trophic level species (Bocquené and Abarnou, 2013; Brázová et al., 2012), even though, the mullet species from the Mondego estuary present differences in their concentration despite their similar food resources.

4.2. PCBs congeners

PCBs 101, 118, 138, 149, 153, 170 and 180 are high chlorinated congeners, and presented higher contribution for PCB contamination in the three mullet species from the Mondego estuary. High chlorinated congeners are slowly eliminated metabolically (Stapleton et al., 2001; Wu et al., 2008), less volatile and more resistant to microbial degradation (Bazzanti et al., 1997; Zhou et al., 2001), therefore more persistent in the environment. So, once in the organism they tend to bioaccumulate, as they are not readily metabolized and excreted (Borga et al., 2001).

The predominant congeners for the three mullet species were PCB 138 and 153, which are, usually, the prevailing in biological samples (Antunes et al., 2007; Bocquené and Abarnou, 2013).

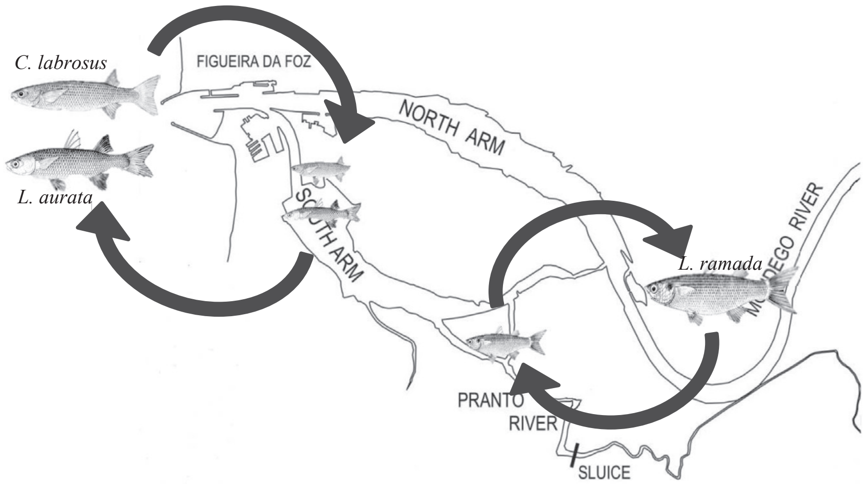

Fig. 5. *C. labrosus*, *L. aurata* and *L. ramada* lifespan location in the Mondego estuary.

According to Ulbrich and Stahlmann (2004) these congeners have great impact in human health. Moreover, PCBs 101, 118, 138 and 153 are toxic for humans, by increasing tumor promoting activity, oxidative stress, and also leading to DNA damage (Marabini et al., 2011).

PCBs 118, 138 and 153 increased their concentration with age in *L. ramada*, due to the species ecology, lifespan and diet. On the other hand, these congeners concentration in *L. aurata* and *C. labrosus* remain constant with age, also due to these species ecology (Marabini et al., 2011).

4.3. Fishing, aquaculture and human impact

Mugilidae family has a great economic importance in many countries, such as Egypt (El-Halfawy et al., 2007), Italy or Greece (Hotos et al., 2002). Mullets are suitable fishes for aquaculture purposes in brackish and fresh water ponds (Arruda et al., 1991). In Italy the aquaculture practice of these three species is very common, using cultured-based fisheries (valliculture) (http://www.fao.org, 2011).

L. ramada is the most common species captured for human consumption, due to its great nutritious value and as an alternative food resource (El-Halfawy et al., 2007), and can achieve high market prices (Hotos et al., 2002; Mousa, 2010). This species is a very abundant despite its the massive fishing. Though, this species accumulate PCBs along its different age groups, which can lead to possible health risks in estuaries more contaminated than the Mondego.

Human exposure by PCBs is mostly through food products (Marabini et al., 2011; Ulbrich and Stahlmann, 2004). Though, PCBs levels in food have been gradually decreasing in the environment, since environmental legislation on use and disposal of PCBs was introduced by the European Union (http://www.efsa.europa.eu, 2011). The European Union has recommended a tolerance limit of 75 ng g^{-1} (wet wt.), for the sum of the six ecological indicators (IUPAC Σ_{6ICES} 28, 52, 101, 138, 153 and 180), for fish muscle and fishery products (Commission Regulation (EU) No 1259/2011). In the Mondego estuary, *L. ramada* presented the higher concentration of the three mullet species. *L. ramada* concentration varied between 1–9 ng g^{-1} (wet wt.), whereas *L. aurata* was around 1 ng g^{-1} (wet wt.) and *C. labrosus* varied between 0.25–1 ng g^{-1} (wet wt.). The human consumption of *L. ramada*, *L. aurata* and *C. labrosus*, and the implementation of aquaculture units in medium contaminated estuaries can be made safely, since these species presented values far below the concentration limit.

5. Conclusions

PCB concentration was measured in three mullet species (*C. labrosus*, *L. aurata* and *L. ramada*) from the Mondego estuary. *L. ramada* presented higher concentration and its concentration increased along its different age groups. *C. labrosus* and *L. aurata* concentration remained stable along the different age groups. This dissimilarity in the PCBs accumulation in the different species emphasizes the importance of studying specific accumulation behavior in different species. The main factors that influenced PCB accumulation in the mullet species were the time spent in the estuary and probably their diet, rather than lipid content. Higher chlorinated congeners contributed more for the accumulation in the mullet species, and the congeners with higher concentration were PCB 138 and 153.

Mullet species are often used in aquaculture, and can achieve high market prices. PCB concentration in all samples of the studied mullet species were found to be below the limits stated by EU regulations (75 ng g^{-1},wet wt.).

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