



## Short communication

Influence of sex and age on PCBs accumulation in the commercial fish *Chelon labrosus*Joana Baptista <sup>a,\*</sup>, Pedro Pato <sup>b,c</sup>, Sílvia Tavares <sup>a</sup>, Armando C. Duarte <sup>b</sup>, Miguel A. Pardal <sup>a</sup><sup>a</sup> CFE, Center for Functional Ecology, Department of Life Sciences, University of Coimbra, Apartado 3046, 3001-401 Coimbra, Portugal<sup>b</sup> Department of Chemistry & Center for Environmental and Marine Studies (CESAM), University of Aveiro, 3810-193 Aveiro, Portugal<sup>c</sup> School of Technology and Management, Polytechnic of Viana do Castelo, Avenida do Atlântico, 4900-348 Viana do Castelo, Portugal

## ARTICLE INFO

## Article history:

Received 16 May 2012

Received in revised form 2 January 2013

Accepted 28 January 2013

Available online 14 February 2013

## Keywords:

PCBs

Fish

Bioaccumulation

*Chelon labrosus*

Sex

Age

## 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 was 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<sup>-1</sup> (lipid wt.) and in females from 32 to 62 ng g<sup>-1</sup> (lipid wt.). In male liver sample concentrations ranged from 106 to 158 ng g<sup>-1</sup> (lipid wt.), while female concentrations ranged from 88 to 129 ng g<sup>-1</sup> (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.

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## 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 Cousins, 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?; and D) Is this species safe for human intake in estuaries with low contamination?

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## 2. Material and methods

### 2.1. Study site and laboratory work

The Mondego estuary is a small estuary (area 8.6 km<sup>2</sup>), located in the western coast of Portugal (40°08' N, 8°50' 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 (−20 °C), 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 −20 °C until analyzed. For comparison with the European Union limits, *C. labrosus* wet weight concentration was calculated using the formula:  $\sum_6 \text{PCB concentration (dry wt.)} * (100 - \text{water (\%)})$ .

### 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 g of muscle and 1 g 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 ( $\sum_{13}\text{PCB}$ ) content (ng g<sup>−1</sup>, 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



Fig. 1. The Mondego estuary and sampling stations location.

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