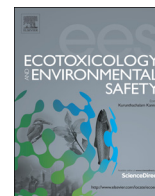




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## Uptake and depuration of PCB-153 in edible shrimp *Palaemonetes varians* and human health risk assessment

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### ABSTRACT

A medium-term mesocosm exposure study was conducted to elucidate bioaccumulation and depuration of polychlorinated biphenyl congener 153 (PCB-153) in edible shrimp *Palaemonetes varians*. Over the 15-day exposure period, shrimp under different exposure concentrations exhibited a significant increase in PCB-153 concentration compared with control organisms. Distinct bioaccumulation patterns and uptake rates were observed depending on the exposure concentrations. For low PCB-153 exposure levels ( $0.25 \mu\text{g L}^{-1}$ ), accumulation followed a saturation model, reaching an apparent steady state after fifteen days exposure. For intermediate ( $2.5 \mu\text{g L}^{-1}$ ) and high PCB-153 levels ( $25 \mu\text{g L}^{-1}$ ), accumulation was faster and linear. In addition, the bioaccumulation rate was not proportional to PCB-153 concentration, and the bioaccumulation was higher at intermediate exposure concentrations. Regarding the depuration phase, *P. varians* lost up to 30% of PCB-153 after 72 h and levels continued slowly to decrease until the end of the 30-d experimental period. However, PCB-153 levels in shrimp did not reach background values, and those exposed to moderate and high PCB-153 concentrations presented contamination levels much higher than the regulatory limit for human food consumption ( $75 \text{ ng g}^{-1} \text{ ww}$  for  $\Sigma_6 \text{ PCB}$ ).

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### 1. Introduction

Among persistent organic pollutants (POPs), polychlorinated biphenyls (PCBs) are recognized as one of the largest groups of widespread anthropogenic contaminants, comprising a total of 209 congeners with different physicochemical and toxicological properties.

By the 1970s, owing to serious concerns pertaining to their human toxicity, suspected carcinogenicity and environmental persistence, several countries limited the use and production of PCBs, but it was only in 1985 that their use and marketing in the European Community was heavily restricted. Since PCBs are highly lipophilic, they can bioaccumulate in the adipose tissues of exposed organisms. They are present in various compartments, both biotic (from plankton to humans) (Wang et al., 1998; Voorspoels et al., 2004; Helm et al., 2008; Croes et al., 2012) and abiotic (air, water, sediments, soil) (Howell et al., 2008; Han et al., 2010; Cachada et al., 2012). Furthermore, biomagnification through the food chain has been well documented, posing serious risks for both wildlife and humans (Borga et al., 2001; Skarphedinsdottir et al., 2010; Nunes et al., 2011).

Since the main pathway of human exposure to PCBs is through dietary intake, namely by ingestion of edible marine species (Nunes et al., 2011; Fillos et al., 2012), the public and the food regulatory agencies are particularly concerned about the potential health effects associated with their consumption (Bodin et al., 2007). Crustaceans are a diverse group of marine organisms with enormous importance in the food webs, tending to accumulate large amounts of lipophilic contaminants in their tissues (Voorspoels et al., 2004; Bodin et al., 2007). Within crustaceans, shrimp have singular interest since they are an important prey for both ichthyofauna and avifauna and represent a highly consumed food item worldwide. The Food and Agriculture Organization estimated a global annual production of 3.4 million tons of cultivated marine shrimp commonly fed on fresh food items, farm-made and/or commercially compounded aquafeeds, valued at more than US\$ 14 million (FAO, 2011). The ditch shrimp *Palaemonetes varians* (Leach 1814) is a small decapod caridean species, commonly found in salt ponds and estuaries worldwide, demonstrating high tolerance to a broad range of temperatures and salinities (Oliphant et al., 2011). It is a species of great economic importance, used as bait for fishery species and for human consumption. In Portugal, its production in intensive systems reaches  $200\text{--}300 \text{ kg ha}^{-1} \text{ yr}^{-1}$  with an average price of nearly  $10 \text{ eur kg}^{-1}$ .

Since coastal areas are subjected to great human pressure and are frequently under episodic contaminant events, it is crucial to

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understand the bioaccumulation trends in marine species, such as *P. varians*, and their ability to detoxify after a hypothetical industrial discharge. For this purpose, the most abundant PCB congener in marine biota was used, PCB-153 (2, 2', 4, 4', 5, 5'-hexachlorobiphenyl). This congener has been shown to be a good indicator in monitoring programs (Danis et al., 2005). It has been suggested that this congener induces neurobehavioral deficits via gestational and lactational transfer, being also known as a hepatotoxin and carcinogenesis promoter (Lee et al., 2002; Chubb et al., 2004). The selection of this specific congener was based on its high abundance and prevalence in the environment and human serum and tissues (Chubb et al., 2004; Johansen et al., 2011). It is often considered to be a congener representative of all the others, constituting nearly 10% of the total amount of PCBs (Ilyina, 2007) and about 33% of the summed six-indicator non-dioxin-like PCBs measured in different fish and seafood products (VKM, 2008).

Studies on PCB uptake and depuration by edible decapod crustaceans are rather scarce or even inexistent, being mostly restricted to experiments using laboratory-contaminated sediments as a source of contamination (Rubinstein et al., 1983; Tatem, 1986; Danis et al., 2005 and references therein). In addition, few studies have taken into account essential species, upon which depends the ecosystem structure and functioning. Further investigation is required in order to estimate biomagnification through the food chain and consequently to assess the risks to human health. Therefore, the major aim of this work was to study the bioaccumulation and decontamination processes of the ditch shrimp *P. varians*, when subjected to different PCB-153 concentrations through a mesocosm experiment. It was also our goal to determine if the organisms were able to detoxify and reach the legal limits allowed for food consumption after a 15-day depuration period.

## 2. Materials and methods

### 2.1. Biological samples collection

The organisms (*P. varians*) and the water used in the experiment were collected in the Gala, Mondego estuary (40°08' N, 8°50' W), located on the Atlantic coast of Portugal (see in detail Cardoso et al. (2013)). Since no local source of PCB contamination is known (Pereira et al., 2005; Nunes et al., 2011), this location was considered as a reference condition. PCB-153 baseline concentrations were minimal, both in the water ( $< 5 \text{ ng L}^{-1}$ ) and in the shrimp *P. varians* ( $2 \text{ ng g}^{-1}$  wet weight).

In the laboratory, the shrimp were measured in terms of total length and all the organisms used in the experiment ranged between 2 cm and 3 cm. These organisms were held in clean seawater at 20 °C for four days to acclimate and depurate. Oxidic conditions were ensured by air-bubbling the water. Shrimp were fed daily with *Artemia* sp. during both acclimation, exposure and decontamination periods. Before being used as a food source, *Artemia* sp. was previously tested for possible residues of PCB-153, which were negligible and below  $0.5 \text{ ng cmg}^{-1}$  dry weight.

### 2.2. Experimental set-up

The experiment was performed in pre-washed glass containers of 3 l (Ø: 14 cm), free of any PCB contamination residues. The water collected in the Mondego Estuary (reference condition) was filtered through 0.45 µm pore size Millipore filters, in order to remove particles. Four different treatments were applied, comprising a total of 96 replicates, 24 by each treatment: control condition without PCB-153; treatment I—low PCB-153 contamination, corresponding to  $0.25 \text{ µg L}^{-1}$  (half the maximum limit stated by the Environmental Protection Agency for PCBs in drinking waters), treatment II—intermediate PCB-153 contamination ( $2.5 \text{ µg L}^{-1}$ ) and treatment III—high PCB-153 contamination ( $25 \text{ µg L}^{-1}$ ), corresponding to a concentration 50 times higher than the legal limit allowed for drinking waters. Those concentrations seemed to be reasonable and realistic by reflecting contamination levels observed in the field. For example, in areas where no exceptional industrial spill events were known, like Daya Bay (China) or the Lake Bosomtwe (Ghana), relatively high PCB water concentrations were recorded:  $1.4 \text{ µg L}^{-1}$  (Zhou et al., 2001) and  $7.2 \text{ µg L}^{-1}$  (Afful et al., 2013), respectively. The highest PCB contamination treatment aims to reflect a hypothetical industrial PCB spillage. All the solutions were prepared using a PCB-153 (2, 2', 4, 4', 5,

5' – hexachlorobiphenyl) OEKANAL<sup>®</sup> standard solution (Sigma-Aldrich) diluted in acetone. Since PCBs are hydrophobic, acetone was used as a carrier solvent to facilitate their addition to seawater (Roesijadi et al., 1976; McLeese et al., 1980).

Mesocosms were established by transferring 1 L of solution, according to the different treatments to each glass container, always maintained under oxic conditions. The 96 glass containers were randomly distributed in an acclimated room, with temperature maintained at 20 °C and water salinity in the range of 30–32. The containers were maintained in these conditions for 24 h to stabilize before introducing the organisms. In each of the glass containers fifteen individuals of *P. varians* were introduced, which was similar to the mean densities observed in the field (Gregg and Fleeger, 1998).

The experiment was divided in two phases: exposure and decontamination. For each of the four treatments (reference – non-contamination, low, intermediate and high PCB-153 contamination) two different sampling times were chosen during the exposure phase ( $t_1$ —three days;  $t_2$ —fifteen days). At each contamination condition and sampling time, six replicates were collected, which were posteriorly combined in pairs, in order to obtain three composite replicates (consisting of a maximum of 30 individuals each composite replicate) and consequently sufficient body mass to perform subsequent PCBs quantification. In parallel, the other 48 flasks, each one with fifteen individuals, were maintained under the same conditions of PCB-153 accumulation and used afterwards for the decontamination study. Therefore, the experimental setup (exposure and decontamination) included 96 ( $6 \times 4 \times 4$ ) containers (sub-experiments). After fifteen days, the organisms were transferred from the contaminated containers to others filled with clean seawater, free of contamination residues. During this phase two sampling times were chosen ( $t_1$ —three days;  $t_2$ —fifteen days). Over the entire experimental period (fifteen days of exposure + fifteen days of decontamination), the medium of each flask was totally renewed twice a week, to avoid re-contamination by the feces.

At the pre-defined sampling times, the organisms were removed from the glass containers, rinsed with estuarine water and placed in constantly aerated clean estuarine water at 20 °C for 24 h to allow depuration (to remove fecal material from the digestive tract) (Blust et al., 1992; Cardoso et al., 2013 and references therein). Afterwards, the organisms were freeze-dried for subsequent PCB-153 analysis and quantification.

PCB-153 quantification in the flasks of water was performed regularly to ensure that concentration remained constant throughout the experiment. Any decrease of PCB-153 concentration due to adsorption and absorption mechanisms was compensated by addition of PCB-153 solution, in accordance with other studies (Cardoso et al., 2013 and references therein).

### 2.3. PCB-153 extraction and quantification

Water samples (aliquots of 100 mL) spiked with PCB-209 were extracted by liquid–liquid extraction with hexane (US EPA Method 3510C). The three organic extracts of each sample were combined, dried with anhydrous sodium sulfate, followed by evaporation to dryness using a rotary evaporator and a stream of nitrogen. The extract was stored at  $-20 \text{ °C}$  and before instrumental analysis, internal standard (PCB-131) was added to the extract and reconstituted to 200 µL of iso-octane. Shrimp samples were extracted by sonication (Selecta<sup>®</sup>) with a *n*-hexane:acetone (1:1) mixture (30 mL), for 20 min. Extract was decanted and the process repeated three times. Solvent volume was reduced through a rotary evaporator. Lipid content was gravimetrically determined using 10% of the extract. A gentle stream of nitrogen was used to reduce solvent volume by evaporation. Lipids were removed with sulfuric acid (97%). Afterwards, the extract passed through a multilayered column packed with florisisil (1 g of Supelclean<sup>®</sup> Florisisil) and anhydrous sodium sulfate and eluted with 15 mL of hexane. All samples were dried, under a gentle stream of nitrogen, before instrumental analysis internal standard (PCB-131) was added and solvent exchanged to iso-octane before analysis.

The quantification was performed using a gas chromatographic system (Shimadzu Corporation GC/MS-QP5050A) equipped with a ZB5-MS column ( $30 \text{ m} \times 0.25 \text{ mm}$  i.d., 0.25 µm film thickness), a mass spectrometry detector using electron impact ionization and selected ion monitoring (SIM) acquisition. Helium was used as the carrier gas (constant flow-rate of 0.9 ml/min). The PCBs were separated with the following GC oven temperature program: 2 min at 40 °C, first ramp at  $10 \text{ °C min}^{-1}$  to 180 °C, second ramp at  $6 \text{ °C min}^{-1}$  to 310 °C (held for 10 min). The injector was held at 280 °C and the interface at 310 °C. Detector acquisition was programmed to monitor  $m/z$  360 for quantification of PCB-153 and the internal standard and  $m/z$  362 for confirmation.

### 2.4. Quality assurance and quality control (QA/QC) procedures

The method performance was assessed by daily check of calibration curves, method blanks, fortification of samples and recoveries of surrogate. Method blanks were done in every fourth sample in order to detect possible interferences from the reagents, glassware and other processing hardware. Recoveries of PCB-209 in water samples were  $78 \pm 6\%$  (95% confidence interval) and the method performance was also assessed by the recoveries of PCB-153 in fortification experiments, which were  $85 \pm 5\%$  (95% confidence interval). For tissue samples, recoveries obtained for the

NIST certified material SRM 2977 (mussel tissue) were  $81 \pm 7\%$  (95% confidence interval).

### 2.5. Data analysis

Uptake kinetics were expressed in terms of change of PCB-153 concentration over time. Variations were described using either a simple linear regression model [Eq. (1)] or by a simple first order exponential kinetic model (Eq. (2)) when the observed pattern tended to reach a steady-state:

$$C_t = k_u t \quad (1)$$

$$C_t = C_{ss} - (C_{ss} - C_0) \exp(-k_u t) \quad (2)$$

where  $C_t$  and  $C_{ss}$  are the concentrations at time  $t$  (d) and at steady-state, respectively;  $k_u$  is the uptake rate constant ( $d^{-1}$ );  $C_0$  is the concentration at time 0 (Cardoso et al., 2013 and references therein).

For treatment I, a biological half-life (the time it takes to reach half of the equilibrium value) was calculated ( $T_{b1/2}$ ) from the corresponding constant uptake rate constant ( $k_u$ ), according to the relation  $T_{b1/2} = \ln 2/k_u$ .

Bioconcentration factors ( $BCF_{15d}$ ) were determined by dividing the total accumulated concentration of PCB-153 in the shrimp by the concentration of that contaminant in the water.

To infer differences between treatments, all data were initially checked for normality using the Kolmogorov–Smirnov test and for homogeneity of variances using the Levene's test (Zar., 1996). However, whenever data did not meet these criteria, even after transformation, non-parametric tests were applied. The Scheirer Ray–Hare test (equivalent to two-factor ANOVA (time  $\times$  treatments)) was applied in order to determine differences in PCB-153 concentrations between treatments and sampling times (Dytham, 2011). Differences in shrimp survival among treatments were investigated through a parametric one-way ANOVA test. Statistical analyses were performed using the SigmaPlot 11.0 software package and the IBM SPSS Statistics 20.0.

## 3. Results

### 3.1. PCB-153 bioaccumulation and decontamination rates

Over the entire exposure period, shrimp under different contamination treatments exhibited increased PCB-153 concentrations compared to control organisms, which maintained vestigial concentrations all over the time (Fig. 1). However, distinct accumulation patterns were observed regarding contamination treatments. Organisms subjected to low PCB-153 contamination were best fitted using a first order kinetic model (Fig. 1A), while shrimp experiencing intermediate and high PCB-153 levels were best fitted using simple linear regressions (Fig. 1B, C). Uptake rate constants ( $k_u$ ) increased with PCB-153 contamination, being around 100 times faster in the organisms exposed to treatment II than in the shrimp under treatment I (Table 1). In addition, shortly before 2 days of exposure, shrimp subjected to treatment I reached half of the equilibrium concentration (Table 1). Significant differences in PCB-153 concentrations were found between contamination treatments and the controls, as well as between sampling times (Table 2).

For all the treatments, high PCB-153 accumulation was observed within the first three days of exposure (treatment I: 800%; treatment II: 6000%; treatment III: 45,500%). During the following twelve days of exposure, the accumulation corresponded to values around 30% in the treatment I and 500% for both treatments II and III. After fifteen days of PCB-153 exposure, concentration factors (CFs) were highest for intermediate concentrations ( $2.5 \mu\text{g L}^{-1}$ ) and lowest for lower contamination levels ( $0.25 \mu\text{g L}^{-1}$ ) (Table 3).

During the depuration phase, significant declines in PCB-153 concentrations were observed after 72 h, for all the contamination treatments (Fig. 1). This loss was identical in the shrimp exposed to the treatments I and III (25%) and slightly higher in the organisms subjected to intermediate PCB-153 levels (30%). Concentrations of PCB-153 continued to decrease until the end of the experiment, however the initial concentration values were never

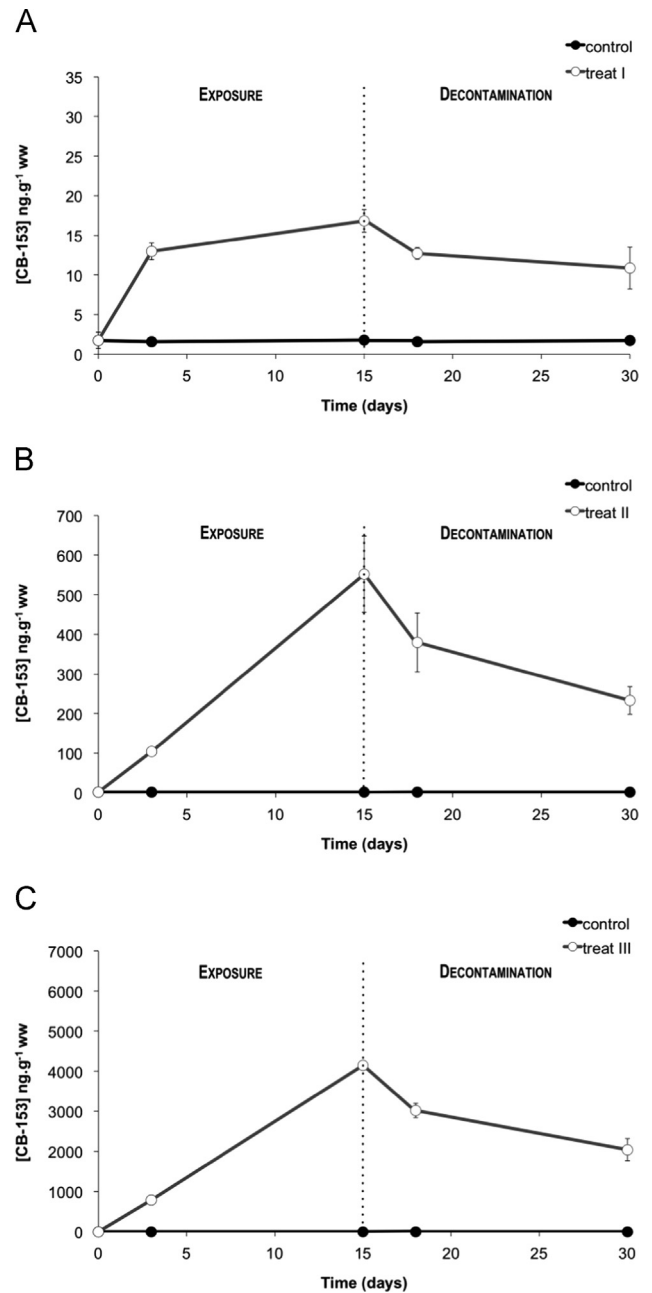


Fig. 1. Uptake and decontamination kinetics of the shrimp *Palaemonetes varians* under PCB-153 treatments (mean  $\pm$  SE): (A) Treatment I; (B) Treatment II; (C) Treatment III.

Table 1

Estimated uptake kinetic parameters for the shrimp *Palaemonetes varians*, exposed for fifteen days to PCB-153 in seawater.  $C_{ss}$ : concentration at steady state;  $k_u$ : uptake rate constant ( $d^{-1}$ );  $R^2$ : determination coefficient;  $T_{b1/2}$ : biological half-life (d).

	Uptake period			
	$C_{ss}$	$k_u$	$R^2$	$T_{b1/2}$
Treat. I ( $0.25 \mu\text{g L}^{-1}$ )	16.90	0.36	0.99	1.94
Treat. II ( $2.5 \mu\text{g L}^{-1}$ )	–	36.84	0.99	–
Treat. III ( $25 \mu\text{g L}^{-1}$ )	–	277.01	0.99	–

reached. Thus, even after fifteen days in clean seawater, the organisms exposed to different contamination treatments still retained substantial PCB-153 levels compared with controls and



**Table 2**  
Statistical results of the Scheirer Ray–Hare test.

	Scheirer Ray–Hare test			
	SS	SS/MS <sub>total</sub>	df	P-value
A	1919.7	12.7	4	-
B	2942.4	19.5	3	**
A × B	775.3	5.1	12	ns

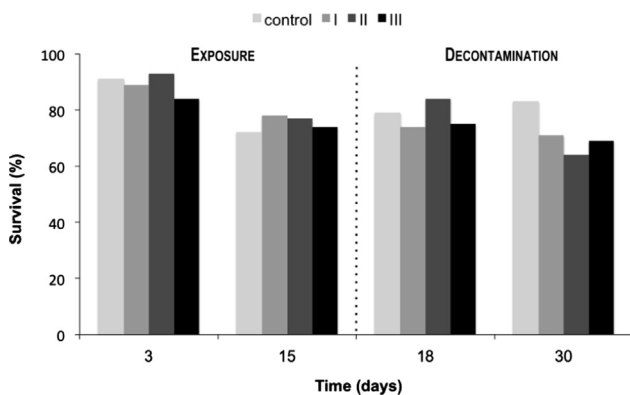
Factor A—time, factor B—treatments, A × B—interactions—not significant

\*  $p < 0.05$

\*\*  $p < 0.001$

**Table 3**  
Bioconcentration factors (BCF<sub>15d</sub>) of *Palaemonetes varians* in the three contaminated treatments following fifteen days exposure. BCF—ratio between CB-153 concentration in the organism/water. Values are the mean of three replicates with standard error in brackets.

Bioconcentration factors		
Treat. I (0.25 µg L <sup>-1</sup> )	Treat. II (2.5 µg L <sup>-1</sup> )	Treat. III (25 µg L <sup>-1</sup> )
67.3 (± 4.9)	220.6 (± 38.4)	165.7 (± 1.6)



**Fig. 2.** Survival rates of *P. varians* under different PCB-153 treatments (control, treatment I, treatment II, treatment III).

inclusively higher than those allowed by the European legislation (Commission Regulation (EU), 2011 Regulation no 1259/2011) for muscle meat of fish and fishery products (75 ng g<sup>-1</sup> wet weight).

### 3.2. Shrimp survival rates

Survival decreased during PCB-153 exposure in all the treatments (Fig. 2). After being transferred to clean seawater, survival at day eighteen was identical to the observed at day fifteen and slightly higher than the registered at the end of the experiment. Overall survival was around 80% in both control and PCB-153 treatments and no significant differences were observed between them (one-way ANOVA,  $p > 0.05$ ). Thus, shrimp mortality did not seem related with PCB-153 toxicity.

## 4. Discussion

Toxic effects inherent to direct exposure to PCBs or indirectly by ingestion of contaminated food constitute an important concerning issue for both humans and the environment. In fact, several studies have demonstrated that exposure to PCBs may interfere with normal physiology and biochemistry of the organisms. These compounds may produce immune, oxidative and

genotoxic effects in aquatic organisms of different trophic levels, such as bivalves, crabs and fishes (Orbea et al., 2002; van der Oost et al., 2003). In humans, for example, these compounds have been linked with adverse health alterations, including skin lesions, neuro and immunotoxicity, endocrine disruption, carcinogenicity and chronic reproductive effects, with an increasing risk of infertility (Çok and Şartiroğlu, 2004; Jaikanlaya et al., 2009; Meeker and Hauser, 2010). Thus, it is crucial to understand the mechanisms underlying bioaccumulation and detoxification of the marine fauna, particularly of species playing key role in the ecosystems' structure and functioning and simultaneously with great socio-economic interest, like the edible shrimp *P. varians*. This species lives in close association with aquatic plants and occasionally browses on the substrate surface. Although, it is more representative of the water column habitat, being primarily susceptible to water-mediated uptake (Rubinstein et al., 1983).

Grass shrimp *Palaemonetes* spp. is considered a good estuarine bioindicator of anthropogenic impacts (Key et al., 2006), being more sensitive to heavy metals compared to estuarine fish (Downs et al., 2001). Heavy metals are usually stored in detoxified form, being unavailable to interact with metabolic sites where they might exert a toxic effect (Rainbow and Luoma, 2011). In contrast to heavy metals, the toxicity of organic pollutants is typically related to the total accumulated concentration (Rainbow and Luoma, 2011). This in turn is closely associated with the local bioavailability of that contaminant (McLeese et al., 1980).

The present study revealed that *P. varians* exhibited different kinetics of accumulation depending on the concentration of PCB-153. For intermediate and higher PCB-153 levels, shrimp uptake followed a linear accumulation kinetics, while for low PCB-153 contamination the organisms followed a saturation model. However, independent of the contamination treatments at which the shrimp were subjected, significant accumulations occurred during the first 72 h of exposure. Shrimp exposed to low PCB-153 levels demonstrated a slower constant uptake rate, reaching an apparent steady state after fifteen days exposure, contrarily to organisms exposed to intermediate and high PCB-153 concentrations.

Comparisons with other studies focusing on the accumulation of PCBs on marine and freshwater fauna are normally difficult, since they are relatively scarce. In addition, the contaminants (Aroclors mixtures and/or different individual congeners), their intrinsic toxicity and the sources of contamination are in most cases very distinct. Nevertheless, results similar to those observed in the present study were previously demonstrated in the grass shrimp *Palaemonetes pugio* after exposure to different water contaminated treatments (Nimmo et al., 1974). For lower concentrations of Aroclor 1254 (0.09 µg L<sup>-1</sup>), accumulation became more evident on the third day, appearing to stabilize after 21 days of exposure. On the other hand, for a higher concentration of Aroclor 1254 (0.62 µg L<sup>-1</sup>), accumulation began earlier, after only 8 h exposure, and tended to follow a linear model. This indicates that somewhere in the range of 0.09–0.62 µg L<sup>-1</sup>, accumulation of Aroclor 1254 follows a more linear pattern instead of a saturation model. The same analogy may be transposed to our results.

In addition, observations reported by Tatem (1986), investigating bioaccumulation of PCBs from contaminated sediments by the freshwater *Palaemonidae* prawn *Macrobrachium rosenbergii*, also corroborate our results. When exposed to sediments containing low concentrations of Aroclor 1254, *M. rosenbergii* followed an accumulation pattern described by a first order kinetic model, reaching a steady state after sixteen days of exposure. Moreover, the uptake rate ( $k_{11}$ ) from a mixture of 10% contaminated sediment and 90% of clean sand was identical to that obtained in the present study, for the lowest PCB-153 treatment.

On the contrary, Rubinstein et al. (1983) reported contrasting results from those described above. By using four different PCB

contaminated sediments, in which *P. pugio* were exposed for 58 days, Rubinstein et al. (1983) observed that accumulation in shrimp did not show a well-defined pattern, varying erratically, independently of the sediment at which they were exposed. Also, organisms under high PCB levels, reached maximum concentrations in their tissues after approximately 26 days of exposure. From that time onwards, the PCB concentration decreased, possibly indicating a detoxification mechanism (Rubinstein et al., 1983).

Bioconcentration factors suggest that PCB-153 accumulation after a fifteen-day period is not proportional for all levels of contamination, since shrimp exposed to treatment II, which corresponds to a concentration 10 times higher than those of treatment I, reached accumulations 30 times higher than those of treatment I. Likewise, organisms exposed to treatment III, corresponding to a concentration 10-fold higher than the treatment II and 100 times higher than the treatment I, achieved accumulations of 7.5 and 250 times higher than those of treatments II and I, respectively.

Apparently, none of the PCB-153 concentrations was lethal for the shrimp. However, increases in respiratory activity of shrimp exposed to PCB-153 treatments might have occurred, since toxic stress is almost instantaneous, even though the PCB concentration was very low (Reyes et al., 2003). Furthermore, several authors have suggested that shrimp show great susceptibility to PCBs, especially during molt (Duke et al., 1970; Nimmo et al., 1971).

The concentration of a chemical in air or water, which is expected to cause death in 50% of the test animals in a given time period is known as  $LC_{50}$ . This measure has been estimated for several shrimp species to assess their response to a wide range of contaminants. For example, the  $LC_{50}$  of Aroclor 1254 for *Palaemon adspersus* is  $1 \text{ mg L}^{-1}$  (Reyes et al., 2003) while for the shrimp *Palaemonetes kadiakensis* is considerably smaller, only  $3 \mu\text{g L}^{-1}$ . Furthermore, a mortality of 51% was observed in pink shrimp (*Penaeus duorarum*) within fifteen days of continuously exposure to  $0.94 \mu\text{g L}^{-1}$  of Aroclor 1254 (Stalling and Mayer, 1972). This means that response to Aroclor 1254 may be species specific and dependent on the physiological susceptibility of each species. With this respect, the behavior demonstrated by *P. varians*, when exposed to different PCB-153 levels, appeared to be more similar to the one observed in *P. adspersus*, since they can tolerate higher concentration ranges.

Regarding the decontamination period, shrimp transferred to clean seawater began immediately to lose PCB-153, as observed by Tatem (1986). This decline was faster in the first three days, especially for treatment II. Overall, after fifteen days of decontamination, shrimp lost around 35%, 60% and 50% of the PCB-153 previously accumulated, corresponding to treatments I, II and III respectively. Thus, higher losses were recorded in shrimp under intermediate contamination levels, which is consistent with results of Tatem (1986) for a depuration period slightly longer (twenty days) and for both Aroclors 1242 and 1254. In opposition, Nimmo et al. (1974) reported higher losses of Aroclor 1254 in *P. pugio* after 14 days of decontamination (50–80%).

Despite great declines in PCB-153, it is important to highlight that even after 15 days in clean seawater, the organisms were not able to recover completely and at the end of the experiment maintained levels comparable to those after three days of exposure. According to Bodin et al. (2007) the high levels and prevalence of PCBs in crustacean tissues reflect their low rates of biotransformation, and particularly the inability of these organisms to metabolize congeners with chlorine atoms in positions 2, 4, and 5 in one (PCB-118 and 138) or both rings (PCB-153 and 180). Even after decontamination, shrimp that were exposed to the lowest PCB-153 concentration of  $0.25 \mu\text{g L}^{-1}$ , which represent half the maximum limit allowed for PCBs congeners in drinking waters, still maintained  $11 \text{ ng g}^{-1}$  ww in their tissues.

The Norwegian Scientific Committee for Food Safety stated that PCB-153 corresponds roughly to 1/3 of the summed six indicator congeners ( $\Sigma_6$  PCB) (VKM, 2008), which in turn comprise about 50% of the amount of total non-dioxin-like PCBs (ndl-PCBs) ( $\Sigma_{197}$  PCB) present in food (EFSA, 2010). Taking this into account, the organisms accumulated about  $33 \text{ ng g}^{-1}$  ww and  $66 \text{ ng g}^{-1}$  ww, extrapolating directly as  $\Sigma_6$  PCB and  $\Sigma_{197}$  PCB, respectively. This evidence is of critical significance, since exposure of shrimp to half the PCB concentration allowed for drinking waters, caused an accumulation of approximately 50% the maximum limit of  $\Sigma_6$  PCB established by the European Union (Commission Regulation (EU), 2011 Regulation no 1259/2011) for muscle meat of fish and fishery products ( $75 \text{ ng g}^{-1}$  ww). Similarly, for intermediate and high levels of PCB-153, organisms largely exceeded that limit, incorporating around  $700/1400 \text{ ng g}^{-1}$  ww and  $6135/12,270 \text{ ng g}^{-1}$  ww, by direct conversion as  $\Sigma_6$  PCB and  $\Sigma_{197}$  PCB, respectively. Based on these formulations and knowing that accumulation of PCB-153 was not proportional, it is reasonable to infer that for a PCB-153 concentration equal to the threshold for drinking waters, shrimp will likely exceed the maximum limit of  $\Sigma_6$  PCB imposed for human consumption. Nevertheless, there is no consensus among European and United States agencies relatively to regulations of PCBs in food. For instance, according to the United States Food and Drug Administration (US-FDA) only concentrations achieved by shrimp under high PCB-153 levels may be harmful to human health, by far exceeding tolerance level of  $2000 \text{ ng g}^{-1}$  for PCBs in shellfish.

These findings raise concerns, since most organisms did not return to safety values (for food consumption). Facing a hypothetical PCBs leakage, here simulated by the high PCB-153 treatment, the consequences for the ecosystem and ultimately to human health might be dangerous and the recovery of the habitat would be compromised. Further research on the transfer of PCBs from abiotic compartments (e.g. water and sediments) to marine edible species is needed, in order to estimate levels of contaminants that will accumulate in their predators and consequently to assess their impact on the biomagnification process through the food webs.

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