Discussion
DISCUSSION

PCBs and DDT and its metabolites are persistent organic pollutants, that bioaccumulate in animals and may present deleterious effects to wildlife, and to humans. PCBs are no longer produced and DDT is only applied in punctual situations, nevertheless, these compounds, or their degradation products, remain in the environment for long periods, become widely distributed geographically, accumulate in the fatty tissue of living organisms and are toxic to humans and wildlife particularly in marine environments (Geyer et al., 2000). Their study is therefore a matter of concern for all the environmental protection and food safety agencies, and the World Health Organization (WHO). This thesis presents the bioaccumulation of PCBs and DDTs in two fish species: sardine, from natural environment; and seabass from natural environment and from fish farming.

The bioaccumulation in sardine will be discussed over an annual reproductive cycle, taking into account the chemical structure of PCBs, namely the number and position of the chlorines in the molecule. Besides reporting the organochlorines levels in seabass and water, it will evaluate the accumulation mechanisms, uptake and elimination pathways through bioaccumulation factors and bioaccumulation models. Metabolization also will be checked by quantification of hydroxylated PCB metabolites, and biological effects will be evaluated by accessing phase I and phase II biotransformation enzymes.

This work can also be useful as a model for other fish species and other persistent organic pollutants (POPs), such as the emerging contaminants (polybrominated diphenyl ethers – PBDEs, polyfluorinated compounds – PFCs) (Barber, 2003). In fact, due to financial, practical and ethical constraints most species and substances of interest for environmental management are not monitored at all relevant locations and periods. To allow risk assessment for many species and substances, and different locations and periods, results from monitoring programs should be checked for consistency with data from other studies, and for this reason accumulation models were developed (Veltman et al., 2005). In the present thesis two kinds of models were applied: bioaccumulation factors that correlated bioaccumulation in fish with one source of contaminants; and mass balance models that allowed evaluating the uptake and elimination of organochlorines through several pathways at the same time. Both models were adjusted and validated to seabass, in Ria de Aveiro and in fish farming. The mass balance model OMEGA presented by Hendriks et al. (2001) and Hendriks and Heikens (2001), was validated to seabass in laboratorial conditions and applied to the coastal lagoon with good results.

It has been recognised for the last years by international organisations and environmental agencies that risk assessment can not be solely based on chemical analysis of
environmental samples, because this approach does not provide any indication of the deleterious effects of contaminants on the biota (Cajaraville et al., 2000, Martín-Díaz et al., 2004). Various biochemical parameters in fish have been tested for their responses to toxic substances and their potential use as biomarkers of effect (Handy, 2003). The biomarkers were tested as a faster and cheaper tool to monitoring environmental contaminants, such as PCBs, PAHs and others (Goksoyr and Forlin, 1992; Whyte et al., 2000; Ferreira et al., 2004; Ferreira et al., 2006). Biomarkers applied in both laboratory and field, can provide an important linkage between laboratory toxicity and field assessment (Handy et al., 2003). In this thesis the biological effects of PCBs were assessed by evaluating biomarkers (hepatic EROD and GST activities) in seabass exposed to sublethal concentrations of PCBs.

**Influence of gender and reproductive cycle in organochlorines accumulation in sardine**

To study the influence of the reproductive cycle in organochlorines accumulation, sardine (*Sardina pilchardus*) was selected because it is known that the reproductive cycle of sardine is associated to a large variation in lipid content (Bandarra et al., 1997; Soares 1999). The annual fluctuation of the levels of PCB congeners in muscle, liver and gonads was analysed in sardine from the Portuguese coast, and it was examined the effect of the chemical structures on the mobility of PCB congeners in sardines, males and females.

To identify the reproductive cycle of sardine the maturity phases of gonads were evaluated by morphological analysis and gonado-somatic index (GSI). Both males and females exhibited a pronounced seasonal variation in GSI (Fig. 2, page 34), the increase in GSI occurred sharply in October (males) and November (females) and remained relatively constant until January. It was verified that the high values of GSI are indicative of the spawning period (Ferreira et al., 2004), showing that the spawning period of the sardine used in this study was from the end of October to the end of January. In both genders, lipids increased before the spawning period (Fig. 3, page 35), and reached a maximum in September (in liver and in gonads) and November (in muscle). Muscle lipids constitute an energy reserve to be consumed during the maturation period and are representative of the other reserves of the organism. The evolution of GSI and lipid content in muscle was in accordance with a previous study which related lipid content in muscle to the reproductive cycle, and attributed lower levels after spawning due to fat mobilization (Bandarra et al., 1997).
Seasonal variation of total PCB concentrations in muscle showed low values from February to May, a gradual increase in the following months reaching a maximum in November, and a significant decrease in January. During the accumulation period, the increase in PCB levels was proportional to increase in lipids content, in all tissues, but when sardine started to consume their energy reserves different patterns of eliminations were found. The PCB concentrations in muscle of females were significantly lower than in males in February, showing a higher elimination of PCBs by females at the end of spawning period. The PCB composition observed in sardine samples was comparable to results obtained with various marine organisms, which reported the hexachlorobiphenyls CB-153 and CB-138 as major contributors, and lower chlorinated congeners being present at very low concentrations (Bayarri et al., 2001).

Individual congeners concentrations normalised to the dominant CB-153, as usually followed in several works (Bayarri et al., 2001; Hoekstra et al., 2002; Li et al., 2003), allows to compare the differences in individual congeners without the interference of seasonal variations on total PCBs. During the annual period of this study, the CB153-normalized values of lower chlorinated congeners in liver were highly dispersed, possibly reflecting mobility from other tissues. The higher mobility of lower chlorinated PCBs was also described by Debruyn et al. (2004) and Antunes et al. (2007). In addition, levels in liver were significantly (p<0.05) higher than in muscle and gonads at the end of spawning period, reinforcing the hypothesis of higher mobility of lower chlorinated PCBs, when lipids are metabolized for energy production. In what concerns the higher chlorinated CBs, similar mean proportions in the three analysed tissues were found, which is in accordance with the limited mobility due their chemical structure.

To evaluate possible differences in PCB metabolization by sardine the quantified PCB congeners were divided in four groups as proposed by Boon et al. (1994) and Weisbrod et al. (2001). The metabolization of PCBs is dependent on the number and position of the chlorines in the molecule, congeners of group I (G(I) and II (GII) are characterised by higher number of ortho Cl atoms, which cause a non-planar configuration of their molecules. The products of their metabolization were not found in marine mammals unlike congeners of group III (GIII) and IV (GIV) which elimination is favoured by vicinal H and lower number of ortho Cl atoms (Boon et al., 1994).

To the sardines analysed in this study, muscle was the major contributor to the total PCB body burden (45-92%) and since sardines presented a broad seasonal fluctuation in lipids, it was expected a considerable mobility of PCBs from muscle. The CB concentrations in females varied logarithmically with lipids (Fig. 4, page 46), implying that after the increase of CBs until November levels decreased rapidly to the initial values.
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This seasonal fluctuation means that female sardines regulate the excess of congeners sequestered during fatten period. The study of CB groups points that, in females, elimination did not vary substantially with their chemical structures. However, a different behaviour was found in males. Indeed, most of the points corresponding to GIII+GIV were projected close to the female fitting line, but points of GI+GII were far above the respective line (Fig. 4, page 46). This indicates that mobility of compounds included in GI+GII was slower in males than in females as lipids were consumed during gonad maturation. Moreover, at low lipid conditions, levels of GI+GII were still high, which suggests that after an annual cycle their concentrations do not reach the initial values. The analysed sardines were expected to be 4-7 years old (Silva et al., 2008), and thus the differences observed between the two genders may result from accumulated residues in males after consecutive annual cycles.

In liver the CB concentrations showed opposite variation to lipid content (Fig. 4, page 46). This was in contrast with Hebert and Keenleyside (1995) who showed that PCBs fluctuations should be analogous to lipid contents, due to their lipophilicity. Poor correlations, both in males and in females, were found between levels of GI+GII, or GIII+GIV, and lipid content. However, males contained in general higher GI+GII than females in periods of lower lipids. This enhancement suggests that release from the liver during the final period of spawning was insufficient to compensate the compounds arriving to the liver. This insufficiency was more accentuated for the compounds included in GI+GII in males (Fig. 3, page 45).

A similar tendency of higher PCBs levels in the period of lower lipid content was observed in gonads, but gonads of males and females had comparable concentrations of PCBs during the final period of spawning. The few studies that presented gender related differences in the accumulation of PCBs fish, attributed the differences to the higher lipid content of female gonads and to egg release. Johnston et al. (2002) quantified PCB and p,p'-DDE levels in walleye (Stizostedion vitreum) populations, and concluded that males generally had higher burdens than females at large body sizes but not at small body sizes and justified the differences by the successive annual egg releases by females. Bodiguel et al. (2008) applied a bioaccumulation model to hake (Merluccius merluccius). This model includes a differentiated elimination in males and females due to spawning. Hake from the Gulf of Lion presented a evolution of CB153 with fish length very distinct between genders. Bodiguel et al. (2008) obtained a good agreement between simulated and measured concentrations of CB153, indicating that spawning had a significant influence in elimination of CB153. Vives et al. (2005) studied the evolution of PCBs and DDTs with age in brown trout (Salmo trutta) and found that concentration of these contaminants
increased with fish age, and after some years some compounds presented higher concentrations in males than in females, justified by a slightly higher organochlorines detoxification capacity of females, e.g. during spawning. But these differences could be metabolic and not only a consequence of eggs release, females may have higher capacity to transform $p,p'$-DDT to $p,p'$-DDE than males.

All these results showed that spawning was very significant in organochlorines elimination, presenting females a distinct capacity comparing to males. In sardine the differences in elimination between genders can not be entirely explained by eggs release, since there were no differences in the gonads PCB patterns, suggesting metabolic differences, as reported for trout (Vives et al., 2005).

In the same sardine samples, where PCBs were studied, it was also quantified the $p,p'$-DDT and its metabolites. The seasonal variation of total DDT (tDDT calculated as the sum of $p,p'$-DDT, $p,p'$-DDD and $p,p'$-DDE) concentrations followed the same pattern as total PCB: lower values between February and May, a gradual increase in the following months reaching a maximum in November, and a significant decrease in January. The tDDT concentrations in females muscle were significantly higher than in males in December, showing a faster elimination of DDT compounds by females at the beginning of spawning period. As discussed for PCBs, females appear to have a higher capacity to eliminate these compounds.

Concentrations of PCBs and DDTs in muscle of sardines caught near by the cape of Peniche were 3 to 5 times lower than levels reported for the same species in Adriatic Sea, (Perugini et al., 2004), and lower than the reported in sardine acquired in Spanish markets in January and February 2002 (Bordajandi et al., 2006) and in March to April 2005 (Bocio et al., 2007). Residues in sardines from Adriatic Sea were considered a consequence of the wide pollution by OCs in this shelter water body (Perugini et al., 2004; Stefanelli et al., 2004). Values were also considerably lower than those reported for the Atlantic Iberian waters in two studies, although the comparison was limited by different analytical procedures (Fernandez and Franco, 1976; Magalhães and De Barros, 1987), and comparable to tDDT concentrations observed in the Mediterranean Spanish coast for the period 1989-90 (MEDPOL Database).

In the environment $p,p'$-DDT is metabolized to $p,p'$-DDE. Vives et al. (2005) verified this transformation in brown trout, producing higher bioaccumulation factors of $p,p'$-DDE in older fish. Tsydenova et al. (2004) confirmed that when this compound accounts to more than 60% to the total DDT is interpreted as absence of recent inputs of pollution of $p,p'$-
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DDT. Values in the analyzed sardines from Peniche ranged from 68 to 98%, confirming the lack of recent inputs of \( p,p' \)-DDT to the coastal region.

**Organochlorines accumulation in seabass**

Seabass (\textit{Dicentrarchus labrax}, Linnaeus, 1758) was chosen for this study because it is one of the main species produced in aquaculture, reaching a production of 1 300 ton in 2003 in Portugal (official data from DGPA) and above 50 000 ton per year in the Mediterranean (FAO, 2006). Moreover, this species can also be caught in the Portuguese coast which allowed us to study PCB bioaccumulation behaviours in fish of different sizes and from different environments: a semi intensive fish farming, and a natural coastal lagoon. And in addition, a controlled laboratory exposure was also conducted with cultured seabass.

**Bioaccumulation of PCBs in seabass, cultured and from natural environment**

PCB congeners were quantified in three weight classes of seabass (Class I with about 15 g, Class II about 35 g and Class III about 250 g) from the semi intensive fish farm. The food and water were also analysed for the content of PCB congeners.

The concentrations of PCBs in water and SPM were not significantly different between tanks, and showed higher concentrations of lower chlorinated PCBs (tri- and tetrachlorobiphenyls). In fish tissues and food the congeners with higher concentrations were CB153 and CB138, and the total PCB concentrations were not significantly different between the three analyzed size classes. However, the lower chlorinated congeners (CB18, 44, 49 and 52) showed higher accumulation in smaller fish than in the adult fish. The dry weight concentrations of tPCB in the three size classes of seabass ranged from 24.8 ± 7.8 to 31.3 ± 10.1 ng g\(^{-1}\) in muscle, and in the diet pellet supplied to these fish was 22.0 ± 4.7 ng g\(^{-1}\), (representing a biomagnification of 1.1 to 1.4 fold). Other studies evaluating PCB contamination in cultured seabass, found the sum of the 7 indicator PCBs (N\(^{\circ} 28, 52, 101, 118, 138, 153\) and 180) up to 19 ng g\(^{-1}\) dry weight, representing a biomagnification of PCB from commercial feed concentrations from 1.9 to 3.4 fold (Serrano \textit{et al.}, 2003). Fernandes \textit{et al.} (2008) reported values from 7.0 to 19.1 ng g\(^{-1}\) dry weight, representing a biomagnification of PCBs from feed of about 10 fold. Our result showed similar accumulation levels, but significantly lower biomagnification from the commercial pellets.

The levels of organochlorines have been measure in farmed fish for some years, in particular in salmonid cultures. Easton \textit{et al.} (2002) found mean concentrations of PCBs
(sum of 112 congeners) of 171 ng g\(^{-1}\) dry weight in farmed salmon, while mean concentration in wild salmon was 17.7 ng g\(^{-1}\) dry weight. This difference was attributed to the high level of contaminants found in commercial salmon feed. These levels were considered of concern for individuals who consumed farmed salmon on a regular weekly basis, according to WHO (1998) and Environment Canada (2000) guidelines. Jacobs et al. (2002) in a similar study, found comparable results (total PCB concentrations from 8.8 to 347 ng g\(^{-1}\) dry weight). Hites et al. (2004) reported values up to 167 ng g\(^{-1}\) dry weight in farmed salmon, and the lowest values of 7 ng g\(^{-1}\) dry weight in wild salmon. The report of these higher levels of organochlorines in farmed salmon lead to a temporary reduction of sales, and the industry was forced to introduce changes in feed production, in order to reduce organochlorines levels. The contents reported in farmed salmon before 2004, that revealed being of concern to public health, were higher than the ones in this thesis. For wild salmon the levels were at the same order that the values obtained in this study for farmed and wild seabass.

In order to examine the influence of different uptake pathways to seabass PCB contamination, several bioaccumulation factors can be used (as eg. Mackay and Fraser, 2000; Burkhard et al., 2003): The bioaccumulation factors (BAF), biota-SPM bioaccumulation factors (BSMAF) and biomagnification factors (BMF), that were applied in this study. In general, log BAF and log BSMAF showed good correlations with log \(K_{\text{ow}}\), for congeners with log \(K_{\text{ow}} > 6.1\). In the three fish size classes, PCB congeners with log \(K_{\text{ow}} < 6.1\) were found at concentrations higher than what was expected based on the BAF calculated for the more hydrophobic congeners. The calculation of fugacity (Campfens and Mackay, 1997) of dissolved fraction and SPM showed that both compartments reached equilibrium, and therefore the accumulation pathway from both SPM and water are identical, in accordance to what is usually assumed that the uptake through gills is only possible if the PCBs are dissolved in water (Gobas et al., 1986; Gobas, 1992). The BMF values were, in general, higher than 1 (1 – 5.6) and for compounds with log \(K_{\text{ow}} > 6.1\) were not significantly different. PCBs with log \(K_{\text{ow}} < 6.1\) had higher BMF (2.5 – 5.6 against 1 – 3.5), suggesting a different contribution of the accumulation pathways for different congeners. However, these empirical models do not explain the differences, once they reflect only one of the accumulation mechanisms (Mackay and Fraser, 2000; Burkhard et al., 2003).

The mass balance model presented by Clark et al. (1990) and Mackay (2001) describes the bioaccumulation of organic chemicals by fish from food and water, using species-dependent parameters. The model was applied for accumulation of five PCB congeners in seabass, as example. Estimated concentrations were 3 to 38 fold higher than the
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measured values, probably by overestimating gill absorption (Fig. 3, page 54). The model was applied with other scenarios in order to maximize uptake from food, which may have a significant influence in accumulation, and not only from diet pellets. These scenarios produced estimated BAF of 0.90– to 2-fold the measured values (Fig 4, page 55). Even in the scenario with higher influence of food, the diet pellets had low relative contribution to fish contamination. The relative importance of the pathways changes with chlorine content of PCBs, showed for highly chlorinated PCBs that they may contribute with a maximum of 54-64%, and for lower chlorinated PCBs its contribution was less than 20%. Our results points to the need to control the water quality, if necessary to reduce the contamination in farmed fish.

To confirm if the bioaccumulation and relative importance of accumulation pathways established to the semi-intensive fish farm will be valid to wild seabass, a similar study was conducted in the coastal lagoon of Ria de Aveiro, where seabass were captured and divided by size classes close to the ones from fish farm (Class I with about 13 g, Class II about 50 g and Class III about 150 g).

In seabass from Ria de Aveiro, lipids explain 69% of the variability in dry weight tPCB concentrations, and lipid normalized PCB concentrations in muscle had no significant differences between the analyzed fish. This dominant influence of lipid content on organochlorine compounds concentrations in aquatic biota is well established in the literature (Hebert and Keenleyside, 1995; Bentzen et al., 1996; Pastor et al., 1996; Kucklick and Backer, 1998). In fact, there were differences in individual congeners pattern of the three size classes, in juveniles the lower chlorinated congeners (CB18, 44, 49 and 52) showed higher accumulation than the more chlorinated CBs. These differences followed the same pattern (even more evident) found in the fish farm, the wild juvenile seabass presented higher accumulation of lower chlorinated PCBs than juveniles in the semi-intensive culture.

To explain the differences between contribution of the two accumulation pathways, and the variation of the accumulation with fish size, we used a model that takes into account these two factors. The OMEGA model simulates the bioaccumulation of lipophilic organic compounds in fish, based on compounds properties, species-dependent parameters, and on allometric parameters of fish (including fish growth rate, and feeding rate) (Hendriks, 1995; Hendriks et al., 2001; Hendriks and Heikens, 2001; Van der Linde et al., 2001). The OMEGA model predicted a systematic overestimation of levels, suggesting that the measured dissolved concentration was not correct, probably because it included the
PCBs adsorbed in some particles smaller than filter pore size increasing the measured values in relation to the real ones. Dissolved concentrations were recalculated, based on suspended particulate matter (SPM) concentrations and the sediment-water partition coefficient (Kp). The estimated concentrations ranged from 0.84 to 2.2 of the measured in Ria de Aveiro, showing that the OMEGA model is valid to seabass from this site.

Papers reporting model application usually just compare the estimated concentrations with the measured ones (as e.g. Gobas and Mackay, 1987; Morrison et al., 1995; Coristine et al., 1996; Campfens and Mackay, 1997; Veltmann et al, 2005; Brambilla et al., 2007). Our results bring new Information on the relative importance of the different accumulation pathways. In the wild seabass, PCBs with seven or more chlorine atoms (CB 170, 180, 183, 187 and 194) accumulated almost exclusively from food, the lower chlorinated PCBs (CB18, 44, 49 and 52) was mainly derived from the water, and for the other CBs contributions from water and food were similar (Fig. 4 page 65).

The only work found that quantified this different contributions is from Moermond et al., (2004) that determined the contribution of water, food and sediment to the uptake of PCBs in Carp (Cyprinus carpio) a benthivorous fish, and they found from 5 to 60% of PCBs uptake from water, being the contribution higher for lower chlorinated PCBs. These results are in accordance with the ones of our study. Other authors indicate that the most important contribution to accumulation is usually the food (Oliver and Niimi, 1985; Kucklick et al., 1996; Harding et al., 1997; Shawn et al., 2006; Berntssen et al., 2007; Maule et al., 2007), although other studies postulated a significant contribution of water (Fernandes et al., 2008).

**Uptake and elimination of organochlorines after exposure to sublethal concentrations in seabass**

To evaluate, the differences in the accumulation mechanisms, and elimination capacity of PCBs in juvenile and in adult seabass, a controlled exposure was performed. Fish of two size classes (juveniles weighting approximately 15 g and adults 250 g) were exposed to sublethal concentrations of selected PCB congeners in controlled conditions.

**Adults**

In the adult seabass PCB exposure produced a significant uptake in all tissues after one day. At the end of exposure (12 days), PCB concentrations presented two orders of magnitude higher than the initial values, being higher in liver. The absorption of PCBs by fish tissues revealed an average efficiency of about 80%, in agreement with the values
reported by Hendriks et al. (2001) and Veltman et al. (2005), and higher than those reported by Buckman et al. (2004). After the exposure period, PCB levels in muscle and liver still increased, before they started to eliminate PCBs.

The model OMEGA was also applied in the controlled exposure to allow a quantification of the different accumulation mechanisms. It produced estimations of the total body concentrations, within a factor about 1.5 to 7.0 fold higher than the measured in the individual tissues. The overestimation was in the accumulation period, during elimination the model estimations presented similar deviation to day 12.

The internal redistribution of PCBs between fish tissues appeared to be very relevant in adult seabass during the experiment. The amount of some PCBs in the analyzed tissues increased after the end of the exposure, up to day 40. The only explanation for the lower assimilation rate constants calculated in tissues is the possible accumulation of PCBs in visceral tissues that were not analyzed, and their posterior mobilization to internal tissues. For the more hydrophobic PCBs the increase of concentrations in muscle after the end of exposure period was more intense and evident, which indicates that higher chlorinated PCBs may present higher accumulation in visceral tissues, and have a slower internal distribution than lower chlorinated PCBs.

In opposition to our results, O'Connor and Pizza (1987) did not find significant differences in the PCBs' elimination rate constants for tissues and the entire body elimination rate constants, probably because their experiment was carried out with small striped bass (bellow 5 g) with low fat content. However, other studies presented similar evidences of internal redistribution of PCBs and other persistent organochlorines in fish. Debruyn, et al. (2004) and Kelly et al. (2007) have reported that salmon, during the pre-spawning period (when they migrate to reach native spawning grounds and fulfil reproduction, travelling several hundred of kilometres without feeding), consume their lipid reserves for energy, and consequently the lipophilic compounds present in that lipids are redistributed to the other tissues, namely muscle and gonads. Brambilla et al. (2007), verified that after exposing rainbow trout (Oncorhynchus mykiss) to food contaminated with PCBs and other organochlorines, the compounds revealed different accumulation within the tissues, and posterior redistribution. In a previous work with mullet (Antunes et al., 2007) a similar effect was observed during a starvation period. All these authors verified that the effect of internal distribution of PCBs in the fish was more significant for higher chlorinated PCBs, as described in the present study. We also verified that a high fat content in tissues and viscera, and bigger fish size produced higher differences in PCB accumulation in the tissues, due to the kinetics of internal PCB mobilization.
There are a great number of studies presenting BAF values of organochlorines in fish, with a range that varies from 0.15 to 50 (as e.g. Kucklick et al., 1996; Fisk et al., 1998; Serrano et al., 2008), and different correlations with hydrophobicity (Fisk et al., 1998; Kucklick and Baker, 1998; Maruya and Lee, 1998). These studies generally present the BAF in lipid basis without considering the physical condition of the fish and the possible influences of lipid mobilization in the organism. The present study indicates that internal organs (liver and viscera) can act as a contaminant sink, presenting a variation pattern different from the entire body. In case of increase of environmental levels of PCBs, the viscera accumulate organochlorines faster than the muscle, leading to a slower accumulation in edible tissue, factor that is enhanced if the organism has high lipid content. They also can release PCBs to the muscle if those levels in the environment decrease or if the fish consumes its fat reserves for energy (Debruyn, et al., 2004; Antunes et al., 2007; Brambilla et al., 2007; Kelly et al., 2007).

During this elimination period it was possible to estimate several elimination rate constants ($k_2$) (Fig. 4, page 73). From these elimination rate constants we may calculate half-life times ($t_{1/2}$) using the equation: 

$$t_{1/2} = \frac{\ln(2)}{k_2}$$

Values presented some variations with tissues, in muscle (the tissue that better represents the overall body concentrations) values ranged from 51 to 171 days.

Therefore, results from the present work may be compared with the half-life times reported by several authors. In fish the elimination of PCBs was studied in rainbow trout (Salmo gairdneri) by Niimi and Oliver (1983). These authors found that the biological half-life of PCBs elimination, in whole fish, ranged from 5 days (for dichloro-biphenyls) to no apparent elimination with the increase of the number of chlorines. Coristine et al. (1996) in the same species found half-lives from 47 (for CB77) to 210 (for CB126) days. In both cases elimination was faster for PCBs with lower chlorines content, with no chlorine substitution in the ortho positions, and those with two unsubstituted carbons that are adjacent (vicinal) in the biphenyl. Fisk et al. (1998) found half-life times in the same species that was according to the previous reported, but described that the highly chlorinated PCBs, log $K_{ow}$ > 7.0, presented a faster elimination than the expected, probably due to slower kinetics of these compounds inside the fish body. Also in rainbow trout, Buckman et al. (2007) verified that the water temperature influenced the elimination of recalcitrant PCB congeners, reported half-life times in the same magnitude as the previous, but reduced from 263 days at 8°C, to 190 days if water was at 16°C. Some other studies conducted with other species, Wang (1998) studied PCB elimination in striped bass (Morone saxatilis), fish when exposed to PCBs by different uptake pathways and
verified that, depending on the uptake pathway, in 20 days they could eliminate 31% (water exposure) or 71% (food exposure) of PCBs, for water exposure and combined exposure in water and food, respectively. Goerke and Weber (2001) studied *Platichthys flesus* and obtained half-life times from 9 to 69 days, they compared the PCB pattern found in wild fish with half-life times of the compounds, obtaining a good agreement. Paterson *et al.* (2007) studied elimination of PCBs in yellow perch (*Perca flavescens*) during an annual temperature cycle, and demonstrated that in summer the elimination kinetics were similar to those reported to other species. During the fall and winter, however, elimination of PCBs was not verified.

The elimination of PCBs verified in seabass tissues, was in accordance with the reported values, and, taking into account the large variations in reported $t_{1/2}$, this work is a contribution to better understanding the factors that play a role to these variations.

**Comparing elimination kinetics of juvenile and adult seabass**

Juvenile seabass presented higher elimination rates than adults, and differences between tissues elimination rate constants were lower than in adults, and closer to the total body concentrations estimated by OMEGA model (Fig. 1, page 79). However, it was still visible a trend for more hydrophobic PCBs to have faster elimination in blood and liver.

There are few studies comparing elimination rates between adult and juveniles. Buckman *et al.* (2006) found faster elimination in smaller rainbow trout. The lower differences observed between juvenile tissues was in accordance with O’Connor and Pizza (1987) that did not observed significant differences between tissues elimination rates in striped bass below 5 g, from the Hudson river.

Comparing the elimination rate constants of juveniles with the ones of adults we can conclude that the internal distribution in the smaller fish produce less differences than in the adults. This was expected if we consider that transport is controlled by passive diffusion of the compounds between tissues in the organism (Gobas, 1992; Meironyté Guvenius *et al.*, 2003; Soechitram *et al.*, 2004). PCBs elimination was faster in the juveniles, as expected by the theoretical model OMEGA, due to the higher respiration and food ingestion rate constants (*i.e.* higher respiration and food ingestion per fish mass). The transport of organochlorines from water and food, and the growth dilution of compounds are function of Weight $^{-0.25}$ (Hendriks, 1999), therefore all these elimination mechanisms are relatively higher for smaller fish.
Discussion

Metabolization

In general, the estimated concentrations were higher than the ones measured in tissues, and the differences increased with log $K_{ow}$. The relation between PCB metabolization and molecular structures is known, and it was verified that metabolization is faster in compounds with vicinal hydrogen atoms, in particular if one is in meta position, and for lower number of ortho chlorines (Boon et al., 1994; Weisbrod et al., 2001). The selected compounds for this study presented a theoretical metabolization that decreased with the chlorination degree, therefore if deviations from the model were produced by PCB metabolization, they would be higher for the less chlorinated PCBs in opposition to the verified. We can therefore conclude that metabolization was not significant when compared to the other elimination mechanisms.

Fish have a limited capacity to metabolize polyhalogenated contaminants, including PCBs, which tend to be eliminated largely as unchanged compounds to water via gills or faeces (Niimi, 1996; Fisk et al., 1998; Mackay, 2001). However, the role of metabolic biotransformation on the total elimination rate of polyhalogenated contaminants in fish remains unclear, in the way that most authors consider it negligible (Oliver and Niimi, 1985; van der Oost et al., 1996a; Hendriks and Heikens, 2001; Van der Linde et al., 2001; Arnot and Gobas, 2004). The developments in analytical techniques in recent years allowed to identify PCB metabolites in fish (Letcher et al., 2000), in particular the accumulation of OH-PCBs plasma. Li et al. (2003) and Valters et al. (2005) reported OH-PCBs in thirteen species of fish, OH-PCB ranged from about 1% to 157% the PCB concentrations.

In the PCB exposure all the metabolites investigated were detected in plasma of seabass, at concentrations below the quantification limit. The only metabolite that we could quantify was 4-OH-CB107, derived from CB105 (Hovander et al., 2002; Sandau et al., 2002). This showed that seabass was also able to metabolize PCBs, but due to the low concentrations found the metabolization rates were not possible to determine.

In the present study the ratio between OH-PCBs and total PCBs in plasma was lower than the described in other species (Li et al. 2003; Valters et al. 2005). In this experiment the accumulation of PCB metabolites in the plasma did not have a proportional increase with the PCB concentrations, showing probably that the metabolic enzymes were not able to metabolize PCBs in same proportion. When fish are in natural environment the OH-PCBs may accumulate progressively in fish plasma, despite the slow metabolization of PCBs (Li et al., 2003; Valters et al., 2005).
Phase I and Phase II enzymes

To confirm the metabolization of PCBs by seabass and to evaluate the possible application as biomarkers to PCB contamination, the activities of a phase I enzyme (ethoxyresorufin O-deethylase – EROD) and a phase II enzyme (glutathione S-transferases – GST) were measured. These biomarkers are among the most studied enzyme systems involved in metabolism of endogenous lipophilic compounds, as well as different xenobiotics (Bernhoft et al., 1994; reviewed by Whyte et al., 2000).

When exposed to the PCBs the seabass of the two size classes showed different responses in enzymes activities. In juveniles EROD activity was reduced when PCB concentrations increased in tissues, in opposition GST activity was induced and related to CB levels. In adults, despite the higher PCB concentrations, EROD activity was not affected by exposure; and GST activity was increased with exposure, although induction has been lower than in juveniles.

The absence of EROD induction in seabass exposed to high PCB concentrations was contrary to the expected. The presence of OH-PCBs in seabass demonstrates that P450 enzymes metabolized a small fraction of the PCB present in the fish. Also, most of the authors found an induction of hepatic EROD activity when fish were exposed to PCBs, in controlled laboratorial conditions (Brumley et al., 1995; Hylland et al., 1996; Guosheng et al., 1998; Mariottini et al., 2003; Buckman et al., 2006; Coimbra et al., 2007). However, in field conditions this response has been associated to a mixture of several compounds, like PCBs, PAHs, dioxins, and metals (Beyer et al., 1996; Stien et al., 1998; Schmitt et al. 2005; Ferreira et al., 2006; Mayon et al., 2006). Schmitt et al. (2005) demonstrated that the response of hepatic EROD activity was more intense to PAHs than to PCBs. The response is also species-dependent, as Bernhoft et al. (1994) showed with a positive induction of EROD in rainbow trout, but no response in cod (Gadus morhua). Kuzyk et al. (2005) reported, in shorthorn sculpin (Myxocephalus scorpius), a direct correlation between PCBs and EROD activity with a threshold concentration of about 50 ng g⁻¹ wet weight.

Despite the evidence of P450 system on PCB hydroxylation, we can not evaluate if it was the CYP1A1 (indirectly quantified by EROD), or other P450 related enzyme (CYP2B, CYP2C, CYP3A, etc.) that acted in PCB metabolization (Letcher et al., 2000). From the small amount of OH-PCB produced, and its low proportion in relation to total PCB, we may conclude that the contribution of CYP1A was not significant in PCBs metabolization in seabass.
An increase in hepatic GST activity usually benefits the organism since it is a detoxification enzyme and this study showed that, in seabass, this enzyme has an active participation in detoxifying these compounds, as it was also suggested by other authors (reviewed by Hayes and Pulford, 1995; Gallagher et al., 2001). In this exposure, it was possible to relate the GST activities with PCB concentrations. Despite there was not a direct correlation of GST activity with PCB levels, the GST was negatively correlated with the variation of CB concentrations in muscle, showing that the activity was higher when contaminant levels decreased. Correlations obtained with the lower chlorinated CBs were better than the correlations with higher chlorinated PCBs, other authors have demonstrated that the lower chlorinated PCBs are easier to metabolise (Boon et al., 1994; Goerke and Weber, 2001; Weisbrod et al., 2001; Buckman et al., 2006).

Some other studies lead to similar conclusions. In a depuration experiment with mullet Ferreira et al. (2006) and Antunes et al. (2007) verified that the GST activity increased at 4 and 8 month, when the levels of PCBs were decreasing. Forlin et al. (1996) obtained a marked induction of GST activity 15 or 20 weeks after the exposure of rainbow trout to PCBs, in a controlled laboratory experiment. However, Mayon et al. (2006) did not found any relation between GST and PCB levels in wild fish, and Gallagher et al. (2001) demonstrated a decrease in GST activity in Lake Apopka brown bullheads (Ameriurus nebulosus) exposed to several organochlorines including PCBs.

Finally seabass showed to be able to metabolize some PCBs, at a reduced extent. Despite of that, the interpretation of results of EROD and GST activity is ambiguous, as they are not specific to these contaminants. In field studies they might be useful to evaluate the general state of fish contamination, although they cannot be directly correlated to the presence of PCB.