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Organochlorine compounds in liver and concentrations of vitellogenin and 17β-estradiol in plasma of sea bass fed with a commercial or with a natural diet

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Abstract

Results from previous experiments directed to determine the effect of different nutritional factors or the effect of xenobiotics on hormonal control of reproduction, lead to the hypothesis that hormonal perturbations repeatedly observed in sea bass (*Dicentrarchus labrax*) broodstock feeding commercial diets could have been caused by the presence of aryl hydrocarbon receptor (AhR) ligands, such as dioxins, furans and polychlorinated biphenyls (PCBs) in the diet. To evaluate this hypothesis, dioxins and related compounds were analysed in liver of female sea bass fed with a commercial or with a natural diet consisting of trash fish (bogue, *Boops boops*), and concentrations of vitellogenin (VTG) and 17β-estradiol (E2) were determined in plasma obtained previously in monthly samplings of these animals. As observed in other experiments, females fed with a commercial diet exhibited lower VTG and higher E2 plasma levels than females fed with the natural diet. In liver, sea bass fed with the commercial diet exhibited a profile clearly dominated by high-chlorinated dioxins while in fish fed with the natural diet this profile was dominated by low chlorinated furans. However, typical AhR ligands, such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin showed no differences between groups or, as is the case of planar PCBs, showed higher concentrations in the liver of fish fed with the natural diet. These results do not permit to explain the observed hormonal alterations by a possible antiestrogenic effect caused by dioxins and related compounds.

Keywords: Reproduction; Dioxins; PCBs; Vitellogenin; Estradiol; Sea bass

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1. Introduction

A wide variety of chemicals are able to interact with the endocrine systems of humans and wildlife leading to endocrine disruption (Colborn and Clement, 1992; Kime, 1995; Kavlock et al., 1996; Jiménez, 1997; Vos et al., 2000). These compounds can mimic or antagonize the action of hormone receptors, or could also disturb the hormone metabolism. All these chemicals, known as endocrine disrupters, can finally lead to alterations of growth, development, sexual differentiation and reproduction.

Special attention has been paid to the interference of chemicals with the action of estrogens, particularly with that of the prototypical estrogen 17β-estradiol (E2), due to the importance of these hormones in regulation of developmental and reproductive processes in vertebrates. Compounds with estrogenic properties exert their hormonal activity via the classical receptor pathway of steroid hormone action, i.e. they bind as ligands to the estrogen receptor (ER) and the ligand–receptor complex subsequently activates transcription of target genes (Tsai and O'Malley, 1994). One of these genes is the gene for vitellogenin (VTG), a glycolipophosphoprotein that serves as precursor of the egg reserves. VTG is synthesized in the liver of oviparous females under the stimulation of estrogens, particularly that of E2 (Wallace, 1985). The presence of VTG in plasma of male fish can be caused by an estrogenic effect of chemicals present in aquatic environments (Harries et al., 1996).

Contrary to chemicals exerting estrogen-like activities, a variety of non-steroidal substances possesses antiestrogenic features, i.e. these compounds antagonize or inhibit estrogen-dependent processes in the target cells (Navas and Segner, 1998).

Antiestrogenicity can be caused by direct interaction of chemicals with the ER, when these chemicals act as antagonists and/or partial agonists of the ER. This is the case of tamoxifen (Tzukerman et al., 1994). In addition, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), and related compounds such as other polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), some polychlorinated biphenyls (PCBs) and some polycyclic aromatic hydrocarbons (PAHs) also exhibit antiestrogenic activity (Safe, 1995). All these compounds activate the aryl hydrocarbon receptor (AhR), which then interacts with the ER-dependent pathway (Safe et al., 1991; Safe, 1995; Ohtake et al., 2003). Antiestrogenicity caused by these compounds in fishes has been normally detected through reductions in the production of vitellogenin (VTG) in vivo (Anderson et al., 1996a) or in fish hepatocytes in vitro (Anderson et al., 1996b; Navas and Segner, 2000). However, antiestrogenic compounds do not only affect the production of VTG but they can also disrupt the global hormonal control of reproduction in fishes. For instance, in sea bass (*Dicentrarchus labrax*), the ingestion of β-naphthoflavone (βNF) through the diet caused an increase in plasma levels of E2 and luteinizing hormone (LH), concomitantly with a reduction in the plasma concentration of VTG (Navas et al., 2004).

Similar hormonal alterations, i.e. an increase in plasma levels of E2 simultaneously with a decrease in plasma levels of VTG, have also been observed in sea bass fed with a commercial diet (Navas et al., 1998). On the contrary, sea bass feeding a natural diet consisting of trash fish (bogue, *Boops boops*) exhibited higher plasma levels of E2 and lower plasma concentrations of VTG than fish fed with the commercial diet. These differences were associated with differences in the fatty acid composition of the diet that also influenced the fatty acid composition of the eggs (Navas et al., 1998).

In a later experiment, designed to determine if the xenobiotic β-naphthoflavone (βNF) was able to cause in vivo antiestrogenic effects (Navas et al., 2004), as had been previously detected in vitro (Navas and Segner, 2000), it was also observed that βNF-treated broodstock sea bass exhibited lower plasma VTG levels and higher plasma concentrations of E2 with respect to control animals. These results lead to the hypothesis, that hormonal differences detected between fish fed with trash fish and fish fed with a commercial diet could be caused by the presence of antiestrogenic chemicals in the commercial diets inducing similar effects as βNF. In order to evaluate this possibility, VTG and E2 were analysed in sea bass plasma samples taken monthly from two broodstock groups fed with trash fish or with a commercial diet, and PCDDs, PCDFs and PCBs concentrations were analysed in liver of females at the end of the experiment after 2 years of treatment.

2. Material and methods

2.1. Fish and feeding treatments

Fifty male and female 3-year-old sea bass (mean weight and length ± standard error of the mean: 567.8 ± 20.3 g and 35.7 ± 0.3 cm, respectively) were reared at the Instituto de Acuicultura de Torre de la Sal, CSIC (East coast of Spain, 40°N, 0°). During rearing, larvae were first fed with *Artemia salina* nauplii, and then with commercial food during the first 6 months of life. After that, animals received natural food (bogue, *Boops boops*, considered as trash fish, obtained from the local fish market and freezed. They were cut to little pieces just before feeding the sea bass broodstock) without a concrete feeding schedule until the beginning of the experiment. At the beginning of the summer, the fish were divided into two groups. Each group contained 24–26 fish and the male/female ratio was approximately 1.5/1. They were kept in 2000 l tanks supplied with aerated flow-through sea water (37.8 ‰ salinity) and maintained under natural photoperiod and temperature (temperature range: 10 °C in January to 26 °C in August).

In the experiment, one group was fed with whole trash fish (*Boops boops*). The other group was fed with commercial diet pellets (9 mm pellet, BOCM Pauls, Renfrew, Scotland). The composition of this diet (Table 1) was provided by the manufacturer, who controlled it regularly. Fish were fed five days a week at a ration of 2.2% of tank biomass per day, from June to October, and 1.2% of tank biomass the rest of the year. The experiment started in July and lasted for 22 months.

2.2. Sampling procedure

All animals were sampled at monthly intervals until the beginning of the second spawning season. Before sampling, the fish were anaesthetised with ethyl *m*-aminobenzoate metanesulfonate (MS-222, 0.1 g l^{-1}) after one day of starvation. Weights were recorded and the tank biomass was calculated in order to adjust the ration for each group. Blood was withdrawn from the caudal vessels with a heparinized syringe and dispensed in ice-cold heparinized tubes. Blood samples were always collected at the same time of the day (between 11 and 14 h). Plasma was obtained by centrifugation (1500 × *g* for 30 min at 4 °C) and stored at −20 °C until analyzed.

Sea bass are pelagic spawners and during the spawning season, females and males released their ova and sperm spontaneously into the tanks. The eggs were collected from the outflow water of the tanks in fine meshed nets. The nets were checked daily and the incidence of spawning recorded. The period between the first and the last spawning was considered as the spawning season, and the mean spawning date was calculated as the mean of the number of days passed between the first and the successive spawnings; i.e. if the first spawning occurred on the 2nd of December, the second on the 15th and the third on the 1st of January, the series of numbers used to calculate the mid spawning time was 1, 14, and 31 (Zanuy et al., 1995). Spawning period and the mean spawning time are represented in Figs. 1 and 2, in order to make the results of VTG and E2 plasma concentrations clearer.

After 2 years with the described feeding regimes, i.e. when animals were 5 years old, some females were anaesthetised by immersion in ice and sacrificed. A sample of liver was taken, immediately frozen in liquid nitrogen, and stored at −80 °C for the chemical analysis of organochlorine compounds.

Table 1
Proximate composition (percentage of dry weight) of the pelleted diet and the natural diet (trash fish, *Boops boops*) used in the experiment

	Natural diet	Commercial diet
Dry weight (%)	23	91
Proteins	71	60
Carbohydrates	Not detected	19
Lipids	20	10
Ash	9	10

2.3. Vitellogenin and 17β-estradiol analysis

Vitellogenin and E2 analysis were performed in a total of seven or eight female plasma samples every month (the plasma from the same females was always analysed). Plasma VTG levels in females were measured using a homologous ELISA, as described by Mañanós et al. (1994). The range of the standard curve was 1 to 60 ng/ml, corresponding to 85–20% of binding, respectively. Depending on the reproductive stage of the females, appropriate dilution factors (from 10-fold to 100,000-fold dilution) were used for the plasma

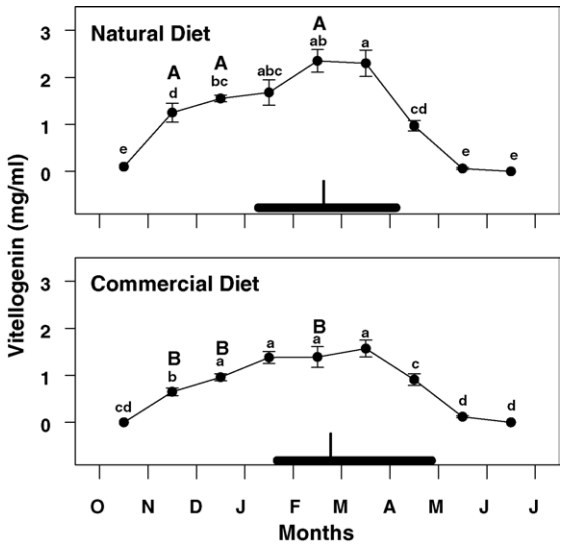

Fig. 1. Plasma vitellogenin levels throughout the reproductive cycle in female sea bass fed with a natural diet (trash fish) or with a commercial diet. Data are expressed as mean ± S.E.M. ($n = 7$ or $n = 8$ fish per sampling point). Horizontal bars at the bottom of each graph represent the spawning period in each group and the little vertical bar indicates the mid spawning time. Different letters indicate significant differences ($P < 0.05$). Statistics were performed between groups for the same sampling point (*t*-test was used and differences are indicated by uppercase letters), and between sampling points for the same group (ANOVA followed by Student–Newman–Keuls was used and differences are indicated using lowercase letters).

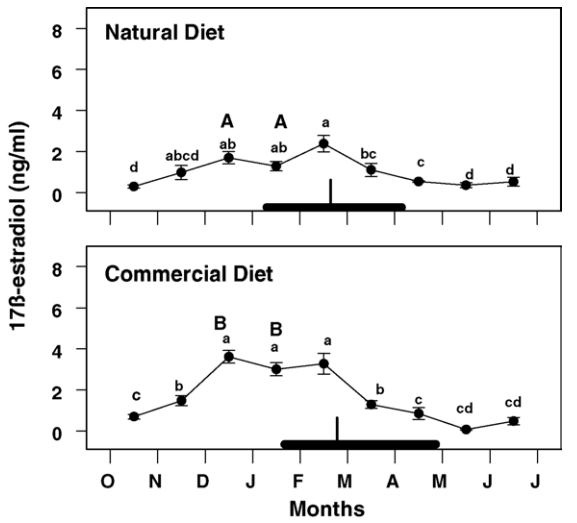

Fig. 2. Plasma 17β-estradiol levels in female sea bass along an entire reproductive cycle, in fish fed with a natural diet (trash fish) or with a commercial diet. Data are expressed as mean ± S.E.M. ($n = 7$ or $n = 8$ fish per sampling point). Legend and signs of the figure are similar to those in Fig. 1.

samples, in order to place all measurements within the confidence range of the standard curve.

E2 was extracted from 100 μl of plasma using cyclohexane:ethylacetate (1:1, v/v) and E2 concentrations were determined by radioimmunoassay (RIA), according to Prat et al. (1990). The range of the standard curve was 150–8000 pg/ml, corresponding to 85–20% of binding, respectively.

2.4. Analysis of organochlorine compounds in liver samples

Due to the complexity of the extraction procedure of the organochlorine compounds from the biological samples, the analysis of these compounds was performed first in four and three liver samples of females fed with the commercial or with the natural diet, respectively. These samples were randomly selected without taking into account the previous physiological status of the female. Since analyses were performed following standardized methods (see below) and no strong variations between samples were found for the majority of the compounds used, it was decided to limit the analyses to these first samples.

For residue analysis, freeze-dried liver samples were homogenised and spiked with known amounts of mixtures of $^{13}C_{12}$-PCDDs/PCDFs and $^{13}C_{12}$-dioxin-like PCBs. Samples were extracted in a Soxhlet apparatus for 24 h with toluene:cyclohexane (1:1). Extracts were treated with sulphuric acid in order to remove organic components, fat and other interfering substances. Further cleanup was based on solid-liquid adsorption chromatography. To this end, an automated Power Prep™ system (FMS Inc., MA, USA) (using pre-packaged columns containing multilayer silica, basic alumina and PX-21 carbon as adsorbents) was employed to remove the bulk and other interferences as described by Abad et al. (2000). Finally, analytes were collected in two single fractions containing firstly the mono-*ortho* PCBs and secondly non-*ortho* PCBs jointly with the PCDDs/PCDFs.

The analysis of planar chlorinated hydrocarbons was carried out using high resolution gas chromatography-high resolution mass spectrometry (HRGC-HRMS) using a Micromass Ultima NT mass spectrometer coupled with an Agilent gas chromatograph equipped with a PAL CTC autosampler (Water Instruments, Manchester, UK). The masslynx 4.0 software version of the workstation was employed. Two microliters sample extracts were injected on a DB-5 ms (J&W Scientific, CA, USA) fused capillary GC column (40 m × 0.18 mm i.d. and 0.25 μm thickness) in the splitless mode at 280 °C. The sample was kept at the initial oven temperature of 140 °C for 1 min, thereafter, the temperature increased to 200 °C at 20 °C/min and was maintained at 200 °C for another min. Finally, the oven temperature increased to 310 °C at 5 °C/min and was maintained at that temperature for 6 min. The mass spectrometer was operated in the selected ion-monitoring (SIM) mode using a positive electron impact (EI+) source. The electron energy was 40 eV, the current trap was 600 μA, the acceleration voltage 8000 V and the source temperature 250 °C. MS power resolution was tuned at 10,000 (10%) by measuring perfluorokerosene (PFK) reference peaks.

Quantification was based on the isotopic dilution method (USEPA, 1994). Relative response factors (RRF) for the individual 2,3,7,8-PCDDs/Fs and dioxin-like PCBs were obtained by analysing multi-analyte standard solution mixtures. The recoveries of labelled standards were calculated using a mixture of two labelled PCDDs and four PCBs, added before the HRGC-HRMS analysis.

To evaluate the accuracy of the procedure a number of quality control (QC) and quality assurance (QA) measures were applied. This included the analysis of procedural blanks, certified reference materials (CARP-2), and the participation in intercalibration exercises on food and related matrices (Malisch et al., 2003). Other common practices such as specific GC separation, sensitivity, resolution power of mass spectrometer, retention times, and acceptable recovery rates were routinely attended.

2,3,7,8-TCDD equivalents (TEQs) were estimated for PCDD/F congeners and dioxin-like PCBs with an assigned toxic equivalency factor (TEF) value, based on the Fish TEFs reported in 1998 by the World Health Organisation (Van den Berg et al., 1998).

2.5. Statistical analysis

Results are expressed as mean ± standard error of the mean (S.E.M.). For statistical analysis the Sigma Stat software from Jandel Scientific (USA) was used. The program tests automatically the normality of the data using Kolmogorov Smirnoff test, and the equal variance by checking the variability of the group means. VTG and E2 data were parametric. Significant differences between both groups were determined monthly using the *t*-test ($P < 0.05$). Significant differences ($P < 0.05$) among months over a reproductive cycle were compared for each group using one-way analysis of variance (ANOVA) followed by Student–Newman–Keuls test.

3. Results

3.1. Spawnings

In the group fed with a natural diet, the first spawning was recorded on 11th of January, and the last one on 3rd of April. In group fed with commercial food, the spawning period began on 22nd of January and finished on 27th of April. The mid spawning time was on 21st and 26th of February, respectively. These spawning dates are represented in Figs. 1 and 2, for a better understanding of the hormonal variations.

3.2. Vitellogenin plasma levels

The vitellogenic period in sea bass is characterized by a gradual increase of plasma VTG concentrations, from October until the beginning of spawnings in December/January. The high levels of VTG reached at this date are maintained normally until the end of the spawning period, showing a sharp decrease around May/June. In the present experiment, an increase in plasma levels on VTG between October and November was observed in the group fed with trash fish (Fig. 1). Concentrations of VTG increased then slowly to reach maximal levels in February and March. From March, a sharp decrease in plasma levels of VTG was observed, to reach basal levels in May. The group fed with a commercial diet showed a similar profile in the variation of VTG plasma levels, but concentrations detected in November, December, and February were significantly

($P < 0.05$) lower than those observed in the group fed with trash fish.

3.3. 17β-Estradiol plasma levels

In reproducing female sea bass, E2 plasma levels increase from the beginning of vitellogenesis in October until the beginning of spawnings in December/January. In the present experiment, the group fed with natural food exhibited a very soft increase in plasma E2 levels from October to January (Fig. 2). In February and March E2 plasma concentrations were similar to those of January, and from March on began to decrease reaching basal plasma levels in April. In the case of the group feeding a commercial diet, an increase in plasma concentrations of E2 was observed between October and December, reaching at this point a maximal concentration of about 4 ng/ml. These high levels were maintained in January and February. As a consequence, plasma E2 levels in December and January were significantly ($P < 0.05$) higher in the group fed with a commercial diet than in the group fed with fish. In March, plasma concentrations of E2 showed a decline that was maintained during April and May. Thus, for the group fed commercial diet, basal values of E2 were reached in May.

3.4. Liver concentrations of organochlorines

Total PCDD/Fs concentrations were low in both groups, not exceeding a value of 20 pg/g (Table 2). The mean concentrations of each 2,3,7,8-substituted congener in liver of fish fed with natural food or with a commercial diet are presented in Fig. 3. A clear difference in both profiles can be observed between the groups. While sea bass fed with fish exhibited a profile clearly dominated by low chlorinated furans (2,3,7,8-TCDF, 1,2,3,7,8-PnCDF, 2,3,4,7,8-PnCDF and 1,2,3,4,7,8-HxCDF), animals fed with a commercial diet were dominated by a high prevalence of higher chlorinated dioxins (OCDD and 1,2,3,4,6,7,8-HpCDD) as well as for some low chlorinated PCDFs (2,3,7,8-TCDF and 2,3,4,7,8-PnCDF).

Regarding PCBs, average levels were always higher than PCDD/F concentrations. Non-*ortho* PCBs (77, 126 and 169) were higher in sea bass fed with fish than in the specimens fed with commercial diet, with average levels of 428 pg/g, which is almost the double of the concentration found in fish fed with the commercial diet (Table 2). *Ortho* PCBs showed the highest values, especially in sea bass fed with trash fish, reaching 60,000 pg/g. Levels in sea bass fed with a commercial diet where much lower, with a mean value of 39,000 pg/g.

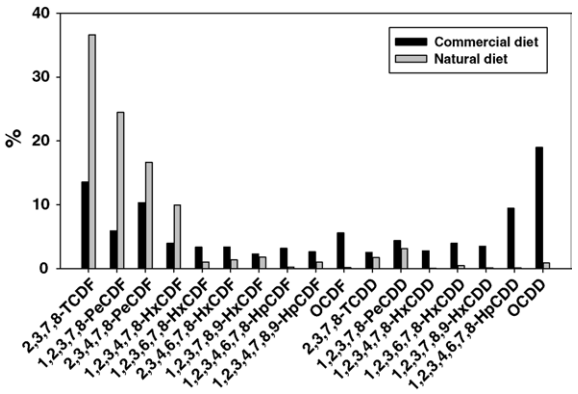

Fig. 3. Percentage distribution of each 2,3,7,8-substituted congener in liver of fish fed with a natural diet consisting on trash bogue (black bars) or with a commercial diet (grey bars).

Calculated TEQs were low in both groups: 4.7 in sea bass fed with trash fish and 2.7 in sea bass fed with a commercial diet (Table 2). PCDDs and PCDFs were the most important contributors to total TEQs, followed by non-*ortho* PCBs. The contribution of *ortho* PCBs to total TEQs was almost negligible.

4. Discussion

In the present experiment, sea bass broodstock fed with a commercial diet exhibited lower plasma levels of VTG and higher plasma levels of E2 than animals fed with a natural diet. This effect was also observed in a previous experiment (Navas et al., 1998). Since production of VTG is dependent on high plasma E2 levels, the results obtained in both studies are indicative of alterations in the synthesis of VTG. In a previous study (Navas et al., 1998), it was hypothesized that lower plasma levels of VTG observed in sea bass fed with a commercial diet were consequence of an inadequate dietary balance of fatty acids that could influence the overall fatty acid composition of the liver affecting the synthesis of VTG (Frémont et al., 1984). In accordance

Table 2
Mean PCDD/F and PCB concentrations and calculated TEQs (pg/g fresh weight) in liver of sea bass fed with a commercial diet ($n = 4$) or with a natural diet ($n = 3$)

Congener	Natural diet (Trash fish)			Commercial diet (Fodder)		
	Mean	S.E.M.	TEQs	Mean	S.E.M.	TEQs
2,3,7,8-TCDF	7.31	0.85	0.37	1.86	0.39	0.09
1,2,3,7,8-PeCDF	4.88	0.86	0.24	0.81	0.18	0.04
2,3,4,7,8-PeCDF	3.32	0.48	1.66	1.42	0.31	0.71
1,2,3,4,7,8-HxCDF	1.98	0.36	0.02	0.54	0.11	0 06
1,2,3,6,7,8-HxCDF	0.20	0.03	0.02	0.46	0.10	0.05
2,3,4,6,7,8-HxCDF	0.27	0.02	0.03	0.46	0.11	0 05
1,2,3,7,8,9-HxCDF	0.36	0.35	0.04	0.31	0.07	0.03
1,2,3,4,6,7,8-HpCDF	0.04	0.01	0.00	0.43	0.11	0 00
1,2,3,4,7,8,9-HpCDF	0.20	0.18	0.00	0.36	0.07	0.00
OCDF	0.03	0.00	0.00	0.77	0.29	0.00
2,3,7,8-TCDD	0.34	0.04	0.34	0.35	0.05	0.35
1,2,3,7,8-PeCDD	0.62	0.09	0.62	0.60	0.13	0.60
1,2,3,4,7,8-HxCDD	0.01	0.00	0.00	0.38	0.12	0 19
1,2,3,6,7,8-HxCDD	0.09	0.01	0.09	0.54	0.15	0.01
1,2,3,7,8,9-HxCDD	0.03	0.03	0.03	0.48	0.15	0.10
1,2,3,4,6,7,8-HpCDD	0.03	0.00	0.00	1.29	0.57	0.00
OCDD	0.17	0.09	0.00	2.61	0.76	0.00
Sum PCDD/Fs	19.96	2.87	3.53	13.75	3.53	2.19
PCB 77	237.38	24.86	0.02	116.74	25.87	0.01
PCB 126	173.90	22.70	0.87	53.70	16.90	0.27
PCB 169	16.91	2.38	0.00	8.17	2.11	0.00
Sum non-*ortho* PCBs	428.20	49.84	0.89	178.62	42.34	0.28
PCB 123	8550.82	1 738.29	0.04	7691.75	1531.66	0.04
PCB 118	25796.20	1294.15	0.13	15539.00	9268.49	0.08
PCB 114	634.48	132.14	0.01	1546.50	468.01	0.00
PCB 105	8337.95	2 227.22	0.04	4789.50	1202.33	0.02
PCB 167	5437.79	1 057.30	0.03	8415.68	6071.68	0.04
PCB 156	7646.96	1283.39	0.04	23.13	0.00	0.00
PCB 157	1755.14	331.05	0.01	580.50	182.11	0.00
PCB 189	1844.92	396.74	0.01	334.00	94.85	0.00
Sum *ortho*-PCBs	60004.27	8 460.27	0.30	38920.06	18581.48	0.19
Total TEQs			4.72			2.66

with this, a clear influence of the fatty acid composition of the diets on the fatty acid composition of the eggs was observed (Navas et al., 1998). Probably the fatty acid composition of the VTG was also affected, since VTG accounts for the major part of the oocyte growth (Wallace, 1985).

However, when βNF was administered through the diet to sea bass broodstock a reduction of VTG plasma levels associated with high plasma concentrations of E2 was also observed (Navas et al., 2004). The antiestrogenicity of βNF, i.e. its capacity to provoke a dose-dependent reduction in E2 induced VTG production, has been shown in vitro using primary trout hepatocytes (Navas and Segner, 2000). Reduction of E2 induced VTG production has also been reported in rainbow trout in vivo after βNF injection (Anderson et al., 1996b). Some authors have also reported increases in plasma sex steroid concentrations after TCDD treatments in great blue heron (*Ardea herodias*, Janz and Bellward, 1997) and in rainbow trout (*Oncorhynchus mykiss*, Andersson et al., 1993). Intraperitoneal injections of the planar PCB126, 3,3′,4,4′-tetrachlorobiphenyl, caused also an increase of E2 plasma levels simultaneously with a reduction of

VTG plasma concentrations in perch (*Morone americana*, Monosson et al., 1994). In the above-cited studies about βNF antiestrogenicity, the reduction of VTG production was accompanied by an increase in the hepatic content of cytochrome P4501A (CYP1A) protein or in CYP1A dependent enzymatic activities. Since CYP1A induction is mediated by the activation of AhR, a direct implication of AhR can be suggested. Similarly, TCDD and PCB126 are also known AhR ligands and activators (Safe et al., 1991) and exposure of sea bream, *Sparus aurata*, to TCDD in vivo leads to an induction of CYP1A (Ortiz et al., 2002). All these results suggest a direct implication of AhR in the antiestrogenic processes observed.

PCBs and PCDDs are poorly metabolized in fish, and these compounds bioaccumulate and biomagnify through the marine food chain, so that they may reach high concentrations in fish oil used for the manufacture of fish feed. The presence of some *ortho*-substituted PCBs in fish feed and in both commercially and laboratory-cultured sea bass has been recently reported (Serrano et al., 2003). However, these *ortho*-substituted PCBs do not exist in planar conformation (Chana et al., 2002), which is a structural condition necessary for the compound to bind to the AhR and induce antiestrogenic processes mediated by this receptor. In fact, a study carried out in vitro using cultured rainbow trout hepatocytes showed that the antiestrogenic potency of these *ortho*-substituted PCBs is limited (Anderson et al., 1996a).

In this work, several compounds showing high binding affinity for the AhR, and high antiestrogenic capacity have been analysed in liver, including a variety of PCDDs, PCDFs and planar PCBs (Wang et al., 1993; Safe, 1995; Ohtake et al., 2003). Due to the hydrophobicity and lipoaffinity of these compounds, it is expected that other body compartments, such as visceral fat, exhibit higher concentrations than those observed in hepatic tissues (Serrano et al., 2003). During vitellogenesis, fat depots are used and contaminants may be redistributed and transported to liver. Since liver plays a major role in both detoxification processes and vitellogenesis, organochlorines present in this organ could be hampering the synthesis of VTG. Taking that into account, chemical analyses were performed only in the hepatic tissues.

Levels of organochlorine compounds detected in liver of fish feeding a commercial or a natural diet were very low and the toxicity associated to these concentrations should be negligible, as evidenced by the low TEQs values. As a consequence, a direct effect of these chemicals on the hepatic processes related with the production of VTG stimulated by E2 is unlikely. In addition, levels of PCBs, PCDDs and PCDFs were in general higher in liver of female sea bass fed with fish, exhibiting higher VTG levels, than in liver of sea bass fed with a commercial diet. As a consequence, reduction of VTG concentrations observed in sea bass fed with a commercial diet, with respect to sea bass fed with fish, cannot be attributed to the accumulation of these compounds.

The presence of PCBs and some organochlorine pesticides in the tissues of a variety of organisms related with the marine environment has been reported (Jiménez et al., 2000; Borrell et al., 2001; Harris et al., 2003; Kannan et al., 2003; Storelli et al., 2003). However, there exists an enormous lack of information about the levels of PCDDs, PCDFs and some PCB congeners (particularly non-*ortho* substituted PCBs) in commercial marine organisms. Antunes and Gil (2004) found that sea bass from natural environments showed lower PCB levels than farmed fish. Commercial diets had been suggested to be a major source of PCBs and other organochlorine compounds. In addition, the higher lipid content normally found in farmed fish can, at least in part, be the cause of this effect. The results from the present study give some more valuable information about the PCDD, PCDF and PCB content in farmed fish, and, interestingly, these results do not agree with reported results of other authors since sea bass fed with natural food exhibited slightly higher values of PCBs, PCDDs and PCDFs than sea bass fed with commercial food. These results could be related with the fact that bogues serving as food were captured in coastal areas near harbours thus being exposed to pollution caused by sea traffic, so that they accumulate higher levels of the analysed chemicals than the fish used to prepare the commercial diet of the present experiment.

5. Conclusions

It has been repeatedly observed that fish fed with a commercial diet exhibited lower plasma concentrations of VTG and higher concentrations of E2 than fish

fed with a natural diet. These alterations are similar to those observed in female sea bass exposed through the diet to the AhR agonist βNF. In the present work, differences observed in the liver concentrations of typical AhR agonists, such as dioxins, furans and PCBs, between sea bass fed with a commercial diet and sea bass fed with a natural diet, cannot support the hypothesis that the observed hormonal alterations are caused by an abnormally high ingestion of these compounds through the commercial diet.

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