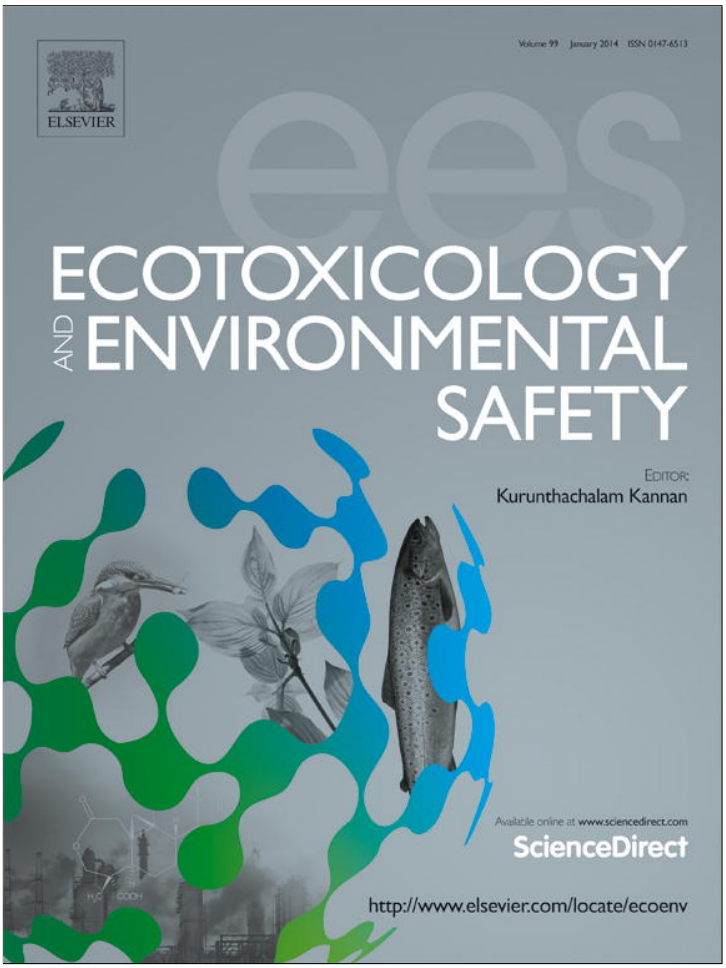

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Assessment of persistent organic pollutants accumulation and lipid peroxidation in two reproductive stages of wild silverside (*Odontesthes bonariensis*)

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ABSTRACT

Persistent organic pollutants (POPs) in streamwater can sometimes exceed the guidelines values reported for biota and human protection in watersheds with intensive agriculture. Oxidative stress and cytotoxicity are some of the markers of exposure to POPs in fish. Accumulation of organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) as well as lipid peroxidation (LPO) was assessed in wild silverside (*Odontesthes bonariensis*) from maturation and pre-spawning stages sampled in a typical soybean growing area. Pollutants were quantified by gas chromatography with electron capture detection and LPO by the method of thiobarbituric acid reactive substances. Concentrations of POPs were in the following order: OCPs > PCBs > PBDEs in all organs and stages. Liver, gills and gonads had the highest OCP concentrations in both sexes and stages with a predominance of endosulfan in all samples. Matured individuals, sampled after endosulfan application period, showed higher endosulfan concentrations than pre-spawning individuals. The predominance of endosulfan sulfate could be due to direct uptake from diet and water column, as well as to the metabolism of the parent compounds in fish. The prevalence of p,p′-DDE in liver would also reflect both the direct uptake and the metabolic transformation of p,p′-DDT to p,p′-DDE by fish. The highest levels of PBDEs and PCBs were found in gills and brain of both stages of growth. The pattern BDE-47 > BDE-100 in all samples corresponds to pentaBDE exposure. In the case of PCBs, penta (#101 and 110) and hexa-CB congeners (#153 and 138) dominated in the maturation stages and tri (#18) and tetra-CB (#44 and 52) in pre-spawning stages, suggesting biotransformation or preferential accumulation of heavier congeners during gonadal development. Differences in LPO levels in ovaries were associated with growth dilution and reproductive stage. Differences in LPO levels in gills were related with pesticide application periods. As a whole, endosulfan, a current-use pesticide, constituted the main pollutant found in wild silverside reflecting the intense agriculture activity in the study area. Moreover endosulfan was positively correlated with LPO.

1. Introduction

Organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) are included in the list of Persistent Organic Pollutants (POPs; Stockholm Convention UNEP, 2004) because they are environmentally persistent, bioaccumulate and undergo long-range atmospheric transport (Wania and MacKay, 1996). PCBs and OCPs, which were used worldwide in industry and agriculture, are currently banned. The flame retardants PBDEs, used in plastics, furniture, textiles and electronic circuitry are similar in its structure to PCBs and behave in the same way in the environment (de Wit, 2002). Due to physicochemical properties, like hydrophobicity and relatively high vapor pressure, OCPs, PCBs and PBDEs residues are often found in remote ecosystems (Wania and MacKay, 1996). Urban discharges, industrial and agricultural activities contaminate the aquatic environment. POPs accumulated on bottom sediments are transported in the aqueous phase either adsorbed to suspended particulate matter (SPM) or dissolved, being available for bioaccumulation. Fish take pollutants directly from water column through gills absorption and by diet ingestion. Pollutant distribution and accumulation within fish depends on internal physiological

Abbreviations: BBB, brain-blood barrier; CF, condition factor; DDTs, dichlorodiphenyltrichloroethanes; GC-ECD, gas chromatograph with electron capture detector; GSI, gonadosomatic index; HCHs, hexachlorocyclohexanes; HSI, hepatosomatic index; LPO, lipid peroxidation; MDA, malondialdehyde, OCPs, organochlorine pesticides; PCBs, polychlorinated biphenyls; PBDEs, polybrominated diphenyl ethers; POPs, persistent organic pollutants; PUFAs, polyunsaturated fatty acids; QGR, Quequén Grande River; ROS, reactive oxygen species; SPM, suspended particulate matter; TAGs, triacylglycerols

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processes such as lipid mobilization during gonadal maturation. So, the reproductive period may also modify contaminants accumulation (Amado et al., 2006, Menone et al., 2000). POPs exhibit low metabolism and cause chronic damage to fish during long-term exposure at low concentrations (Austin, 1999), causing effects at different biological levels. Their accumulation is linked to cytotoxic effects by producing reactive oxygen species (ROS) at levels exceeding antioxidants cell defenses, leading to oxidative damage (Halliwell and Gutteridge, 2007). Consequently, macromolecules modification could produce cell death. Polyunsaturated fatty acids (PUFAs) associated with cell membranes, are one of the more studied ROS target, being prone to undergoing a lipid peroxidation process (LPO; Rikans and Hornbrook, 1997). The principle consequences of this process include decreased fluidity and increased permeability, causing leakage to some molecules and membrane-bound enzymes inhibition (Di Giulio and Meyer, 2008). So, LPO estimation can be used as a damage biomarker (Liu et al., 1997).

The Quequén Grande River (QGR) watershed is a typical agricultural area in the southern Argentine Pampas. Agriculture has increased by 39.6 percent during the last 20 years and transgenic soybean and wheat crops have replaced livestock (Vazquez et al., 2012). POPs, in particular technical endosulfan and PCBs, were found at levels above the recommended values for biota and human protection in streamwater (Gonzalez et al., 2012, 2013). Endosulfan, which is highly toxic to the aquatic biota (Nowak et al., 1995), has been phased out in Argentina since July of 2013 (SENASA, 2011). Furthermore, silverside (*Odontesthes bonariensis*) is an autochthon species of ecological and socio-economical importance in the QGR watershed. Thus, the aim of this work was to study OCPs, PCBs and PBDEs accumulation and distribution in male and female silverside from the QGR watershed, considering two reproductive stages and LPO as a damage measurement.

2. Materials and methods

2.1. Study area

The QGR watershed is located in the southern Buenos Aires Province (38°11′10″ S 59°12′7″W, Argentina) and drains an area of 9990 km^2. The QGR, possessing a length of 180 km, constitutes the most important zonal streamwater. The climate is dry sub-humid and the highest precipitation values are between September and March (Varela and Teruggi, 2002). The most important pesticide application period is from November to March, accompanied by strong, short rains in the zone, causing pesticide transport from the agricultural fields directly to the river (Gonzalez et al., 2012).

2.2. Sample collection and processing

The *O. bonariensis* spawning period takes place in early spring (September–October), with a lesser proportion of spawning appearing during late summer and early fall (March–April; Grosman, 1995). Fish were obtained in June ($n=8$) and September ($n=7$) in 2010 by local fishermen, coinciding with maturation and pre-spawning stages. Samples were immediately frozen and stored at −20 °C. Once thawed, length and weight were determined, gills, brain, muscle, liver and gonads dissected and kept frozen (−20 °C) in aluminum foil until analysis. Gonadal development was determined by the Calvo and Dadone (1972). Condition factor (CF=total weight × 100/total length^3), hepatosomatic index (HSI=liver weight × 100/total weight) and gonadosomatic index (GSI=gonad weight × 100/total weight) were calculated.

2.3. Chemical analysis

2.3.1. Extraction procedure

OCPs, PCBs and PBDEs were extracted according to Metcalfe and Metcalfe (1997) with modifications of Miglioranza et al. (2003). Subsamples of muscle (3 g), liver (0.5–2 g), gonads (1–2 g), gills (0.3–0.5 g) and brain (0.01–0.1 g) were homogenized with anhydrous sodium sulfate and spiked with PCB #103 as surrogate standard. Total lipids and organic compounds were extracted with a 50:50 mixture of dichloromethane and n-hexane in a Soxhlet apparatus for 8 h. Lipids were removed by gel permeation chromatography from the extracts using Bio-Beads S-X3 (200–400 mesh) and evaporated to dryness to calculate the sample lipid content. The fraction containing OCPs, PCBs and PBDEs was further purified by column chromatography with activated silica gel (200 °C for 24 h). Extracts were concentrated to 1 mL and kept in vials at −20 °C prior to gas chromatography analysis.

2.3.2. Analytical procedure

The following compounds were included in the analysis: hexachlorocyclohexanes (α-, β-, γ- and δ-HCH), dichlorodiphenyltrichloroethanes (p,p′-DDT, p,p′-DDE, p,p′-DDD), heptachlors (heptachlor, heptachlor epoxide), dieldrin, chlordanes (α- and γ-chlordane, transnonachlor), endosulfans (α- and β-endosulfan, endosulfan sulfate), PCBs congeners (IUPAC #8, 18, 28, 31, 44, 52, 66, 87, 101, 105, 110, 118, 126, 128, 138, 153, 156, 157, 167, 169, 170, 180, 187, 189, 195, 206 and 209) and PBDEs congeners (IUPAC # 47, 66, 85, 99, 100, 138, 153 and 154). Identification and quantification was performed using Gas Chromatograph, Shimadzu GC-17-A equipped with a ^{63}Ni Electron Capture Detector (GC-ECD) and a capillary column SPB-5 [(5 percent phenyl)-methyl polysiloxane, 30 m × 0.25 mm i.d. × 0.25 μm film thickness; Supelco Inc.]. One microliter was injected on a splitless mode (275 °C) and detector was kept at 290 °C. The oven temperature program was: start at 100 °C and held for 1 min, followed by an increase of 5 °C min^{-1} up to 150 °C, held for 1 min, then 1.5 °C min^{-1} up to 240 °C, and then 10 °C min^{-1} up to 300 °C for 10 min. Ultra-high purity Helium was used as carrier gas (1.5 ml min^{-1}) and nitrogen as make-up gas (Miglioranza et al., 2003). A pesticide mixture from Ultra Scientific, RI, USA and PCBs mixture from Accustandard Absolute Standards, INC, CT, USA, and a Lake Michigan PBDEs mixture, were used for identification and quantification of single compounds, while PCB #103 from Ultra Scientific, USA was used as internal standard.

2.4. Quality control and assurance

Laboratory and instrumental blanks were analyzed throughout the procedure to ensure the absence of contaminants or sample interference. Results indicate that there were no contaminations or interference during laboratory handling. Recoveries, calculated from spiked matrixes, were >90 percent. Instrumental detection limits (Keith et al., 1983), ranged between 0.03 and 0.05 ng ml^{-1} for HCHs and between 0.08 and 0.33 ng ml^{-1} for the rest of compounds. Method detection limits ranged between 0.003 and 0.005 ng g^{-1} for HCHs and between 0.008 and 0.033 ng g^{-1} for the remaining POPs.

2.5. Lipid peroxidation

LPO detection can be estimated by compound quantification such as malondialdehyde (MDA), which are formed by degradation of initial products of free radical attack. The reaction of MDA with 2-thiobarbituric acid (TBA) is one of the most widely used indirect estimators of oxidative stress (Liu et al., 1997). Thus, LPO in subsamples of gills, brain, muscle, liver and gonads was determined, following the Khan and Panda (2008). Briefly, samples were homogenized with KCl and butylated hydroxytoluene (BHT). The assay mixture contained the homogenate, BHT, acetic acid, TBA, double distilled water (ddH_2O) and sodium dodecyl sulfate (SDS). The resulting mixture was vortexed and heated at 95 °C in a water bath for 30 min, cooled for 10 min and vortexed after the addition of ddH_2O and n-butanol. After centrifugation (850*g* for 10 min), the organic layer was fluorimetrically evaluated at 515 nm excitation and 553 nm emission, using a Fluoroskan ASCENT Thermo Labsystems.

2.6. Data expression and statistical analysis

Each data corresponds to the arithmetic mean of four males and four females in maturation stage, and three males and four females in pre-spawning stage. Contaminant concentrations were expressed as ng per gram of wet weight (ng g^{-1} wet wt.) and lipid content was expressed as percentage (%). The lipid peroxides levels were expressed in terms of nmol MDA per gram of wet weight (nmol MDA g^{-1} wet wt.). Gonads, brain and liver burdens of OCPs, PCBs, PBDEs and lipids were calculated by multiplying the organ weight by the compound concentration and values were expressed as ng or gram.

Statistical analyses were carried out using Infostat Software Package (Di Rienzo et al., 2010). Differences between sexes and stages were tested using a one-way analysis of variance (ANOVA), followed by a *t*-Student test for pairs comparisons or a Tukey Test for multiple comparison. When parametric requirements were not fulfilled, a Kruskal Wallis test was applied. Contaminant levels differences among organs/tissues and indexes were tested using a Friedman ANOVA analysis for multiple dependent samples. Spearman correlation coefficient was performed between lipid percentage and contaminant levels (ng g^{-1} wet wt.) and between lipid and contaminant burdens. Significance level was set at a $\alpha=0.05$.

Table 1
Total length (TL) and weight (TW), condition factor (CF), hepatosomatic (HSI) and gonadosomatic (GSI) indexes of silverside in maturation and pre-spawning stages.

	Maturation (III)		Pre-spawning (IV)	
	♂	♀	♂	♀
TL (cm)	27.1 ± 1.5^{a}	27.5 ± 1.5^{a}	26.1 ± 1.2^{a}	27.8 ± 1.1^{a}
TW (g)	160.3 ± 27.3^{a}	169.5 ± 19.2^{a}	151.5 ± 37.6^{a}	196.5 ± 29.5^{a}
CF (%)	0.8 ± 0.02^{a}	0.8 ± 0.1^{a}	0.8 ± 0.1^{a}	0.9 ± 0.1^{a}
GSI (%)	1.0 ± 0.2^{b}	2.3 ± 0.3^{ab}	2.4 ± 0.4^{ab}	10.3 ± 1.9^{a}
HSI (%)	1.4 ± 0.2^{b}	1.7 ± 0.1^{ab}	1.1 ± 0.5^{b}	2.4 ± 0.5^{a}

Data are expressed as Mean ± SD. Different superscript letters (a or b) show statistically significant differences between sexes and/or stages at $p < 0.05$.

3. Results and discussion

3.1. Biological characteristics

Table 1 shows biological characteristics of the silverside individuals analyzed in this work. All fish showed similar size, total weight (g) and total length (cm) ranging between 151.0–196.5 and 26.1–27.8, respectively. The CF is accepted as a quantitative indicator of the fish health (Grosman, 1995). CF values on this work were below the species standard curve (Manzini et al., 2009). This curve was performed with silversides from a lentic environment. In the QGR, a lotic environment, food scarcity (mainly zooplankton) might lead to lower CF values. GSI and HSI represented the percentage of these organs, regarding total fish weight. The main silverside reproductive period takes place in spring (Grosman, 1995). Accordingly, GSI values from both samples dates (June, winter and September, spring) agree with the macroscopically determined reproductive stage (maturation and pre-spawning, respectively). The higher GSI values of pre-spawning individuals were related to the gonadal development during maturation. This process involves a lipid mobilization to the developing gonads, subsequently allowing embryo nutrition (Landrum and Fisher, 1998). This active lipid metabolism was also reflected in the higher HSI observed in females compared to males in pre-spawning stage.

3.2. Total contaminants accumulation in silverside

In general, the contaminant levels distribution in silverside from both stages were in the following order: OCPs > PCBs > PBDEs (Table 2) in accordance with the extensive agricultural activity in the surrounding area. Brain was the exception, being PCBs > OCPs > PBDEs, and PCBs were found in gonads below the detection limit.

Lipid percentage showed the following order: liver > gonads/brain > gills > muscle for males and females of both stages (ANOVA Friedman, $p < 0.05$). No positive correlation was found between lipid percentage and concentrations of OCPs, PCBs and PBDEs in any organ or tissue. Although it is expected that the partition of these hydrophobic contaminants will be subject to lipid levels, it is also important to consider the variability of lipid components. Some of them are strongly polar (phospholipids), while others are generally classified as neutral; wax esters, cholesterol, and the triacylglycerols (TAGs). The highly lipophilic chemicals concentrations, such as PCBs, in biota, are more tightly correlated with neutral lipids than with total lipid (Jorgensen et al., 1997). In this work, the lipid classes were not determined, so some lipid variations could lead to different contaminant partitions, justifying the lack of coincidence between lipid content and contaminants levels.

As shown in Table 2, no significant differences were observed for total OCPs, PCBs and PBDEs concentrations between sexes (except for pre-spawning individual livers where females showed the highest PCBs concentrations). Differences between reproductive stages were found for OCPs concentrations; being higher in samples from maturation individuals in spite of not showing lipid percentage variations (Table 2).

With reference to contaminant distribution, the highest OCPs concentration in both stages was recorded in liver (ANOVA Friedman, $p < 0.05$). Although there was a direct relationship between OCPs and lipid percentage in this organ, it was not significant. It could be suggested that the reproductive stage or compounds bioavailability could be influencing OCPs bioaccumulation.

In addition, gonads and gills also recorded high OCPs concentrations (Table 2). Gills provide an important toxicants entry point, were they are transported through blood to the liver and metabolized, before being distributed throughout the body. Consequently, gills and liver are the pollutants first target and this was reflected in the high contaminant concentrations found in these organs.

PCBs and PBDEs prevailed in gills and brain (Table 2), being higher in the brains of maturation individuals than in pre-spawning. A positive correlation between PCBs and lipid burdens was observed in liver of maturation individuals ($r = 0.85$, $p = 0.01$). In pre-spawning stage this correlation was not statistically significant due to the high values of PCBs burden in female livers (Fig. 1b), although a positive relation was observed. In gonads, higher OCPs (Fig. 1a), PBDEs (Fig. 1c) and lipid burdens (not shown) were observed in pre-spawning stage, in accordance with the correlation observed between burdens of OCPs ($r = 0.91$, $p = 0.001$), PBDEs ($r = 0.93$, $p = 0.0014$) and lipid in this organ. Temporal changes in the lipid percentage and OCPs concentrations during the period of silverside gonadal development were also reported by Menone et al. (2000). During pre-spawning stage, the gonads maximum development was reflected by the higher GSI values found (Table 1), along with a contaminant dilution effect, linked to ovaries growth prior to spawning. Gundersen et al. (2000) described this process, in which the gonad with a high contaminant content becomes diluted as an egg mass develops and increases in size. The lipid reserve is used to produce egg mass that may comprise 15–25 percent of the fish's body weight, resulting in lower contaminant levels. Thus, the lipid transference during gonadal development would also transport the lipophilic OCPs from the energy stores to the gonad (Landrum and Fisher, 1998).

3.3. OCPs groups

In all stage samples, except brain, the endosulfans group predominated representing up to 97.6 percent of the total OCPs (Table 2). DDTs, the second largest group, ranged between 1.2 and 27.6 percent of the total OCPs (regardless of the brain that reached values up to 64.6 percent).

The distribution pattern of endosulfans in organs and tissues coincided with that of the total OCPs, confirming the prevalence of the current-use endosulfan as the main pollutant. Furtheremore, DDTs with a different distribution pattern reflected a chronic exposure to this phased-out pesticide.

Regarding HCHs, chlordanes and dieldrin, there was not a totally clear pattern between stages (Table 2). Similar behavior has been observed in QGR streamwater (Gonzalez et al., 2012, 2013) indicating chronic exposure to these compounds. Particularly, heptachlor, α-, β- and δ- HCH in all samples were below the detection limit (0.027 ng g^{-1} wet wt.) showing evidence of a non-recent pesticide use. Menone et al. (2000), reported pesticide levels in silverside from the Mar Chiquita lagoon (located in the eastern Pampa Region) with a distribution pattern DDTs > heptachlor epoxide > HCHs > endosulfan. The difference between contaminant patterns in QGR and Mar Chiquita lagoon silversides shows

Table 2
OCPs, PCBs and PBDEs groups distribution (ng g^{-1} wet wt.) in organs and tissues of silverside in maturation and pre-spawning stages.

		Gills		**Brain**		**Muscle**		**Liver**		**Gonads**	
		♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Maturation (III)	**Lipids**	0.7 ± 0.2^{a}	0.8 ± 0.4^{a}	5.1 ± 2.2^{a}	1.8 ± 0.5^{b}	0.4 ± 0.04^{a}	0.4 ± 0.1^{a}	8.8 ± 3.4^{a}	4.2 ± 1.2^{a}	2.7 ± 1.1^{b}	3.6 ± 2.7^{ab}
	Σ OCPs	**36.8 ± 12.0^{a}**	**25.2 ± 3.8^{ab}**	**5.4 ± 1.5^{b}**	**20.6 ± 12.0^{ab}**	**12.4 ± 3.6^{a}**	**10.6 ± 3.0^{a}**	**177.7 ± 69.3^{a}**	**165.8 ± 56.7^{ab}**	**37.1 ± 18.6^{a}**	**39.8 ± 23.9^{a}**
	Endosulfans	34.8 ± 11.7^{a}	23.2 ± 3.6^{ab}	0.3 ± 0.5^{b}	< dl	11.2 ± 3.9^{a}	9.8 ± 3.1^{a}	173.2 ± 68.1^{a}	161.8 ± 55.7^{ab}	35.3 ± 18.1^{a}	36.2 ± 22.8^{a}
	DDTs	1.1 ± 0.6^{a}	0.8 ± 0.4^{a}	2.7 ± 1.2^{b}	12.8 ± 10.7^{a}	0.3 ± 0.2^{a}	0.2 ± 0.1^{a}	2.6 ± 1.1^{a}	1.9 ± 1.2^{a}	0.8 ± 0.3^{b}	2.0 ± 1.5^{b}
	Chlordanes	0.2 ± 0.2^{b}	0.6 ± 0.3^{ab}	0.9 ± 1.0^{b}	< dl	< dl	< dl	0.6 ± 0.3^{b}	0.5 ± 0.3^{b}	0.3 ± 0.1^{b}	0.6 ± 0.5^{b}
	Dieldrin	0.4 ± 0.2^{a}	0.4 ± 0.1^{a}	0.9 ± 0.9^{b}	6.5 ± 3.0^{a}	0.1 ± 0.1^{a}	0.2 ± 0.1^{a}	1.2 ± 0.7^{a}	1.3 ± 0.6^{a}	0.5 ± 0.1^{b}	0.9 ± 0.8^{b}
	HCHs	0.3 ± 0.2^{a}	0.2 ± 0.1^{a}	0.6 ± 0.7^{a}	1.3 ± 0.8^{a}	0.8 ± 0.2^{a}	0.4 ± 0.2^{b}	0.1 ± 0.1^{b} *	0.2 ± 0.2^{ab}*	0.2 ± 0.1	< ld
	Σ PCBs	**25.2 ± 6.1^{ab}**	**8.1 ± 4.0^{b}**	**63.0 ± 9.6^{a}**	**85.1 ± 56.2^{a}**	**0.9 ± 0.5^{a}**	**0.8 ± 0.8^{a}**	**2.7 ± 1.1^{b}**	**1.8 ± 0.4^{b}**	**< dl**	**< dl**
	3 Cl	< dl	< dl	< dl	< dl	< dl	< dl	< dl	< dl	< dl	< dl
	4 Cl	0.7 ± 0.2	0.5 ± 0.3	< dl	< dl	< dl	< dl	< dl	< dl	< dl	< dl
	5 Cl	7.6 ± 1.7	3.1 ± 1.0	23.6 ± 10.3	13.3 ± 7.7	< dl	< dl	0.9 ± 0.5	0.7 ± 0.1	< dl	< dl
	6 Cl	16.9 ± 4.6	4.5 ± 2.8	39.4 ± 9.1	71.8 ± 48.6	0.9 ± 0.5	0.8 ± 0.8	1.8 ± 0.7	1.1 ± 0.4	< dl	< dl
	Σ PBDEs	**3.5–9.7^{a}**	**1.4–2.7^{ab}**	**0–15.5^{a}**	**0–20.0^{a}**	**0–1.3^{a}**	**0.1–1.7^{a}**	**0–2.7**	**0–2.4**	**0–0.9**	**0–6.6**
	47	3.5–9.7	1.4–2.7	0–13.9	0–17.4	0–1.3	0.1–1.7	0–2.6	0–1.6	< dl	0–1
	100	< dl	< dl	0–1.7	0–2.6	< dl	< dl	0–0.4	0–0.7	< dl	0–1.4
	154+85	< dl	< dl	< dl	< dl	< dl	< dl	< dl	0–1.7	0–0.9	0–5.3
Pre-spawning (IV)	**Lipids**	0.5 ± 0.1^{a}	0.8 ± 0.3^{a}	4.1 ± 1.4^{a}	4.0 ± 0.4^{ab}	0.3 ± 0.05^{a}	0.4 ± 0.1^{a}	7.5 ± 3.8^{a}	5.8 ± 4.6^{a}	7.4 ± 1.8^{a}	2.3 ± 0.6^{b}
	Σ OCPs	**5.5 ± 3.4^{c}**	**11.4 ± 7.7^{bc}**	**16.2 ± 11.2^{ab}**	**30.4 ± 8.5^{a}**	**2.7 ± 1.5^{b}**	**2.3 ± 0.7^{b}**	**40.4 ± 5.6^{c}**	**55.1 ± 9.4^{bc}**	**39.8 ± 15.9^{a}**	**14.7 ± 1.4^{a}**
	Endosulfans	2.4 ± 2.1^{c}	9.1 ± 6.5^{bc}	8.1 ± 7.2^{b}	20.3 ± 8.4^{a}	2.3 ± 1.2^{b}	1.7 ± 0.7^{b}	31.7 ± 2.6^{c}	49.2 ± 10.5^{bc}	31.6 ± 14.2^{a}	12.7 ± 1.8^{a}
	DDTs	1.5 ± ± 0.9^{a}	1.2 ± 0.8^{a}	3.4 ± 2.0^{b}	5.2 ± 1.7^{ab}	0.2 ± 0.1^{a}	0.2 ± 0.1^{a}	4.77 ± 2.16^{a}	3.0 ± 2.04^{a}	4.7 ± 1.0^{a}	1.3 ± 0.6^{b}
	Chlordanes	1.2 ± 0.4^{a}	0.9 ± 0.4^{ab}	2.2 ± 1.2^{a}	2.2 ± 0.1^{a}	0.1 ± 0.04^{b}	0.2 ± 0.1^{a}	2.05 ± 0.46^{a}	1.0 ± 0.3^{b}	1.6 ± 0.5^{a}	0.4 ± 0.2^{b}
	Dieldrin	0.4 ± 0.2^{a}	0.3 ± 0.1^{a}	1.1 ± 0.6^{b}	1.2 ± 0.2^{b}	0.1 ± 0.05^{a}	0.1 ± 0.03^{a}	1.6 ± 1.3^{a}	1.5 ± 0.5^{a}	1.9 ± 0.4^{a}	0.3 ± 0.2^{b}
	HCHs	< dl	< dl	1.2 ± 0.5^{a}	1.4 ± 0.6^{a}	< dl	< dl	0.3 ± 0.03^{ab}*	0.4 ± 0.2^{ab}*	< dl	< dl
	Σ PCBs	**29.5 ± 11.4^{a}**	**18.2 ± 10.5^{ab}**	**2.4 ± 1.0^{b}**	**5.4 ± 0.9^{ab}**	**0.2 ± 0.1^{a}**	**0.5 ± 0.2^{a}**	**1.8 ± 0.2^{b}**	**5.1 ± 1.6^{a}**	**< dl**	**< dl**
	3 Cl	12.8 ± 4.5	8.0 ± 5.1	< dl	2.9 ± 1.3	< dl	< dl	< dl	< dl	< dl	< dl
	4 Cl	16.4 ± 6.8	10.0 ± 5.3	1.2 ± 0.5	1.2 ± 0.3	< dl	0.3 ± 0.02	1.6 ± 0.2	4.1 ± 1.3	< dl	< dl
	5 Cl	0.3 ± 0.1	0.2 ± 0.1	< dl	< dl	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.04	0.4 ± 0.3	< dl	< dl
	6 Cl	< dl	< dl	1.2 ± 0.5	1.3 ± 0.3	< dl	< dl	< dl	0.6 ± 0.4	< dl	< dl
	Σ PBDEs	**0–6.6^{a}**	**0–0.5^{b}**	**0–2.9^{b}**	**0.4–0.9^{b}**	**0.05–0.3^{a}**	**0–0.1^{a}**	**0.04–0.5**	**0–0.6**	**0.4–1.4**	**0–1.3**
	47	0–6.6	0–05	0–2.9	0.4–0.9	0.04–0.1	0–0.1	0.04–0.5	0–0.6	0.4–1.4	0–1.3
	100	< dl	< dl	< dl	< dl	0–0.1	< dl	< dl	< dl	< dl	< dl
	154+85	< dl	< dl	< dl	< dl	< dl	< dl	< dl	< dl	< dl	< dl

Data are expressed as Mean ± SD. Different superscript letters (a, b or c) show statistically significant differences between sexes and/or stages within each organ or tissue, for each compound at $p < 0.05$. < dl: below the detection limit. HCHs: γ-hexachlorocyclohexane isomer; ∑Endosulfans: α-+β- isomers+endosulfan sulfate; ∑Chlordanes: α-+γ- isomer+trans-nonachlor; ∑DDTs: p,p′-DDT+ p,p′-DDE HCHs: PCBs 3Cl: 18. 4 Cl: 44, 52, 66. 5 Cl: 101, 110. 6 Cl: 138, 153. The congeners # 8, 28, 31, 87, 105, 126, 128, 156, 157, 167, 169, 170, 180, 187, 189, 195, 206 and 209 were below the detection limit. The asterisk (*) means differences at $p < 0.01$.

the influence of the time elapsed since DDTs, HCHs and heptachlor use restriction, in 1998 in the region. Regarding technical endosulfan, actions were recently taken by the National Service of Food Quality (SENASA from Spanish acronyms, Argentina). The importation of active ingredients and formulates of endosulfan was canceled in July 2012, while its use and production was totally banned in July 2013 (SENASA, 2011). Consequently, technical endosulfan was in use on soybean and wheat crops when this study was carried out. Aerial application on agricultural fields, close to the river margins, allowed this pesticide to easily reaches the aquatic environment (Gonzalez et al., 2012, 2013), being the main pollutant in silverside samples. The main application period (November–March) coincides with the frequent rains systems and also when soils are devoid of vegetation (Gonzalez et al., 2012, Varela and Teruggi, 2002), increasing the possibility of pesticide residues reaching the river. Thus, the highest total endosulfans concentrations were found in maturation stage individuals, sampled in a post-application period of technical endosulfan (Table 2). On the basis of endosulfan concentrations found in liver and gills, they would reflect a recent application exposition. Conversely, pre-spawning individuals (sampled in September) showed lower endosulfans concentrations in all samples related to the sampling date, performed during a pre-application period of endosulfan. The occurrence of this insecticide in the QGR streamwater at levels above the limit established by the National Argentine Water Council (INA from Spanish, 7 ng L^{-1} for the sum of α- and β-isomer), and international limits (3 ng L^{-1}, CCME, 2001) for aquatic biota protection, were reported for post-application periods (Gonzalez et al., 2012, 2013). Consequently, endosulfan uptake from the water column would be an important input pathway to fish. Endosulfan concentrations (Table 3) and burden (not shown) in gonads did not show significant differences between stages. However, there was a tendency to a higher endosulfan burden in pre-spawning individuals, suggesting a concentrations dilution together with gonad size in those individuals, as previously mentioned.

Technical endosulfan is a 70:30 mixture of α-: β-isomers and endosulfan sulfate production results mainly by metabolism of the α-isomer (ATSDR, 2012). Levels of endosulfan sulfate in all samples were significantly higher than α- and β-endosulfan (ANOVA Friedman, $p<0.05$, Fig. 2a, b). Gonzalez et al. (2012) registered the presence of endosulfan sulfate in QGR streamwater. Consequently, the predominance of this metabolite in silverside organs and tissues could be due to direct uptake by diet and column water, as well as to the metabolism of the parent compounds into fish. Liver and gonads showed the highest endosulfan sulfate concentrations and burden in both stages (Fig. 2a,b; ANOVA Friedman, $p<0.05$). The toxicants metabolism in teleostean fish, as in the rest of vertebrates, is primarily performed in the liver, which is located in a strategic position within organisms, receiving a large quantity of blood, contributing to the distribution of toxics and metabolites to other organs. As was observed for total endosulfans, the concentration of the endosulfan sulfate in gills, liver and muscle of maturation individuals was significantly greater than in pre-spawning stage, while no difference was observed for gonads (Fig. 2a, b). The α-/β-isomer ratios in gills and liver from maturation individuals gave evidence of acute exposure to aged technical endosulfan. The isomers ratio >1 found in gills agrees with the predominance of α-isomer (70 percent) in streamwater during post-application season (Gonzalez et al., 2013), while the α/β ratio in liver <1 might indicate a faster metabolism of α-isomer, mainly to endosulfan sulfate, related to β-isomer metabolism. On the other hand, both isomers were below detection limit in the pre-spawning individual livers, showing the metabolism of the technical endosulfan, suggesting that no additional endosulfans inputs occurred during pre-spawning period.

DDTs, the second largest group, showed total concentrations in all organs and tissues of both reproductive stages, one order of magnitude lower than endosulfans (Table 2, Fig. 2).

Gonads DDTs concentrations were well correlated with the lipid percentage ($r=0.75$, $p=0.0048$, not shown), which is in agreement

Table 3
Lipid peroxidation levels (nmol MDA mg^{-1}) in organs and tissues of silverside in maturation and pre-spawning stages.

	Gills		Brain		Muscle		Liver		Gonads	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Maturation (III)	0.4 ± 0.2^{a}	0.4 ± 0.1^{a}	0.3 ± 0.1^{a}	0.3 ± 0.1^{a}	0.03 ± 0.02^{ab}	0.1 ± 0.1^{a}	0.3 ± 0.2^{a}	0.1 ± 0.03^{a}	0.3 ± 0.3^{b}	0.9 ± 0.1^{a}
Pre-spawning (IV)	0.04 ± 0.03^{b}	0.2 ± 0.1^{ab}	0.03 ± 0.02^{b}	0.02 ± 0.01^{b}	0.03 ± 0.003^{b}	0.02 ± 0.01^{b}	0.1 ± 0.1^{a}	0.2 ± 0.1^{a}	0.6 ± 0.04^{a}	0.04 ± 0.02^{b}

Data are expressed as Mean ± SD ($n=4$, except pre-spawning males $n=3$). Means not sharing the same superscript (a or b) within groups for each tissue/organ evidence statistically significant differences between sexes and/or stages, at $p<0.05$. Different superscript letters (a or b) show statistically significant differences between sexes and/or stages within each organ or tissue, at $p<0.05$.

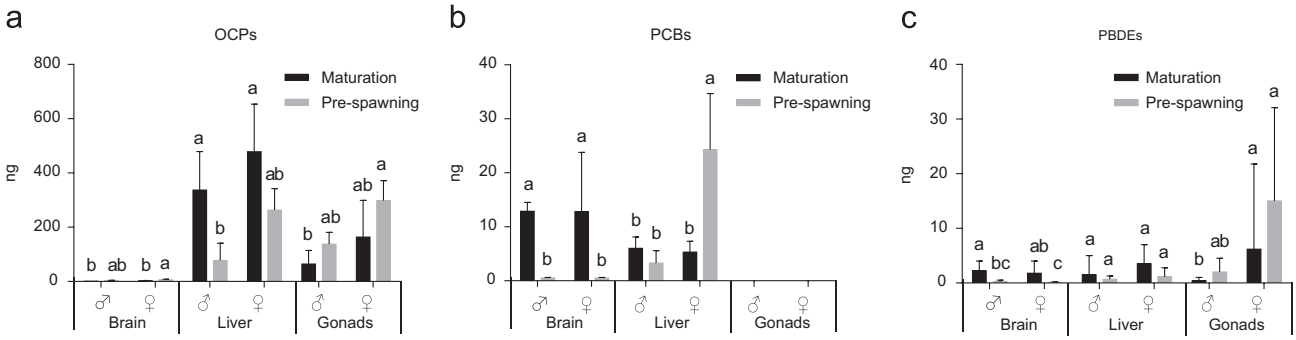

Fig. 1. Total OCPs (a), PCBs (b) and PBDEs (c) burden (ng) in brain, liver and gonads of Odontesthes bonariensis in maturation and pre-spawning stage. Different superscript letters (a or b) show statistically significant differences between sexes and/or stages within each organ or tissue, for each compound at $p<0.05$. ΣOCPs: γ-hexachlorociclohexane+endosulfans (α-+β- endosulfan+endosulfan sulfate)+chlordanes (α-+β- chlordan+trans-nonachlor)+dieldrin+DDTs (p,p′-DDT+p,p′-DDE). ΣPCBs: #44+52+66+101+110+138+153. The CB-congeners # 8, 28, 31, 87, 105, 126, 128, 156, 157, 167, 169, 170, 180, 187, 189, 195, 206 and 209 were below the detection limit. ΣPBDEs: #47+100+154/185.The BDE-congeners # 66, 99, 138 and 153 were below the detection limit.

with the high hydrophobicity of these compounds (p,p′-DDT log K_{ow}=6.91 and p,p′-DDE log K_{ow}=6.96, Sabljic et al., 1995). Furthermore, the higher concentrations of DDTs in pre-spawning gonads are consistent with the lipid mobilization during the gonadal development.

Since DDT is a forbidden pesticide, the presence of fresh p,p′-DDT in silverside could be due to Dicofol acaricide use, which contains DDT traces as manufacturing process impurities (Qiu et al., 2005). The p,p′-DDE/p,p′-DDT ratio >1 indicates a metabolism by fish and/or a direct intake of the metabolite from the diet and water column. Gonads and liver of both stages, and gills of pre-spawning males, showed higher p,p′-DDE concentration relative to p,p′-DDT (ANOVA Friedman, $p<0.05$, Fig. 2c, d). The preferential partition of DDTs to SPM (Gonzalez et al., 2012; 2013), enhance the incorporation of this pesticide by the plankton-feeding silverside. On the other hand, the predominance of p,p′-DDE in liver (Fig. 2c, d) would reflect both the direct uptake and the metabolic transformation of p,p′-DDT to p,p′-DDE by fish, but this cannot be discriminated.

As stated, brain showed a different contaminant distribution pattern between stages. Endosulfan concentrations were lower than DDTs in maturation individuals (Table 2, Fig. 2), while pre-spawning individuals kept the pattern endosulfans > DDTs. Moreover, unlike other organs and tissues analyzed, the brain of pre-spawning individuals showed higher endosulfans concentrations than maturation individuals (Table 2). This result could indicate that endosulfan reaches the brain after being distributed through all other organs. It is possible that the metabolization process and lipid mobilization during gonadal development (Landrum and Fisher, 1998) might redistribute endosulfans in the pre-spawning individuals, resulting in brain accumulation. DDTs levels reflected a chronic exposure scenery with similar levels in both stages. Unlikely, endosulfans pulses, linked to application periods, led to a different contaminant tissues distribution between stages.

As discussed earlier, the classes of lipid could influence contaminant distribution in fish. Other authors have reported a higher content of neutral and polar lipids (cholesterol and phospholids) in brain of *Salvelinus alpines* jointly with an inverse relationship between the lipid content and octachlorostyrene, a lipophilic persistent pollutant (Jorgensen et al., 1997). Therefore, the content of polar lipids in brain could be influencing endosulfan distribution, due to its relatively high water solubility (low K_{ow}).

The presence of endosulfans, DDTs and γ-HCH in the brain indicated that they are crossing the brain–blood barrier (BBB), which is its defense system against toxic substances. This barrier consists of endothelial cells with extremely narrow junctions. Moreover, lipid-rich myelin surrounding axons provide an additional barrier in the peripheral nervous system for hydrophilic xenobiotics (Di Giulio and Hinton, 2008). Consequently, this barrier would not be effective against highly hydrophobic contaminants such as POPs. Thus, endosulfan, DDTs and γ-HCH would cross the BBB due to their physicochemical characteristics, $K_{ow}>3$ and molecule conformation, which in the case of DDT is given by its planarity (Sabljic et al., 1995; Shoeib and Harner, 2002). Likewise, other authors have reported the BBB transfer by compounds with similar molecular weights and K_{ow} as diazinon (Üner et al., 2005).

3.4. PCBs and PBDEs distribution

PCBs were detected in all silverside organs and tissues, but they were below the detection limit in gonads. The absence of PCBs in gonads attracts attention since they tend to be rich in lipids and PCBs have a relatively great affinity for these biomolecules. However, the

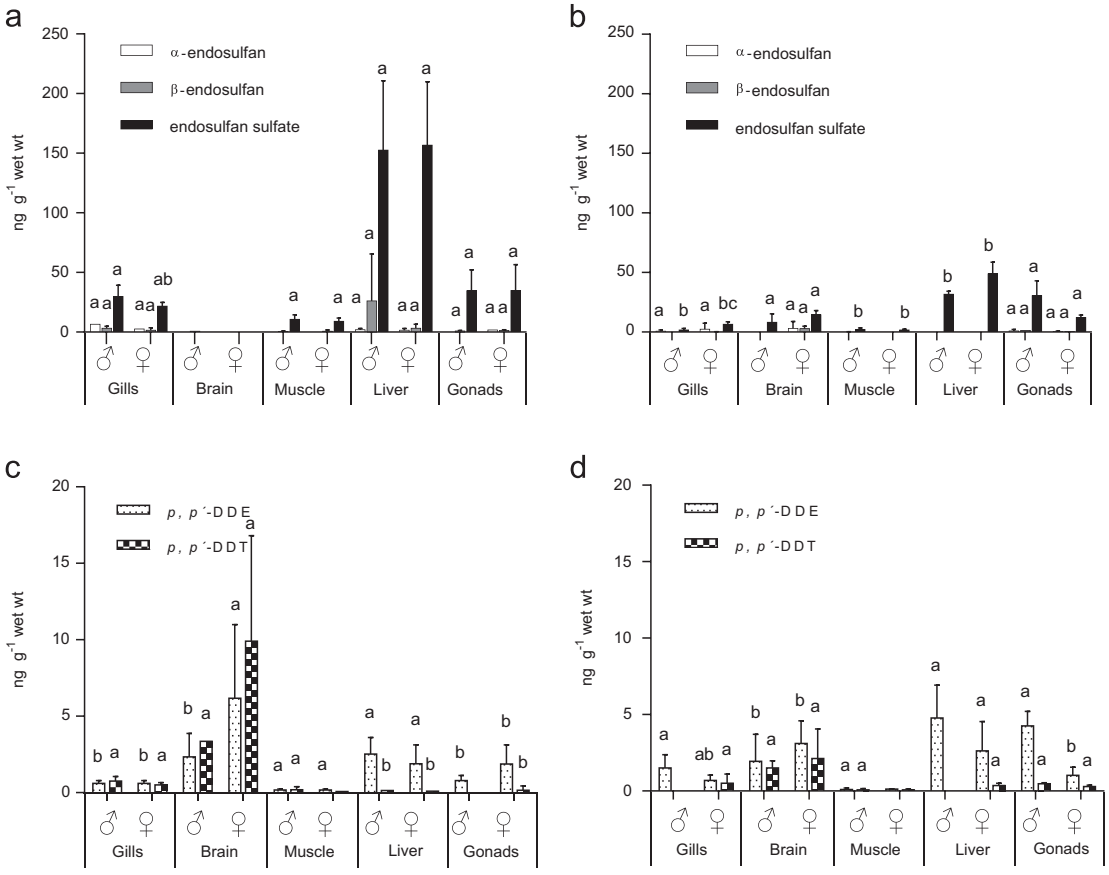

Fig. 2. Endosulfans (α- and β- isomers and endosulfan sulfate) and DDTs (p,p′-DDT and p,p′-DDE) in gills, brain, muscle, liver and gonads of *Odontesthes bonariensis* in maturation (a, c) and pre-spawning (b, d) stage. Different superscript letters (a, b or c) show statistically significant differences between sexes and/or stages within each organ or tissue, for each compound at $p<0.05$.

vitellogenin of teleost female contains twenty percent of lipids, mostly phospholipids which are polar lipids (Evans and Clairborne, 2006). Therefore, this high content of polar lipids and the relatively low levels of PCBs in the environment could lead to a low accumulation in gonads at levels below the detection method limit.

The distribution pattern of PCBs was brain/gills > liver > muscle (ANOVA Friedman, $p < 0.05$). These results are closely related to the entry points of pollutants, the low lipid content in muscle and the PCBs transport to the brain during lipid mobilization.

The congeners # 8, 28, 31, 87, 105, 126, 128, 156, 157, 167, 169, 170, 180, 187, 189, 195, 206 and 209 were below the detection limit in all organs and tissues. In maturation stage, the PCBs profile was dominated by the penta (#101 and 110) and hexa-CB congeners (#153 and 138), accounting for 26 percent and 76 percent of the total PCBs, respectively (Table 2). This pattern is similar to the commercial mixture Arochlor 1254, suggesting the use of this PCB mixture in the study area. In pre-spawning stages, the PCBs profile was dominated by the tri (#18) and tetra-CB congeners (#44 and 52), accounting for 37 percent and 55 percent of the total PCBs. The kinetic of these compounds is influenced by an efficient non-selective uptake process, and a selective elimination process that is based on the chlorine content, and to a lesser degree, chlorine position of the congener (Niimi and Oliver, 1983). Menone et al. (2000) registered a PCB pattern rich in higher congeners (#101, 110, 118, 153 and 138) in silverside from Mar Chiquita lagoon, suggesting that atmospheric deposition was not a major source of these PCBs. Conversely, pre-spawning silverside from QGR showed an inverse pattern with enrichment in the lower chlorinated congeners (#18, 44, 52 and 66, Table 2). Similar results were found in QGR streamwater, and were associated with chronic and diffuse pollution in the watershed (Gonzalez et al., 2013). This enrichment in lower chlorinated congeners could be the consequence of atmospheric transport and deposition in the water during cold months favoured by their high vapor pressure and a preferential desorption from atmospheric particles (Hoff et al., 1992a, b). Urban areas act as potential PCBs sources that move away via atmospheric transport to rural environments (Breivik et al., 2002). Moreover, pre-spawning individuals also showed a loss of higher chlorinated congeners. PCBs biotransformation by fish might occur via an insertion of an OH-group (Buckman et al., 2006). So, during the gonadal development biotransformation could lead to the hydroxylation of higher chlorinated PCBs that were not quantified in this study.

During the last decade, many results have been reported on the occurrence of PBDEs in fish, with a similar worldwide trend of PCBs decline and PBDEs increase over time (Bhavsar et al., 2007; Ondarza et al., 2011; Ondarza et al., 2012; Zhu and Hites, 2004). As shown in Table 2, PBDEs were detected in all silverside organs and tissues and the congener pattern was dominated by the BDE-47 (87 percent of total PBDEs) followed by the BDE-100. This pattern agrees with those reported for other fish species (Erdogrul et al., 2005; Rice et al., 2002) and has been linked to the composition of the "pentabromodiphenyl commercial product" by Strandberg et al., 2001. Gonzalez et al., 2013 reported also the occurrence of BDE-47 and BDE-100 in streamwater, river bottom sediment and SPM of QGR. The predominance of BDE-47 in these abiotic matrices denotes a direct relationship with fish tissues and supports the hypothesis of a direct intake from the environment through diet or gills.

3.5. Lipid peroxidation

LPO is a natural process in organisms and there are many sources of environmental stressors such as variations in temperature, food and oxygen availability, and reproductive development that may generate oxidative stress and increased LPO (van der Oost et al., 2003). Comparison among the levels of LPO in different organs or tissues is not possible due to variations in the content of PUFA in membrane lipids and antioxidant enzymes activities in each organ (Di Giulio and Hinton, 2008). Lipids are an LPO target, so their content might affect oxidative process (Huang et al., 2004). However, in this study no correlation was observed between the lipid percentage and LPO. There were no significant differences in LPO between sexes for any organ or tissue, except gonads (Table 3). Male gonads from maturation stage showed lower LPO levels than pre-spawning males, whereas females registered an inverse pattern (ANOVA, $p < 0.05$, Table 3). These results could be due to the higher endosulfan burden and lipid percentage in pre-spawning individuals. Previous studies demonstrated that endosulfan induces oxidative stress in various fish organs leading to LPO (Ballesteros et al., 2009; Pandey et al., 2001). Referring to female gonads, although the endosulfan burden was higher in pre-spawning individuals, the concentration was lower (not significant differences). These results are a consequence of ovary growth and therefore more pesticides are bioaccumulated, but due to dilution effect lower concentrations are found. During ovary growth, antioxidant responses might be increased by the metabolic processes involved. Enzymes activities fluctuations associated with reproductive cycles have been reported (Viarengo et al., 1991; Palace and Klaverkamp, 1993). This fact could explain why lower LPO levels were found in pre-spawning females. Nevertheless, in this study endosulfan and total OCPs values were lower than the concentrations reported as the cause of reproductive dysfunction in catfishes and carps (Singh and Singh, 2008).

Brain and gills showed higher LPO concentrations in maturation than pre-spawning individuals (Table 3). The higher LPO in maturation stage gills was related to increased endosulfan concentrations. Particularly, the positive correlation between LPO and endosulfan sulfate gills concentration ($r = 0.74$, $p = 0.01$), showed a direct relationship between endosulfan uptake, metabolism and LPO in this organ. On the other hand, acute exposure of *Channa punctatus* to 5 $\mu g\ L^{-1}$ of endosulfan under controlled conditions doubled LPO in gills (Pandey et al., 2001). Even endosulfans levels in QGR streamwater are three orders of magnitude lower than those used by Pandey et al., 2001; a direct connection was also found between endosulfan concentration and LPO in silversides. Therefore, QGR silversides are subject to endosulfan exposure during seasonal fluctuations associated with application periods that lead to LPO damage due to pesticide uptake and metabolism.

3.6. Contaminant levels in muscle: human consumption

The knowledge of contaminant levels in fish muscle is of great concern in the study area because sport and subsistence fishing are frequent activities in the watershed. The quantity of pollutant in a 300 g filet was calculated and the total amount that an individual of 70 kg could ingest per day without risk was obtained on the basis of the reference dose (Table 4). Although none of the studied compounds exceed the allowed value derived from the international reference dose, PCBs content in the filet of maturation individuals (June sampling) accounts for 18.6 percent of the daily allowed ingest for a 70 kg person. Furthermore, PCBs streamwater levels were above the recommended values for aquatic biota considering its use for human consumption (0.004 $ng\ L^{-1}$; Gonzalez et al., 2013). Special attention should be taken since a person can be exposed to other sources of pollution besides fish ingestion. However, when considering fish from pre-spawning stage (September sampling), PCBs fell to 5 percent of the oral Reference Dose. PCBs presence in the QGR is related mainly to diffuse pollution, however daily fish consumption represents a potential risk for human beings, especially fish from maturation

Table 4
Contaminants levels (ng g^{-1} wet wt.) and burden content (μg/1 filet of 300 g) of contaminants in muscle of silverside in maturation and pre-spawning stages and oral reference dose (RfD) values for each contaminant group.

	Silverside muscle							
	Maturation**		Pre-spawning		Maturation**	Pre-spawning		
	Muscle	Burden (mg)	Muscle	Burden (mg)	Percentage of daily allowed ingest for a 70 kg individual	Percentage of daily allowed ingest for a 70 kg individual	RfD (mg kg^{-1} d^{-1})	mg in a 70 kg individual
∑PBDEs*	0.22 ± 0.15	0.07	0.11 ± 0.12	0.03	1.0	0.4	0.1 [(a)]–2[(b)]	7.0
∑PCBs[#]	0.86 ± 1.09	0.26	0.22 ± 0.14	0.07	18.6	5.0	0.02[(c)]	1.4
γ-HCH[#]	0.56 ± 0.30	0.17	< dl	–	0.8	–	0.3	21.0
∑Endosulfans[#]	10.51 ± 3.38	3.15	1.99 ± 0.95	0.60	0.75	0.1	6.0	420.0
∑Chlordans	0.06 ± 0.03	0.02	0.16 ± 0.08	0.05	0.5	1.2	0.06[#]	4.2
Dieldrin[#]	0.15 ± 0.06	0.04	0.11 ± 0.04	0.03	1.1	0.9	0.05	3.5
∑DDTs[#]	0.26 ± 0.13	0.08	0.19 ± 0.11	0.06	0.2	0.2	0.5 [(d)]	35.0

Values represent the mean ± SD of 8 individual (4 males and 4 females) in maturation and 7 individual (3 males and 4 females) in pre-spawning stage. < dl: below the detection limit. ∑PBDEs: #47+100+154/185, 1; ∑PCBs: #44+52+66+101+110+138+153; γ-HCH: γ -hexachlorocyclohexane isomer; ∑Endosulfans: α-+β- isomers+endosulfan sulfate; ∑Chlordanes: α-+γ- isomer+trans-nonachlor; ∑DDTs: p,p′-DDT+ p,p′-; * IRIS EPA database (http://www.epa.gov/IRIS/ accessed may 2012); # ATSDR (http://www.atsdr.cdc.gov/accesed may 2012); [(a)]BDE-47, [(b)]Penta BDE, [(c)]Arochlor 1254/1248, [(d)]p,p′-DDT. ** Results of maturation stage are already published in Gonzalez et al., 2013.

stage. Therefore, fish ingestion is not advisable during the reproductive cycle period.

4. Conclusion

These results demonstrate that distribution patterns and pesticide concentrations in wild silverside were influenced by the environmental levels, and also by internal physiological processes. The current-use pesticide endosulfan, was the main accumulated compound, although phased-out pollutants as DDTs, which possess high environmental persistence, were also bioaccumulated, due to chronic exposure. Endosulfan levels and distribution, particularly in gills and liver, were highly associated with the time elapsed since the pesticide application period. Lipid mobilization and ovary growth during gonadal development lead to a pollutants dilution effect with differences in endosulfan levels between reproductive stages. PCBs distribution pattern was possibly associated with biotransformation processes during gonadal development, enhancing differences between reproductive stages. The absence of PCBs in gonads could be related to the presence of more polar lipids in this organ. In order to understand the relationship between lipid content and contaminant distribution in fish, it would be necessary to analyze lipid tissue composition.

Endosulfan exposure is a key trigger of reactive oxygen species, potentially leading to oxidative stress and consequently causing damage to lipid membranes. Results from this work showed a direct relationship between endosulfan exposure and LPO increase in organs primarily related with the absorption point of pollutants. Moreover, LPO was also related to application periods and gonadal development. The transition from maturation to pre-spawning stages caused a growth dilution effect and could be related with an increment of some antioxidants defenses. So, in order to discriminate the contribution of this stressor and environmental factors, future studies should consider bioassays of endosulfan long-term exposure. Synergism with other pesticides used in the soybean-wheat system together with physiological changes related with reproductive status should also be considered. In addition to the wild silversides results presented in this study, LPO levels could be monitored in wild silverside during the entire year involving different pesticide exposure levels and reproductive stages such as resting. Furthermore, the effect of such variables on juvenile fish could also be assessed under controlled conditions in order to validate the use of LPO measure from field individuals as indicators of damage by pesticides in impacted agriculture watersheds.

Lastly, the current pollutant concentrations in silverside muscle from the QGR do not pose a serious threat to human health. However, pesticide residues regulations in food do not consider particular cases such as the high fish consumption by the local fishing families as is known to occur in QGR watershed. Considering the extensive agricultural land use next to the river and the high probability of pesticide dispersion, prevention measures should be taken up for other current-use pesticides.

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