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Persistent organic pollutants (POPs) in fish with different feeding habits inhabiting a shallow lake ecosystem

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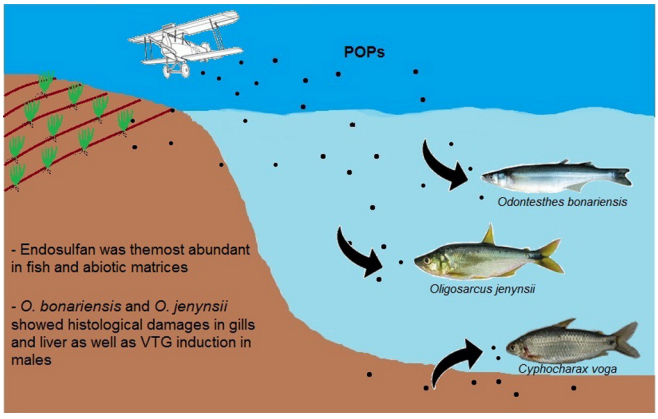
HIGHLIGHTS

- POPs accumulation might be related with fish feeding habits.
- Endosulfan levels in liver and gills might be related with histological damages.
- VTG detection in *O. bonariensis* and *O. jenynsii* males suggested the exposure to estrogenic compounds.

GRAPHICAL ABSTRACT



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ABSTRACT

The occurrence of persistent organic pollutants (POPs) in the environment can affect organisms inhabiting aquatic systems, in particular shallow lakes that are vulnerable to environmental stressors. This study aimed to assess POPs accumulation and changes at histological and physiological levels in tissues of three fish species with different trophic habits. Gills, brain, muscle, liver and gonads of *Odontesthes bonariensis*, *Oligosarcus jenynsii* and *Cyphocharax voga* were collected from the shallow lake La Peregrina, located in an agricultural area from Argentina. In addition, contaminant levels in surface water (SW), suspended particulate matter (SPM) and bottom sediments (BS) were assessed. Histological lesions were evaluated in fish tissues and levels of vitellogenin (VTG) were assessed in plasma of male fish in order to correlate these alterations with the presence of POPs in the environment. Organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) were determined by GC-ECD. Biotic and abiotic samples showed the same POPs distribution pattern: OCPs > PCBs > PBDEs. Although tissue distribution of OCPs was species-specific, muscle showed the lowest levels in all species. The most abundant contaminants were endosulfans, suggesting their widespread use in the area. *O. bonariensis* showed the highest

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endosulfans levels in liver (184.2–219 ng g^{-1} wet w), which was associated with the high SPM levels considering this species is a filter feeder. The occurrence of PCBs and PBDEs shows the ubiquity of these pollutants in the area. Histological lesions in gills and liver of *O. bonariensis* and *O. jenynsii*, might be related with the high levels of endosulfans in these organs. The detection of VTG in males warns about a possible exposure to estrogenic compounds in the environment. In conclusion, the simultaneous exposure of fish to multiple environmental pollutants leads to different alterations, so measures should be taken in order to prevent their occurrence and toxic effects.

1. Introduction

The environmental impact of agriculture is among the predominant global problems of this century. The current production system based on agricultural intensification leads to excessive pesticides application. Argentina is the export leader of soybean oil and meal and has become the third producer and exporter of soybean grains (USDA, 2015). The Pampa region is characterized by the presence of numerous shallow lakes (more than 10,000 larger than 10 ha.; Dangavs, 2005). These environments are highly productive systems (Quirós, 2004) and, due to their small depth and location within extensive agricultural areas, are also highly vulnerable. Agrochemicals and industrial pollutants can often reach waterbodies *via* air drift or surface runoff, affecting abundance and diversity of non-target species, producing complex effects on ecosystems and altering trophic interactions (Islam and Tanaka, 2004). In particular, organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) are classified as persistent organic pollutants (POPs) regulated by the Stockholm Convention (UNEP, 2011). Among OCPs, endosulfan was the last pesticide included as a POP (April 2011) and its use was fully banned in Argentina in July 2013 (SENASA, 2011). Regarding PBDEs, commercial penta and octabromodiphenyl ether are included in the POPs list (UNEP, 2011). POPs are lipophilic and persistent compounds that can be transported atmospherically over long distances, making them ubiquitous pollutants (Wania and MacKay, 1996). However, despite bans or restrictions on production and use, POPs are widely distributed within environmental compartments. It is well documented that fish accumulates POPs, readily taking them up from contaminated food (Fisk et al., 2001; Blocksom et al., 2010; Ondarza et al., 2011; Poma et al., 2014). Therefore, the study of fish species occupying different ecological niches with different feeding habits can provide useful information about dynamics of pollutants in aquatic environments.

Accumulation of POPs might lead to toxic effects in fish, causing endocrine disruption and altering biochemical, physiological, histological and morphological parameters (Noreña-Barroso et al., 2004; Da Cuña et al., 2011, 2013; Hued et al., 2013). Additionally, the presence of vitellogenin (VTG) in male fish can be used as a biomarker of exposure to estrogen mimics that can trigger VTG gene expression, resulting in detectable concentrations of this female-specific protein in blood and body surface mucus (Moncaut et al., 2003; Rey Vázquez et al., 2009; Genovese et al., 2011, 2012; Rautenberg et al., 2015).

The aims of this study were to evaluate the accumulation and tissue distribution of OCPs, PCBs and PBDEs and their relation with changes at histological and physiological levels in three fish species (*Odontesthes bonariensis*, *Cyphocharax voga* and *Oligosarcus jenynsii*) inhabiting different ecological niches of a shallow lake from the Pampa region.

2. Materials and methods

2.1. Study area

La Peregrina shallow lake (37°53′S; 57°54′W), located in southeast of the Pampa region, Argentina, has a total area of 90 ha., with a mean depth of 1.5 m and a muddy bottom. This water body is located in an area with extensive agricultural production (mainly soybean, potato, sunflower and wheat) and is frequently used in sport fishing practices.

2.2. Sampling

The three fish species selected were: *O. bonariensis* (Atheriniformes), a plankton feeder that ingests mainly zooplankton from the water column (Ringuelet et al., 1980, Grosman et al., 2001), *O. jenynsii* (Characiformes) an opportunistic carnivorous, mainly ingesting decapod crustaceans, terrestrial adult insects and fish (Grosman et al., 2002, Barros, 2004), and *Cyphocharax voga* (Characiformes), a bottom dweller organism, feeding on detritus and phytoplankton (Grosman et al., 1996, 2002; González Sagrario and Ferrero, 2013). Fishes were caught following standard fishing procedures using a 20 m-long coastal trawling net. Immediately, fishes were transported to the laboratory for processing. All captures were characterized by a fish taxonomist and individual morphometric parameters were measured (total weight, total and standard length).

Prior to blood collection, fish were softly sedated by immersion in iced water. Peripheral blood was collected by puncture of the caudal vein with a heparin-coated 25 gauge × 1/2 in needle, attached to a 1 mL syringe. Immediately following, fishes were weighed and euthanized by spinal pithing. After dissection, liver, gills, brain, muscle and gonads were quickly removed and weighed. Subsamples of these organs were wrapped in aluminum foil and kept frozen at −20 °C for POPs analysis. Moreover, subsamples of liver, gonad and gills were fixed for subsequent histological examination. Fish handling was conducted in accordance to international standards on animal welfare (Canadian Council on Animal Care, 2005). Finally, condition factor (CF = total weight × 100/total length^3), gonadosomatic (GSI = gonad weight × 100/100/total weight) and hepatosomatic (HSI = liver weight × 100/total weight) indexes were calculated.

Furthermore, to better understand the pollutants behavior in the aquatic system, contaminant levels in surface water (SW), suspended particulate matter (SPM) and bottom sediments (BS) of La Peregrina lake were also analyzed. Samples of SW were collected from the subsurface of the lake in 1 L pre-cleaned amber glass bottles for physicochemical and contaminant analyses and immediately transported to the laboratory (4 °C). SPM was obtained from the water samples at the laboratory by filtering through a 0.45 μm membrane filter under vacuum. BS samples consisted of the upper 0–5 cm of sediments collected with steel core samplers (4 cm inner diameter × 10 cm length). Samples of SPM and BS were air dried at room temperature until they reached a constant weight and kept wrapped in aluminum foil at −20 °C until analytical determination.

Samples of SW, SPM, BS and fish were collected in March 2009 (summer) during a post-pesticide application period.

2.3. Analytical procedures

2.3.1. Physicochemical characteristics of SW

Ions concentrations (CO_3^{2-}, HCO_3^-, Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, SO_4^{2-}, NO_3^-, NO_2^- and NH_4^+), total phosphorus (TP) and total and dissolved solids (TS and DS, respectively) were determined in the laboratory following standardized tests (APHA et al., 1992). Chlorophyll-a

concentration was measured according to Conzonno (1995). Turbidity was calculated by a Secchi disk. Electric conductivity (EC), pH and temperature were measured *in situ* using a HACH Sension 156 multiparameter meter.

2.3.2. OCP, PCB and PBDE extraction and clean up procedure

2.3.2.1. Fish tissues, SPM and BS samples. POPs were extracted according to Metcalfe and Metcalfe (1997), with modifications of Miglioranza et al. (2003). A total of 73 samples were analyzed. Subsamples of SPM (filters), BS (3–3.3 g) and fish organs (2.5–3 g muscle, 0.2–1 g liver, 0.3–3 g gonads, 0.1–1 g gills and 0.05–0.15 g brain), were homogenized with anhydrous sodium sulfate and spiked with 20 ng of PCB #103, as internal standard. They were Soxhlet extracted (8 h) with a mixture of n-hexane-dichloromethane (1:1 *v*/v), and then concentrated under vacuum and nitrogen flow to a final volume of 2 mL. For tissue samples, lipid content was removed by gel permeation chromatography (GPC) using Bio-Beads S-X3 (200–400 mesh). Purification of the contaminant fraction was performed by silica gel chromatography previously activated at 200 °C during 24 h. It was eluted with 150 mL of n-hexane:dichloromethane (2:1 *v*/v) and extracts were concentrated to 1 mL and kept in sealed vials at −20 °C prior to chromatographic analyses. In BS samples, sulfur compounds were removed with activated copper.

2.3.2.2. SW samples. Compounds were extracted from SW according to Gonzalez et al. (2012). Briefly, samples of 1 L of water were spiked with 20 ng of PCB #103 (internal standard), followed by a liquid–liquid extraction using 300 mL of n-hexane:dichloromethane (1:2 *v*/v), shaken during 2 h. The organic layer was concentrated to 2 mL for further clean up by chromatography on activated silica gel (200 °C, 24 h). Extracts were concentrated to 1 mL and kept in sealed vials at −20 °C until gas chromatographic analyses.

2.3.3. Gas chromatographic determination of OCPs, PCBs and PBDEs

The compounds analyzed included: hexachlorocyclohexanes (α-, β-, γ- and δ-HCH), endosulfans (α- and β-endosulfan, endosulfan sulfate), dichlorodiphenyltrichloroethanes (*p*,*p*′-DDT, *p*,*p*′-DDE, *p*,*p*′-DDD), chlordanes (α- and γ-chlordane, *trans*-nonachlor), dieldrin, heptachlors (heptachlor, heptachlor epoxide), PCBs congeners (IUPAC #8,18, 28, 31, 44, 52, 66, 87, 101, 105, 110, 118, 123, 126, 128, 138, 149, 153, 156, 157, 167, 169, 170, 180, 187, 189, 195, 206 and 209) and PBDEs congeners (IUPAC #28, 47, 66, 85, 99, 100,138,153 and 154).

OCPs, PCBs and PBDEs were identified and quantified using a Shimadzu 17-A gas chromatograph equipped with an Electron Capture Detector (GC-ECD). The GC capillary column used was a SPB-5 [(5%phenyl)-methyl polysiloxane], 30 m × 0.25 mm i.d. × 0.25 μm film thickness (Supelco, Bellefonte, PA, USA). 1 μL was injected on a splitless mode (275 °C) and the ECD 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, increase 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 North Kingston, RI, USA, PCB mixture and BDE-LMS (Bromodiphenyl Ethers Lake Michigan Study) from Accustandard Absolute Standards (INC, CT, USA) were used for identification and quantification of single compounds.

2.3.3.1. QA/QC. Laboratory and instrumental blanks were carried out through the same extraction and clean-up procedures as the samples. In this case, only anhydrous sodium sulfate was used. Results indicated that there was no contamination or interference of samples during laboratory handling. Recoveries, calculated by spiked matrices, were greater than 90 ± 15%. Instrumental detection limits, according to Keith et al. (1983), ranged between 0.03 and 0.05 ng mL^{-1} for HCHs (α-, β-, γ- and δ-isomers) and between 0.08 and 0.33 ng mL^{-1} for the rest of the 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.

2.4. Histological analysis

For standard histological analysis, small pieces of gills, liver and gonads were fixed in Bouin's fluid for 24 h and then preserved in 70% ethanol. Samples were dehydrated, embedded in paraffin (Paraplast®, Fisher Brand, USA) and transversally sectioned at 7 μm (Leitz Wetzlar 1212 Leica microtome, Germany). Sections were stained with Hematoxylin–Eosin (H&E). Photomicrographs were taken with a Nikon Microphot FX microscope coupled with a Coolpix 5400 digital camera (Nikon, Japan).

2.5. VTG immunodetection by Western blot

Blood samples with the addition of 2.5 μL of 1 mM PMSF (phenylmethylsulfonyl fluoride, protease inhibitor) were centrifuged at 2500 × *g* for 15 min at 4 °C to obtain plasma which was stored at −20 °C. Plasma samples were analyzed by reducing sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS/PAGE) using a 5% stacking gel and 8% resolving gel followed by Western blot. Samples were diluted in sample buffer containing 0.3 M Tris/HCl, pH 6.8, 10% SDS, 12% glycerol, 11% β-mercaptoethanol and 0.2% bromophenol blue. Equal amounts of protein (20 μg), as measured by Lowry et al. (1951), were loaded into each lane. Molecular mass was estimated using prestained molecular mass standards (Invitrogen). Following separation by electrophoresis, proteins were transferred to a nitrocellulose membrane (Hybond, Amersham Pharmacia) for 90 min at 4 °C and 100 V in transfer buffer (25 mM Tris, 187 mM glycine, 20% (*v*/v) methanol). Non-specific binding of membranes was blocked with 5% non-fat powdered milk in TBST (100 mM Tris–HCl, 0.9% NaCl, 0.1% Tween 20, pH 7.5) overnight at 4 °C. Membranes were then incubated with the primary antiserum, rabbit anti-perch VTG (donated by Dr. B. Allner, Hessisches Landesamt für Umwelt und Geologie, Wiesbaden, Germany) 1:3000, for 90 min at room temperature. After three 5 min washes in TBST, membranes were incubated with a biotinylated anti-rabitt IgG antibody (Vector Lab., CA) diluted 1:1000 for 1 h and washed again, followed by incubation with horseradish peroxidase-conjugated streptavidin (Dako, CA) diluted 1:3000 for 1 h in the dark. Immunoreactivity was developed with 0.1% 3,3′ diaminobenzidine in 0.1 M Tris buffer pH 7.6 (DAB) and 0.02% water peroxide (H_2O_2). Omission of the primary antiserum was performed for Western blot as a negative control. Vitellogenic females for each species were used as positive controls.

2.6. Data expression and statistical analysis

Chemical concentrations were expressed as the average ± standard deviation, and reported as ng L^{-1} for SW, ng g^{-1} dry weight (ng g^{-1} dry w) for SPM and BS and ng g^{-1} wet weight (ng g^{-1} wet w) for fish. Statistical analyses were performed using Infostat Software Package (Di Rienzo et al., 2010). A one way analysis of variance (ANOVA) was used to test significant differences in contaminant levels between fish species followed by Tukey test. When parametric assumptions were not met a Kruskal Wallis analysis followed by Dunn's test was used. Differences in contaminant levels between tissues were tested using a non-parametric Friedman ANOVA for multiple dependent samples. Differences between sexes were tested using a one way ANOVA, followed by a *t*-Student test for pair comparisons or a Mann Whitney test when parametric assumptions were not met. Pearson's correlation coefficient was calculated between lipid percentage and contaminant levels. When the data

were not normally distributed a Spearman's correlation coefficient was used instead.

3. Results and discussion

3.1. Physicochemical characterization of SW

The physicochemical properties of La Peregrina SW (Table S1) were similar to those reported by Díaz and Colasurdo (2008) in the same shallow lake. Nevertheless, turbidity (8.0 cm), together with total solids (TS = 1084 mg L^{-1}) and dissolved solids (DS = 935 mg L^{-1}) were significantly higher than values registered by these authors (turbidity = 16.2 cm, TS = 613.7 mg L^{-1}, DS = 573.7 mg L^{-1}). Differences could be a result of a storm occurring days prior to sampling in the study area, which could have affected sediment stability leading to higher turbidity. These results coincide with those of other Pampean shallow lakes which usually are known as "turbid" lakes. These ecosystems are characterized by high turbidity, abundant development of the phytoplankton and high abundance of planktivorous fish (Quirós et al., 2002). Bicarbonate (595.7 mg L^{-1}) was the dominant anion in SW followed by Cl^- (114.0 mg L^{-1}), whereas Na^+ (340.0 mg L^{-1}) was the dominant cation.

3.2. Biological characteristics

O. bonariensis showed CF values (males = 0.8, females = 0.8) below the species standard curve (Mancini et al., 2008). *O. jenynsii* also showed CF values (males = 0.9, females = 1.0) lower than those reported by other authors (males = 1.0, females = 1.4; Barla et al., 2003). Regarding *C. voga*, literature about CF values is scarce for this species. Therefore, in order to obtain a reference value, CFs were calculated using measurements of total weight and total length values of *C. voga* from a shallow lake (González Sagrario and Ferrero, 2013). The obtained CF ranged between 2.5 and 3.5, being higher than the value obtained at the present work (CF = 2.0). The CF is an indicator of fish health (Grosman et al., 1996) and varies as a function of several biological characteristics such as amount of fat, susceptibility to environmental changes, gonad development and degree of parasitism (Barbieri et al., 1985). Females of *C. voga* showed the highest GSI (18.8) together with the highest gonad lipid content (6.1%) compared to *O. bonariensis* (GSI = 2.3, lipid content = 3.7%) and *O. jenynsii* (GSI = 2.0, lipid content = 3.7%), which could be related with a more advanced reproductive maturity. *O. jenynsii* showed lower HSI values (males = 0.5, females = 0.5) with regard to *O. bonariensis* (males = 1.0, females = 1.5) and *C. voga* (females = 1.1) (see Table S2 in supplementary material). All *C. voga* specimens caught were females.

3.3. Total contaminant levels

POPs concentration showed the following order: OCPs > PCBs > PBDEs in the three fish species tissues (Tables S3, S4 and S5) and the abiotic matrices (SW, SPM and BS; Table S6). In general, pollutants levels were correlated with the tissue lipid content within each fish species, showing brain and liver the highest concentrations (Friedman ANOVA, $p < 0.05$). Brain registered the highest lipid percentage in the three fish species, followed by liver and gonads, whereas gills and muscle showed the lowest values (Friedman ANOVA, $p < 0.0001$). Thus, a good correlation was obtained between OCPs and lipid percentage for *O. bonariensis* ($r = 0.89$, $p < 0.0001$) and *C. voga* ($r = 0.87$, $p < 0.0001$). However, PCBs and PBDEs were not significantly correlated with lipid levels although a positive relation was observed. Similar results were previously observed for *O. bonariensis* and lipid classes have been proposed as influencing factors on contaminant tissue partition (Silva Barni et al., 2014).

3.3.1. OCPs

Endosulfans were the main pollutants in fishes (Fig. 1, Tables S3, S4 and S5) and abiotic matrices (Fig. 2, Table S6), constituting more than 84%, 67% and 89% of OCPs in *O. bonariensis*, *O. jenynsii* and *C. voga*, respectively. The predominance of this insecticide demonstrates that agriculture is the main source of pollutants in the lake. Technical endosulfan use and production was totally banned by the National Service of Food Quality (SENASA from the Spanish acronyms, Argentina) in July 2013 (SENASA, 2011). Consequently, this insecticide was in use during the sampling period. Inter-specific differences in endosulfans levels and tissue distribution between the three fish species were observed. Endosulfans distribution between tissues was as follows: brain > liver > gonads > gills ≥ muscle in *O. bonariensis* (Friedman ANOVA, $p < 0.0001$; Fig. 1 Table S3), liver ≥ gills ≥ brain ≥ gonads > muscle in *O. jenynsii* (Friedman ANOVA, $p = 0.002$; Fig. 1, Table S4) and brain > gonads > gills ≥ liver > muscle in *C. voga* (Friedman ANOVA, $p < 0.0001$; Fig. 1, Table S5). As toxicants are metabolized in the liver, high pollutant levels are expected in this organ. However, the high endosulfans levels in brain indicate that these compounds are able to pass through the brain–blood barrier. Similar results were found in silver carp (*Hypophthalmichthys molitrix*), with higher OCPs concentrations in brain than liver (Zhou et al., 2007). Moreover, individuals of *Jenynsia multidentata* exposed to technical endosulfan for 24 h showed the highest endosulfans levels in liver and brain (Ballesteros et al., 2011). Silva Barni et al. (2014) also reported endosulfans accumulation in brain of *O. bonariensis* from a lothic environment of the Pampa region, although overall levels were lower (males: 5.4 ± 1.5 ng g^{-1} wet w, females: 20.6 ± 12.0 ng g^{-1} wet w during the post-application period) than those of the present work (males = 184.2 ± 26.2 ng g^{-1} wet w, females = 219.1 ± 13.3 ng g^{-1} wet w). Muscle showed the lowest endosulfans levels in the three fish species, in accordance with its lower lipid content.

When comparing species, *O. bonariensis* registered significantly higher endosulfan levels than *C. voga* and *O. jenynsii* in liver (Kruskal Wallis, $p = 0.0068$, Fig. 1) and brain (Kruskal Wallis, $p = 0.0002$, Fig. 1). These results could be associated with the different feeding habits and trophic web levels occupied by the three species (Lavandier et al., 2013). The high endosulfans levels found in liver of *O. bonariensis* could be related with the high levels recorded in SPM (Fig. 2, Table S6), considering that this species filters particles from the water column (Ringuelet et al., 1980). It is generally recognized that direct uptake of contaminants from SPM, as well as from food, serve as sources of hazardous chemicals for fish (Zhou et al., 1999). Even when *O. bonariensis* can develop a piscivorous diet during the adult stage, enabling biomagnification processes, the filter-feeding habits might be favored in Pampean shallow lakes due to its high productivity (Quirós et al., 2002). On the other hand, *O. jenynsii*, an opportunistic carnivorous lacking filter-feeding habits, showed lower bioaccumulation potential (Barros, 2004). Similarly, *C. voga*, a bottom feeder with a high percentage of detritus in its diet (Destefanis and Freyre, 1972; González Sagrario and Ferrero, 2013), showed lower accumulation of this pesticide, as lower endosulfans concentrations were found in BS (Fig. 2, Table S6). Colombo et al. (1990) reported a similar OCPs pattern between *Prochilodus platensis*, a detritivorous fish, and sediments, suggesting that the absorption from sediments could be a dominant process. Additionally, Colombo et al. (2011) found that the biochemical composition of settling material from metropolitan Buenos Aires coast was similar to the stomach contents of fishes, supporting the importance of considering feeding habitat of the organisms. Other many factors related to fish biology such as species, sex, age, reproductive stage and/or trophic level (Rosseland et al., 1999; Rognerud et al., 2002; Vives et al., 2005) can be involved in pollutants accumulation leading to the final pesticide behavior reported here.

Technical endosulfan is a mixture of α- and β- isomers in a 7:3 ratio. As the α-isomer degrades faster than the β-isomer in the environment, the α −/β-ratio enables the estimation of endosulfan application period

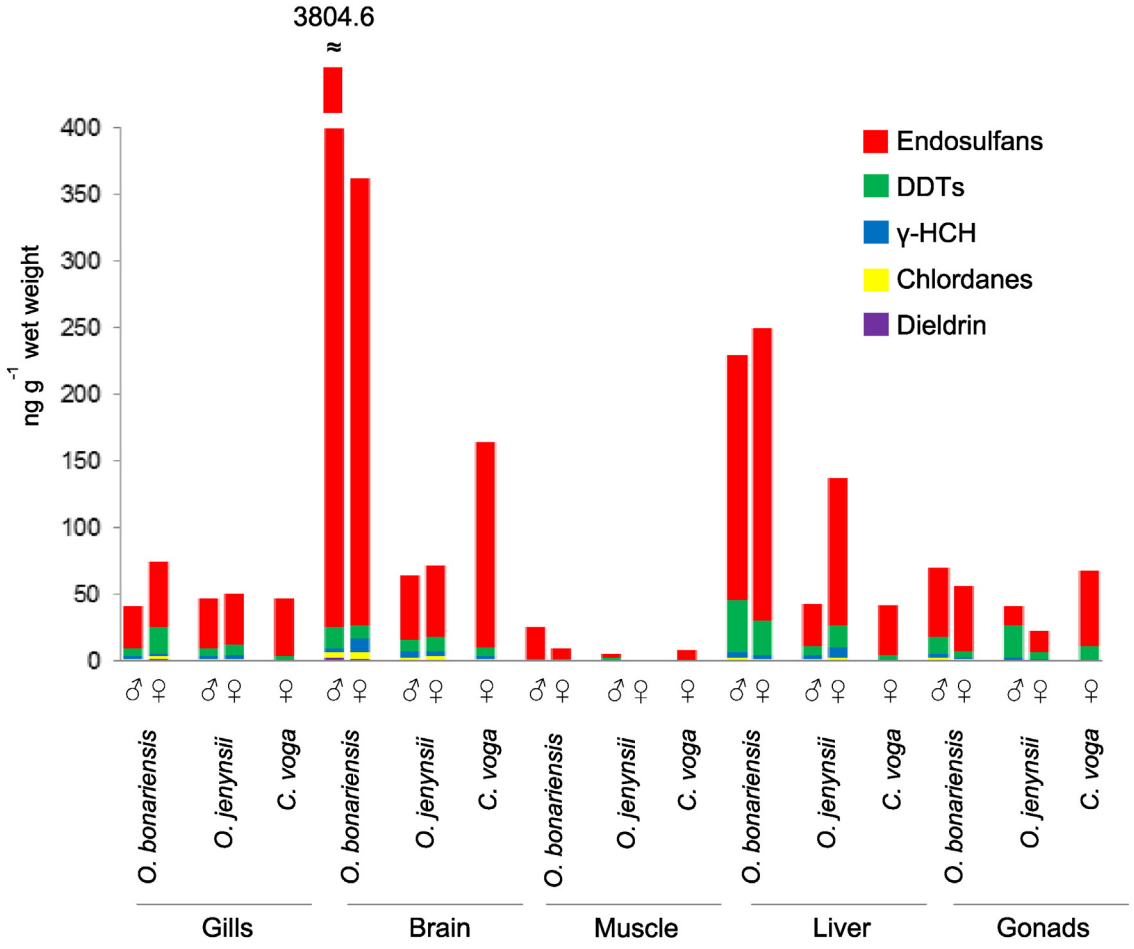

Fig. 1. Organochlorine pesticides (OCPs) in gills, brain, muscle, liver and gonads of *Odontesthes bonariensis*, *Oligosarcus jenynsii* and *Cyphocharax voga*. ΣEndosulfans: α- + β- + endosulfan sulfate; ΣDDTs: *p,p′*-DDT + *p,p′*-DDE + *p,p′*-DDD, HCH: γ-HCH; ΣChlordanes: α- + γ-chlordane and *trans*-nonachlor.

(Schmidt et al., 1997). The α/β ratio obtained and the presence of endosulfan sulfate in SPM and BS suggested the exposure to an aged technical mixture (Walse et al., 2002, Weber et al., 2010; Table S6). *O. jenynsii* and *C. voga* showed predominance of α-endosulfan over the β-isomer and endosulfan sulfate in most tissues (Table S4 and S5), following the pattern observed in abiotic matrices (Table S6). Therefore, following pesticide application, α-isomer could become adsorbed to SPM and BS, being available to be incorporated by fish. On the other hand, *O. bonariensis* showed a predominance of endosulfan sulfate over endosulfan isomers in liver and gonads (Friedman ANOVA, $p < 0.05$). Accordingly, this could indicate a higher endosulfan metabolism in *O. bonariensis* with respect to *C. voga* and *O. jenynsii*. Therefore, different

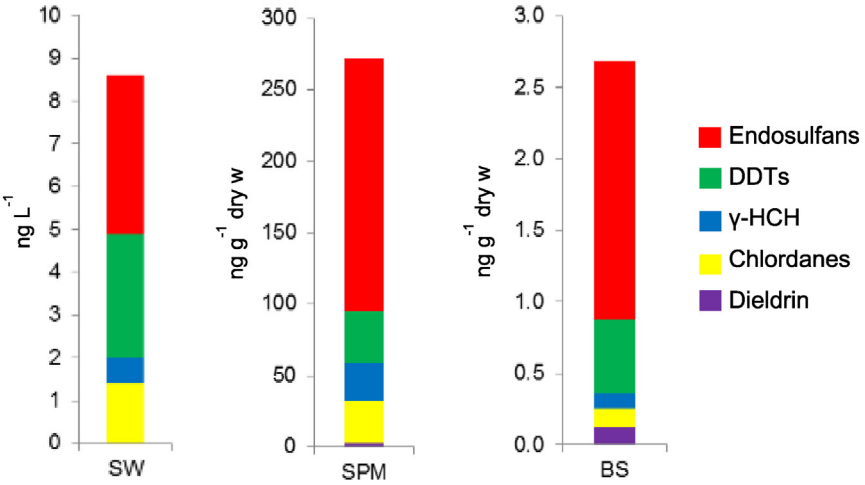

Fig. 2. Organochlorine pesticides (OCPs) in surface water (SW), suspended particulate matter (SPM) and bottom sediments (BS) from the shallow lake La Peregrina. ΣEndosulfans: α- + β- + endosulfan sulfate; ΣDDTs: *p,p′*-DDT + *p,p′*-DDE + *p,p′*-DDD, HCH: γ-HCH; ΣChlordanes: α- + γ-chlordane and *trans*-nonachlor.

species would show different endosulfans distribution patterns in the same environment caused not only to differing feeding habits but also to metabolic differences.

DDTs represented the second predominant pesticide group in fish (Fig. 1) and abiotic matrices (Fig. 2), constituting about 12%, 20% and 8% of OCPs in *O. bonariensis*, *O. jenynsii* and *C. voga*, respectively. DDTs distribution in tissues was species dependent. Liver of *O. bonariensis* showed DDT levels significantly higher than in other tissues (Friedman ANOVA, $p = 0.0018$); *C. voga* presented the highest levels in gonads (Friedman ANOVA, $p < 0.0001$), while *O. jenynsii* showed no significant differences between tissues. A good correlation was observed between DDTs levels and lipid percentage in *O. bonariensis* ($r = 0.78$, $p < 0.0001$) and *C. voga* ($r = 0.86$, $p < 0.0001$), in accordance to the high hydrophobicity of these pesticides (*p,p'*-DDT log $K_{ow} = 6.91$ and *p,p'*-DDE log $K_{ow} = 6.96$, Sabljic et al., 1995). Among species and tissues, the highest DDT and endosulfans levels were found in liver of *O. bonariensis*, coinciding with the highest pesticide levels recorded in SPM. These results could be attributed to the filter-feeding habits of *O. bonariensis* where the uptake of pesticides through SPM ingestion would be an important input pathway.

DDTs levels found in *O. bonariensis* tissues appear as relatively high compared to those registered in the same species from the Quequén Grande River (Silva Barni et al., 2014) while relatively low compared to those registered in *Odontesthes hatcheri* from Negro River, Argentina (Ondarza et al., 2014), in which soils are recognized as a hot spot of *p,p'*-DDE (Gonzalez et al., 2010). DDE/DDT ratio is generally considered to be an index of pollution age (Iwata et al., 1995, Maldonado and Bayona, 2002). All fish tissues showed a *p,p'*-DDE/*p,p'*-DDT ratio > 1 (Table S3, S4 and S5), as was observed in SW and BS (Table S6). Nevertheless, the *p,p'*-DDE/*p,p'*-DDT ratio < 1 in SPM could be associated with dicofol use, since Qiu et al. (2005) have reported the presence of DDT impurities in the technical mixture.

Levels of dieldrin, chlordanes and γ-HCH (Lindane) in the three fish species (Fig. 1) as well as in abiotic matrices (Fig. 2) correspond with a diffuse and historical contamination in the area, considering these compounds were totally banned in 1980 (dieldrin) and 1998 (chlordanes and Lindane).

3.3.2. PCBs and PBDEs

PCB concentrations varied between tissues in the three fish species. The highest PCBs levels were found in brain and the lowest in muscle of all species (Friedman ANOVA, $p < 0.05$, Tables S3, S4 and S5), in agreement with tissue lipid content.

PCBs, especially higher-chlorinated congeners, have a great tendency to adsorb to sediments and remain static for long periods (WHO, 1993; van der Oost et al., 2003). *C. voga* ingests large quantities of sediments and presents a high degree of interaction with the substrate. As a consequence of this behavior, all tissues of *C. voga* showed a predominance of higher chlorinated congeners, mainly penta (#101) and hexa-CBs (#153 and 138; Table S5), which collectively accounted for average 71.7% of total PCBs. Additionally, a similar congener pattern was observed in sediments but in relatively low levels (< dl - 0.4 ng g^{-1} dry w, Table S6). Colombo et al. (2011) found a similar composition of PCBs, with a prevailing contribution of penta, hexa and heptachlorobiphenyls in *Prochilodus lineatus*, a detritivorous fish from the coastal area of Metropolitan Buenos Aires. *Tetra*, penta and hexa-CB congeners prevailed in

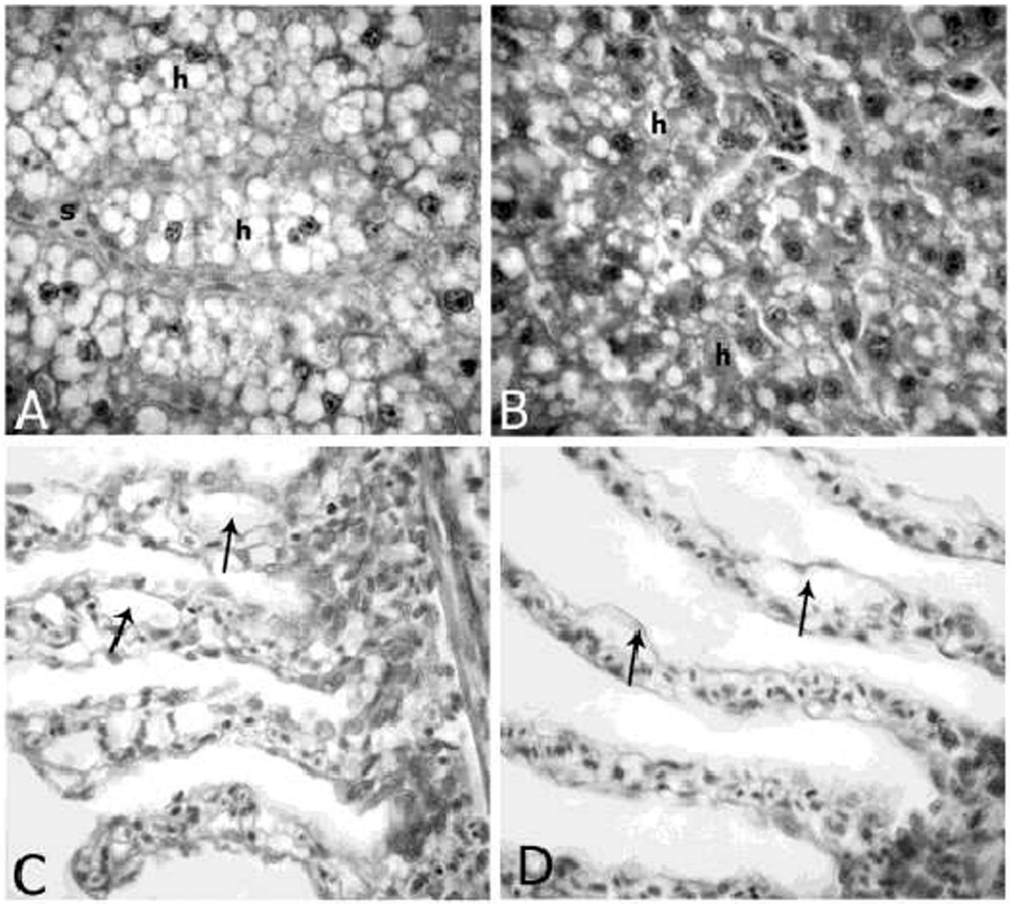

Fig. 3. *O. bonariensis*. Liver sections from a female (A) and a male (B). Gill sections from a female (C) and a male (D) showing edema associated with epithelial lifting (arrow) in secondary lamellae. h: hepatocyte; s: sinusoid.

O. bonariensis (# 52, 44, 66, 101, 153 and 138) and *O. jenynsii* (# 44, 110, 153 and 138), accounting for about 84.2% and 75.7% of the total PCBs, respectively (Tables S3 and S4). *O. bonariensis* showed a major contribution of tetra-CBs (44.3%) than *O. jenynsii* (19.8%) and *C. voga* (23.5%).These results were in accordance with SPM congener pattern (Table S6). Moreover, similar PCB profiles in fish have already been reported for *Salmo trutta* and *O. hatcheri*, from Andean Patagonia and Negro River (Argentina), respectively, with a predominance of penta and hexa-CBs in different tissues (Ondarza et al., 2011, 2014). This pattern reflects pollution by Arochlors 1254 and 1260, which were historically used in the region, as well as the high lipophilicity and persistence of these congeners (Breivik et al., 2002).

Regarding PBDEs, congeners presented low values, in most of cases below the limit of quantification, for all matrices (Tables S3, S4, S5 and S6). BDE-47 and BDE-100 were predominat in *O. bonariensis* and *C. voga*, whilst only BDE-47 was detected in *O. jenynsii*. This pattern of congeners has been also reported in other fish species from freshwater systems (Colombo et al., 2011; Ondarza et al., 2012, 2014; Silva Barni et al., 2014) and is associated to the composition of the commercial penta-BDE formulation that was one of the most used PBDE mixtures in South America.

Several studies have suggested that PBDEs are susceptible to degradation, via debromination, following exposure to UV light (Söderström et al., 2004) as well as a consequence of microbial activity (He et al., 2006) and fish metabolism (Stapleton et al., 2006). Biotransformation of BDE99 to BDE47 has been previously described in whole body, liver, and intestinal tissues of common carp (*Cyprinus carpio*) (Stapleton et al., 2006). Therefore the prevalence of BDE47 in the three fish species found in the present work might be partially due to a biotransformation process.

3.4. Histological analysis

Histological analysis of target organs is very useful to determine possible relationships between pollutants and biological effects and its use has been recommended for monitoring POPs in aquatic ecosystems (STAP/GEF, 2003). In this work, *O. bonariensis* and *O. jenynsii* of both sexes showed histological lesions in liver and gills while no alterations were detected in ovaries or testes.

O. bonariensis liver showed fatty degeneration (nuclei with loose chromatin and scarce free cytoplasm). Gills presented secondary lamellae with edema and epithelial lifting (Fig. 3). In the case of *O. jenynsii*, secondary lamellae fusion and mast cell infiltration, as well as hyperplasia of primary lamellae epithelium, were the most remarkable gill tissue disorders. Basophilic cytoplasm and pycnotic nuclei, indicating a necrotic process, were observed in the hepatocytes. Liver from *C. voga* females showed a high incidence of melano-macrophages centers as the only alteration (Fig. 4).

Gills constitute the primary route for the entry of pesticides from the water column and damages described here such as fusion of secondary lamellae are associated to acute exposure to contaminants (Oliveira Ribeiro et al., 2005). Also, these alterations are considered a defense mechanism against pollutant entry as they decrease the gill surface area that is exposed to toxic substances (Ballesteros et al., 2007).

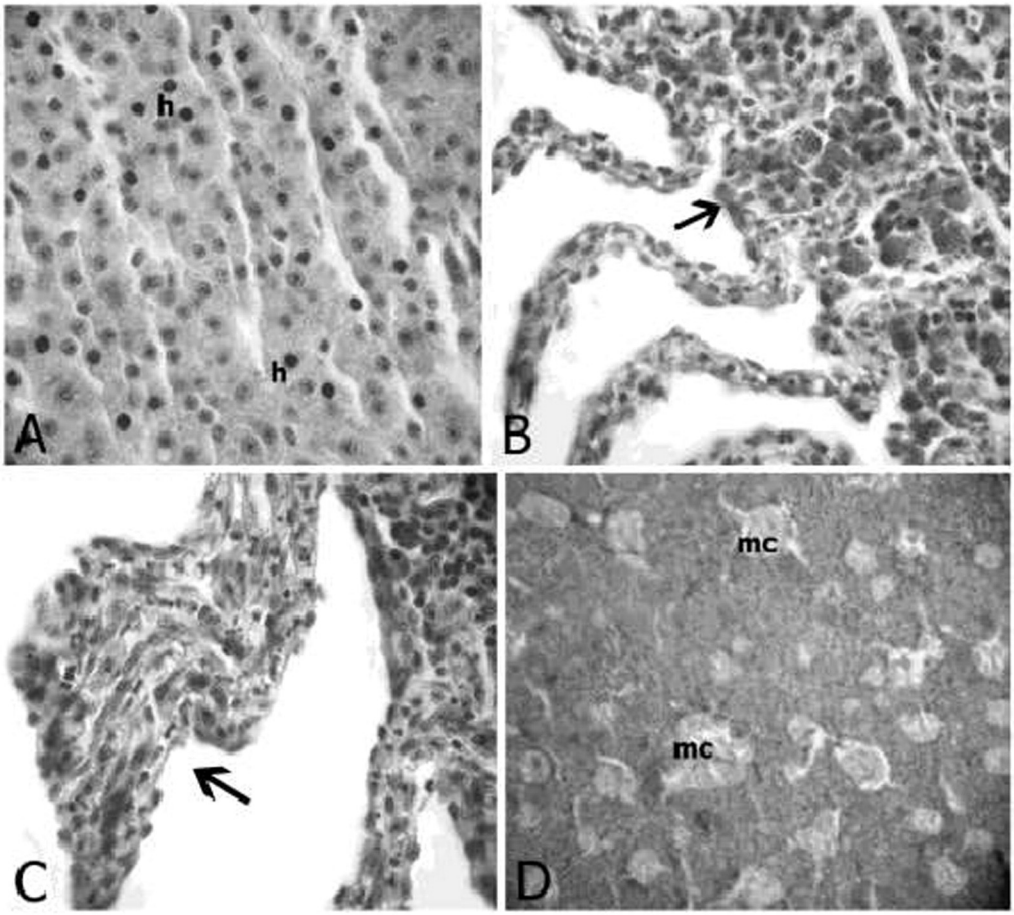

Fig. 4. *O. jenynsii.* A) Liver section from a female showing necrotic process, B) Gill section from a male showing hypertrophy of primary lamellae epithelium (arrow), C) Gill section from a female showing secondary lamellae fusion (arrow). *Cyphocharax voga.* D) Liver section from a female showing a high incidence of melano-macrophages centers. h: hepatocyte; mc: melano-macrophage center.

Conversely, liver as the first organ of detoxification (Velmurugan et al., 2009) is subject to both acute and chronic exposure to organic contaminants. The necrosis and the presence of abundant melano-macrophage centers suggest a continuous exposure to environmental pollutants in *O. jenynsii* and *C. voga*, respectively (Oliveira Ribeiro et al., 2005; Agius and Roberts, 2003).

The histological lesions observed in gills and liver might be related with the predominance and relatively high levels of endosulfans in these organs. Similar histological disorders were reported for other fish species (*Jenynsia multidentata*, *Cichlasoma dimerus*, *Danio rerio*) after laboratory exposure to endosulfan mixture (Ballesteros et al., 2007; Da Cuña et al., 2011; Velasco-Santamaría et al., 2011). Nevertheless, association between these fish lesions and the presence of endosulfans should be carefully studied since fish are exposed to multiple pollutants in the environment.

3.5. VTG immunodetection

VTG synthesis in male fish has been used as a specific biomarker of exposure to xenoestrogens (Sumpter and Jobling, 1995). The estrogenic effects of some POPs are well documented (Flouriot et al., 1995; Christiansen et al., 2000; Okoumassoun et al., 2002). In particular, endosulfan has previously been shown to be capable of inducing VTG synthesis in fish after exposure to concentrations higher than 1 μg L^{-1} (Min et al., 2010; Chow et al., 2013; Lee et al., 2013). Nevertheless, studies with lower concentrations suggest that endosulfan does not act as an estrogen mimic (Hemmer et al., 2001; Berntssen et al., 2010). In addition, nonionic surfactants such as nonylphenol ethoxylate are usually used in combination with or as coadyuvants in pesticide commercial formulations in order to increase chemical penetration in crops, and their degradation products (eg. nonylphenol) have been demonstrated to be estrogenic (White et al., 1994).

SDS-PAGE followed by Western blot of plasma samples revealed one ir-VTG band (70 kDa) *for O. bonariensis* males and two for *O. jenynsii* males (114 and 73 kDa, Fig. 5). Induction of VTG in males of both species suggests that fish are exposed to environmental estrogenic compounds. In the case of *C. voga*, only females were sampled so no results can be mentioned. The prevalence and relatively high levels of endosulfans in liver might lead to VTG synthesis, since this process occurs in hepatocytes in response to the activation of estrogen receptors (Mommsen and Walsh, 1988). DDTs, the second predominant group, could be also responsible of VTG induction in males. Nevertheless, *o,p′*-DDT has been reported as a stronger inducer of VTG than *p,p′*-DDT (Okoumassoun et al., 2002) and only *p,p′*-isomers were measured in this study. Therefore, *o,p′*-isomers could be present in the environment and also induce VTG production in males. Considering that *o,p′*-DDT accounts for more than 80% of current-used dicofol whilst it represents only 18% in the phased-out technical DDT mixture (Qiu et al., 2005), more attention should be paid to the *o,p′*-DDT inputs due to dicofol use.

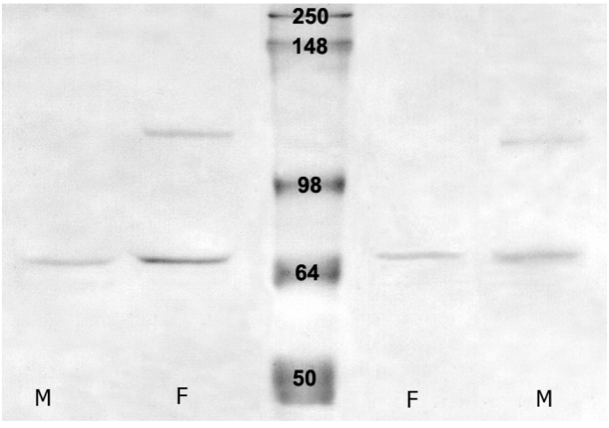

Fig. 5. Immunodetection of VTG in plasma samples from *O. bonariensis* (left) and *O. jenynsii* (right). One ir-Vtg band (70 kDa) was revealed in male *O. bonariensis* while two bands (114 and 73 kDa) were evidenced in male *O. jenynsii*. Numbers: pre-stained molecular weight standards.

4. Conclusion

This work provides an integrative analysis of POPs in freshwater fishes with different feeding habits, demonstrating the utility of analytical and biological approaches for assessing environmental risk to fish species in a typical shallow lake. The distribution pattern of contaminants in fish tissues reflected the importance of considering the ecological niche inhabited by fish to interpret POPs bioaccumulation. A relationship was observed between histological damage and vitellogenesis induction with the levels of POPs in fish organs, mainly liver and gills, which are directly related with POPs exposure. However, it cannot be discarded that other chemicals present in the environment could contribute to such effects. Regardless, the magnitude of these effects appears to not seriously affect the fish vital system, but it could become crucial during reproduction and/or under prolonged exposure periods. Therefore, combining both analytical and physiological studies provides useful information to assess fish exposure in contaminated environments. It has been evidenced that shallow lakes adjacent to agricultural areas are very likely to be affected by pesticides entering via rainfall runoff as well as atmospheric inputs of other contaminants. Since exposure of fish to even low levels of pesticides such as endosulfans can result in several alterations, monitoring programs should be adopted to prevent the occurrence of contaminants in the aquatic environment.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2016.01.176.

References

Agius, C., Roberts, R.J., 2003. Melano-macrophage centres and their role in fish pathology. J. Fish Dis. 26 (9), 499–509.
APHA, AWWA, WEF, 1992. Standard methods for the examination of Water and wastewater. 18th ed. American Public Health Association, Washington.
Ballesteros, M.L., Bianchi, G.E., Carranza, M., Bistoni, M.A., 2007. Endosulfan acute toxicity and histomorphological alterations in *Jenynsia multidentata* (Anablepidae, Cyprinodontiformes). J. Environ. Sci. Health B 42 (4), 351–357.
Ballesteros, M.L., Gonzalez, M., Wunderlin, D.A., Bistoni, M.A., Miglioranza, K.S.B., 2011. Uptake, tissue distribution and metabolism of the insecticide endosulfan in *Jenynsia multidentata* (Anablepidae, Cyprinodontiformes). Environ. Pollut. 159 (6), 1709–1714.
Barbieri, G., Verani, J.R., Pereira, J.A., Barbieri, M.C., Peret, A.C., Marins, Ma, 1985. *Curva* de maturação e fator de condição de *Apareiodon affinis* (steindachner, 1879), *Apareiodon ibitiensis* (Campos, 1944) e *Parodon tortuosus* (eigenman and Norris, 1900) do Rio passa Cinco, Ipeúna - SP (Cypriniformes, Parodontidae). Ciênc. Cult. 37 (7), 1178–1183.
Barla, M.J., Vera, M.S., O'Brien, E.D., 2003. Relative abundance and autecology of three piscivorous fishes in lakes of the upper basin of Salado River (Buenos Aires Province, Argentina). Ecología Austral 13, 205–214.
Barros, S.E., 2004. Alimentación de *Oligosarcus jenynsii* (Characiformes: Characidae) en dos embalses sobre el río juramento, Salta, Subtrópico de Argentina. Aquatic 20, 44–50.
Berntssen, M.H., Tollefsen, K.E., Handy, R.D., Julshamn, K., Aasen, K., Helland, E., Petri, D., 2010. Assessing sensitivity of Atlantic salmon post-smolt to dietary endosulfan using histology and markers of endocrine disruption, oxidative stress, and biotransformation. Aquaculture 303 (1), 86–93.
Blocksom, K.A., Walters, D.M., Jicha, T.M., Lazorchak, J.M., Angradi, T.R., Bolgrien, D.W., 2010. Persistent organic pollutants in fish tissue in the mid-continental great rivers of the United States. Sci. Total Environ. 408, 1180–1189.

Breivik, K., Sweetman, A., Pacyna, J.M., Jones, K.C., 2002. Towards a global historical emission inventory for selected PCB congeners-a mass balance approach. 1. global production and consumption. Sci. Total Environ. 290, 181–198.
Canadian Council on Animal Care, 2005. Guidelines on the Care and the Use of Fish in Research, Teaching and Testing. CCAC 0-919087-43-4, p. 94. http://www.ccac.ca/Documents/Standards/Guidelines/Fish.pdf.
Chow, W.S., Chan, W.K.L., Chan, K.M., 2013. Toxicity assessment and vitellogenin expression in zebrafish (*Danio rerio*) embryos and larvae acutely exposed to bisphenol A, endosulfan, heptachlor, methoxychlor and tetrabromobisphenol A. J. Appl. Toxicol. 33 (7), 670–678.
Christiansen, L.B., Pedersen, K.L., Pedersen, S.N., Korsgaard, B., Bjerregaard, P., 2000. In vivo comparison of xenoestrogens using rainbow trout vitellogenin induction as a screening system. Environ. Toxicol. Chem. 19 (7), 1867–1874.
Colombo, J.C., Cappelletti, N., Williamson, M., Migoya, M.C., Speranza, E., Sericano, J., Muir, D.C.G., 2011. Risk ranking of multiple-POPs in detritivorous fish from the Río de La Plata. Chemosphere 83 (6), 882–889.
Colombo, J.C., Khalil, M.F., Arnac, M., Horth, A.C., Catoggio, J.A., 1990. Distribution of chlorinated pesticides and individual polychlorinated biphenyls in biotic and abiotic compartments of the Rio De La Plata, Argentina. Environ. Sci. Technol. 24 (4), 498–505.
Conzonno, V., 1995. Métodos para análisis químicos. In: Lopretto, E., Tell, G. (Eds.), Ecosistemas de aguas continentalesMetodologías para su estudio. Ediciones Sur, La Plata, pp. 231–249.
Da Cuña, R., Pandolfi, M., Genovese, G., Piazza, Y., Ansaldo, M., Lo, Nostro F., 2013. Endocrine disruptive potential of endosulfan on the reproductive axis of *Cichlasoma dimerus* (perciformes, cichlidae). Aquat. Toxicol. 126, 299–305.
Da Cuña, R., Rey Vázquez, G., Piol, N., Verrengia Guerrero, N., Maggese, M.C., Lo, Nostro F., 2011. Assessment of the acute toxicity of the organochlorine pesticide endosulfan in *Cichlasoma dimerus* (teleostei, perciformes). Ecotoxicol. Environ. Saf. 74, 1065–1073.
Dangavs, N., 2005. Los ambientes acuáticos de la provincia de Buenos Aires. In: de Barrio, R., Etcheverry, R., Caballé, M., Llambías, E. (Eds.), Geología y Recursos Minerales de la Provincia de Buenos Aires. Actas del XVI Congreso Geológico Argentino Chapter XIII, pp. 219–235 (La Plata).
Destefanis, S., Freyre, L., 1972. Relaciones tróficas de los peces de la laguna de Chascomús con un intento de referenciación ecológica y tratamiento bioestadístico del espectro trófico. Acta Zool. Lilloana 29, 17–33.
Di Rienzo, J., Casanoves, F., Balzarini, M., Gonzalez, L., Tablada, M., CW, Robledo, 2010. INFOSTAT Software.
Díaz, O., Colasurdo, V., 2008. El agua revela sus secretos Química de las Lagunas pampeanas. cap. III 2008. In: F., Grosman (Ed.), Espejos en la llanura. Nuestras lagunas de la región pampeana. Universidad Nacional del Centro de la Provincia de Buenos Aires, In. ISBN: 978-950-658-213-5 (54pp., 174pp.).
Fisk, A.T., Hobson, K.A., Norstrom, R.J., 2001. Influence of chemical and biological factors on trophic transfer of persistent organic pollutants in the northwater polynya marine food web. Environ. Sci. Technol. 35, 732–738.
Flouriot, G., Pakdel, F., Ducouret, B., Valotaire, Y., 1995. Influence of xenobiotics on rainbow trout liver estrogen receptor and vitellogenin gene expression. J. Mol. Endocrinol. 15 (2), 143–151.
Genovese, G., Da Cuña, R., Towle, D., Maggese, M.C., Lo, Nostro F., 2011. Early expression of zona pellucida proteins under octylphenol exposure in *Cichlasoma dimerus* (Perciformes, Cichlidae). Aquat. Toxicol. 101 (1), 175–185.
Genovese, G., Regueira, M., Piazza, Y., Towle, D., Maggese, M.C., Lo, Nostro F., 2012. Time-course recovery of estrogen-responsive genes of a cichlid fish exposed to waterborne octylphenol. Aquat. Toxicol. 114, 1–13.
González Sagrario, M.A., Ferrero, L., 2013. The trophic role of *Cyphocharax voga* (Hensel 1869) according to foraging area and diet analysis in turbid shallow lakes. Fundam. Appl. Limnol. 183 (1), 75–88.
Gonzalez, M., Miglioranza, K.S.B., Aizpún, J.E., Isla, F.I., Peña, A., 2010. Assessing pesticide leaching and desorption in soils with different agricultural activities from Argentina (Pampa and Patagonia). Chemosphere 81, 351–358.
Gonzalez, M., Miglioranza, K.S.B., Shimabukuro, V.M., Quiroz Londoño, O.M., Martinez, D.E., Aizpún, J.E., Moreno, V.J., 2012. Surface and groundwater pollution by organochlorine compounds in a typical soybean system from the south pampa, Argentina. Environ. Earth Sci. 65, 481–491.
Grosman, M.F., Gonzalez Castelain, J.R., Usunoff, E.J., 1996. Trophic niches in an Argentine pond as a way to assess functional relationships between fish and other communities. Water SA 22 (4), 345–350.
Grosman, F., Gonzalez, G., Sanzano, P., Agüería, D., 2002. Alimentación, nichos tróficos y competencia interespecífica de peces de la laguna de Monte, Argentina. Actas del Congreso Iberoamericano Virtual de Acuicultura CIVA 2002. Facultad de Ciencias Veterinarias, Universidad Nacional del Centro de la Provincia de Buenos Aires, Tandil (Argentina), pp. 129–140.
Grosman, F., Sanzano, P., González, G., Agüería, D., Sergueña, S., 2001. Ecología reproductiva, edad, crecimiento, condición y alimentación del pejerrey *Odontesthes bonariensis* en un ambiente del SO de la provincia de Buenos Aires, Argentina. Aquatic 12, 1–24.
He, J., Robrock, K.R., Alvarez-Cohen, L., 2006. Microbial reductive debromination of polybrominated diphenyl ethers (PBDEs). Environ. Sci. Technol. 40 (14), 4429–4434.
Hemmer, M.J., Hemmer, B.L., Bowman, C.J., Kroll, K.J., Folmar, L.C., Marcovich, D., Hoglund, M.D., Denslow, N.D., 2001. Effects of p-nonylphenol, methoxychlor, and endosulfan on vitellogenin induction and expression in sheepshead minnow (*Cyprinodon variegatus*). Environ. Toxicol. Chem. 20 (2), 336–343.
Hued, A.C., Lo Nostro, F.L., Wunderlin, D.A., Bistoni, M.A., 2013. Reproductive impairment of a viviparous fish species inhabiting a freshwater system with anthropogenic impact. Arch. Environ. Contam. Toxicol. 64 (2), 281–290.
Islam, MdS, Tanaka, M., 2004. Impacts of pollution on coastal and marine ecosystems including coastal and marine fisheries and approach for management: a review and synthesis. Mar. Pollut. Bull. 48, 624–649.
Iwata, H., Tanabe, S., Ueda, K., Tatsukawa, R., 1995. Persistent organochlorine residues in air, water, sediments and soils from the lake Baikal region, Russia. Environ. Sci. Technol. 29, 792–801.
Keith, L.H., Crummett, W., Deegan, J., Libby, R.A., Taylor, J.K., Wentler, G., 1983. Principles of environmental analysis. Anal. Chem. 55, 2210–2218.
Lavandier, R., Quinete, N., Hauser-Davis, R.A., Dias, P.S., Taniguchi, S., Montone, R., Moreira, I., 2013. Polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) in three fish species from an estuary in the southeastern coast of Brazil. Chemosphere 90, 2435–2443.
Lee, S.E., Young-Woong, C., Mo, H.H., Son, J., Park, K., Cho, K., 2013. Endosulfan-induced biomarkers in Japanese Rice fish (*Oryzias latipes*) analyzed by SELDI-TOF-MS. Int. J. Biol. Sci. 9 (4), 343–349.
Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with folin phenol reagent. J. Biol. Chem. 193, 265–275.
Maldonado, C., Bayona, J.M., 2002. Organochlorine compounds in the Northwestern Black Sea water: distribution and water column process. Estuar. Coast. Shelf Sci. 54, 527–540.
Mancini, M., Nicola, I., Salinas, V., Bucco, C., 2008. Biología del pejerrey *Odontesthes bonariensis* (Pisces, atherinopsidae) de la laguna Los charos (Córdoba, Argentina). Rev. Peru. Biol. 15 (2), 65–72.
Metcalfe, T.L., Metcalfe, C.D., 1997. The trophodynamics of PCBs including 462 mono and non-Ortho congeners in the food web of north-central Lake Ontario. Sci. Total Environ. 201, 245–272.
Miglioranza, K.S.B., Aizpún, J.E., Moreno, V.J., 2003. Dynamics of organochlorine pesticides in soils from a SE region of Argentina. Environ. Toxicol. Chem. 22, 712–717.
Min, S.K., Kim, J.H., Kim, S., Kim, B.S., Yeom, D.H., 2010. Various biomarker and bioindicator responses in muddy loach (*Misgurnus anguillicadatus*) exposed to endosulfan for 21 days. Toxicol. Environ. Heal. Sci. 2 (2), 125–131.
Mommsen, T.P., Walsh, P.J., 1988. Vitellogenesis and oocyte assembly. Fish Physiol. 11 (part A), 347–406.
Moncaut, N., Lo Nostro, F., Maggese, M.C., 2003. Vitellogenin detection in surface mucus of the southamerican cichlid fish *Cichlasoma dimerus* (Heckel, 1840) induced by estradiol-17b. effects on liver and gonads. Aquat. Toxicol. 63, 127–137.
Noreña-Barroso, E., Simá-Álvarez, R., Gold-Bouchot, G., Zapata-Pérez, O., 2004. Persistent organic pollutants and histological lesions in Mayan catfish *Ariopsis assimilis* from the bay of Chetumal, Mexico. Mar. Pollut. Bull. 48, 263–269.
Okoumassoun, L.E., Averill-Bates, D., Gagné, F., Marion, M., Denizeau, F., 2002. Assessing the estrogenic potential of organochlorine pesticides in primary cultures of male rainbow trout (*Oncorhynchus mykiss*) hepatocytes using vitellogenin as a biomarker. Toxicology 178 (3), 193–207.
Oliveira Ribeiro, C.O., Vollaire, Y., Sanchez-Chardi, A., Roche, H., 2005. Bioaccumulation and the effects of organochlorine pesticides, PAH and heavy metals in the eel (*Anguilla anguilla*) at the Camargue Nature Reserve, France. Aquat. Toxicol. 74 (1), 53–69.
Ondarza, P.M., Gonzalez, M., Fillmann, G., Miglioranza, K.S.B., 2011. Polybrominated-diphenyl ethers and organochlorine compound levels in brown trout *Salmo trutta* from Andean Patagonia. Chemosphere 83, 1597–1602.
Ondarza, P.M., Gonzalez, M., Fillmann, G., Miglioranza, K.S.B., 2012. Increasing levels of polybrominated diphenyl ethers (PBDEs), endosulfans, DDTs and polychlorinated biphenyls (PCBs) in rainbow trout (*Oncorhynchus mykiss*) following a mega-flooding episode in the Negro River, Argentinean Patagonia. Sci. Total Environ. 419, 233–239.
Ondarza, P.M., Gonzalez, M., Fillmann, G., Miglioranza, K.S.B., 2014. PBDEs, PCBs and organochlorine pesticides distribution in edible fish from Negro River basin, Argentinean Patagonia. Chemosphere 94, 135–142.
van der Oost, R., Beyer, J., Vermeulen, N.P.E., 2003. Fish bioaccumulation andbiomarkers in environmental risk assessment: a review. Environ. Toxicol. Pharmacol. 13, 57–149.
Poma, G., Volta, P., Roscioli, C., Bettinetti, R., Guzzellaom, L., 2014. Concentrations and trophic interactions of novel brominated flame retardants, HBCD, and PBDEs in zooplankton and fish from Lake Maggiore (Northern Italy). Sci. Total Environ. 481, 401–408.
Qiu, X., Zhu, T., Yao, B., Hu, J., Hu, S., 2005. Contribution of dicofol to the current DDT pollution in China. Environ. Sci. Technol. 39, 4385–4390.
Quirós, R., 2004. Sobre la morfología de las lagunas pampeanas. Documento de Trabajo del Área de Sistemas de Producción Acuática N°3. Departamento de Producción Animal, Facultad de Agronomia. Universidad de Buenos Aires.
Quirós, R., Rennella, A.M., Boveri, M.A., Rosso, J.J., Sosnovsky, A., 2002. Factores que afectan la (structura y el funcionamiento de las lagunas pampeanas. Ecol. Austral 12 (2), 175–185.
Rautenberg, G.E., Amé, M.V., Monferrán, M.V., Bonansea, R.I., Hued, A.C., 2015. A multilevel approach using *Gambusia affinis* as a bioindicator of environmental pollution in the middle-lower basin of Suquía River. Ecol. Indic. 48, 706–720.
Rey Vázquez, G., Meijide, F., Da Cuña, R., Lo Nostro, F., Piazza, Y., Babay, P., Trudeau, V., Maggese, M.C., Guerrero, G., 2009. Exposure to waterborne 4-tert-octylphenol induces vitellogenin synthesis and disrupts testis morphology in the South American freshwater fish *Cichlasoma dimerus* (Teleostei, Perciformes). Comp. Biochem. Physiol. C 150, 298–306 (ISSN 1532-0456).
Ringuelet, R.A., Iriart, R., Escalante, A.H., 1980. Alimentación del pejerrey (*Basilichthyss bonariensis bonariensis*, atherinidae) en laguna de Chascomús (Buenos Aires, Argentina). relaciones ecológicas de complementación y eficiencia trófica del plancton. Limnobios 1, 447–460.
Rognerud, S., Grimalt, J.O., Rosseland, B.O., Fernández, P., Hofer, R., Lackner, R., Lauritzen, B., Lien, L., Massabuau, J.C., Ribes, S., 2002. Mercury and organochlorine contamination in Brown trout (*Salmo trutta*) and artic charr (*Salvelinus alpinus*) from high

mountain lakes in Europe and the Svalbard archipelago. Water Air Soil Pollut. 2, 209–232.
Rosseland, B.O., Massabuau, J.C., Grimalt, J., Hofer, R., Lackner, R., Rognerud, S., Lien, L., 1999. The ecophysiology and ecotoxicology of fishes as a tool for monitoring and management strategy of high mountain lakes and lakes and rivers in acidified areas. Zoology 190, 90–100.
Sabljic, A., Gusten, H., Verhaar, H., Hermens, J., 1995. QSAR modelling of soilsorption. improvements and systematics of log K_{oc} vs. log K_{ow} correlations. Chemosphere 31, 4489–4514.
Schmidt, W.F., Hapeman, C.J., Fettinger, J.C., Rice, C.P., Bilboulian, S., 1997. Structure and asymmetry in the isomeric conversion of β- to α-endosulfan. J. Agric. Food Chem. 45 (4), 1023–1026.
SENASA, 2011. Servicio Nacional de Sanidad y Calidad Agroalimentaria. Endosulfán: nuevas medidas para la importación, elaboración y uso en Argentina (Resolution 511/2011).
Silva Barni, M.F., Gonzalez, M., Miglioranza, K.S.B., 2014. Assessment of persistent organic pollutants accumulation and lipid peroxidation in two reproductive stages of wild silverside (*Odontesthes bonariensis*). Ecotoxicol. Environ. Saf. 99, 45–53.
Söderström, G., Sellström, U., de Wit, C.A., Tysklind, M., 2004. Photolytic debromination of decabromodiphenyl ether (BDE 209). Environ. Sci. Technol. 38 (1), 127–132.
STAP/GEF, 2003. Workshop on the use of bioindicators, biomarkers and analytical methods for the analysis of POPs in developing countries. 10–12 December, Tsukuba, Japan, 2003.
Stapleton, H.M., Brazil, B., Holbrook, R.D., Mitchelmore, C.L., Benedict, R., Konstantinov, A., Potter, D., 2006. In vivo and in vitro debromination of decabromodiphenyl ether (BDE 209) by juvenile rainbow trout and common carp. Environ. Sci. Technol. 40 (15), 4653–4658.
Sumpter, J.P., Jobling, S., 1995. Vitellogenesis as a biomarker for estrogenic contamination of the aquatic environment. Environ. Health Perspect. 103 (7), 173–178.
UNEP, 2011. Stockholm convention, the 9 new POPs under the Stockholm convention.
USDA database. Foreign Agricultural Service, 2015. www.fas.usda.gov
Velasco-Santamaría, Y.M., Handy, R.D., Sloman, K.A., 2011. Endosulfan affects health variables in adult zebrafish (*Danio rerio*) and induces alterations in larvae development. Comp. Biochem. Physiol. Part C: Toxicol. Pharmacol. 153 (4), 372–380.
Velmurugan, B., Selvanayagam, M., Cengiz, E.I., Unlu, E., 2009. Histopathological changes in the gill and liver tissues of freshwater fish, *Cirrhinus mrigala* exposed to dichlorvos. Braz. Arch. Biol. Technol. 52 (5), 1291–1296.
Vives, I., Grimalt, J.O., Ventura, M., Catalan, J., Rosseland, B.O., 2005. Age dependence of the accumulation of organochlorine pollutants in brown trout (*Salmo trutta*) from a remote high mountain lake (Redó, Pyrenees). Environ. Pollut. (Barking, Essex : 1987) 133 (2), 343–350.
Walse, S.S., Shimizu, K.D., Ferry, J.L., 2002. Surface-catalyzed transformations of aqueous endosulfan. Environ. Sci. Technol. 36, 4846–4853.
Wania, F., MacKay, D., 1996. Tracking the distribution of persistent organic pollutants. Environ. Sci. Technol. 30, 390A–396A.
Weber, J., Halsall, C.J., Muir, D., Teixeira, C., Small, J., Solomon, K., Hermanson, M., Hung, H., Bidleman, T., 2010. Endosulfan, a global pesticide: are view of its fate in the environment and occurrence in the Arctic. Sci. Total Environ. 408, 2966–2984.
White, R., Jobling, S., Hoare, S.A., Sumpter, J.P., Parker, M.G., 1994. Environmentally persistent alkylphenolic compounds are estrogenic. Endocrinology 135 (1), 175–182.
WHO, 1993. Polychlorinated biphenyls and terphenyls. Environmental Health Criteria. World Health Organization, Geneva, p. 140.
Zhou, H.Y., Cheung, R.Y.H., Wong, M.H., 1999. Bioaccumulation of organochlorines in freshwater fish with different feeding modes cultured in treated wastewater. Water Res. 33 (12), 2747–2756.
Zhou, R., Zhu, L., Kong, Q., 2007. Persistent chlorinated pesticides in fish species from Qiantang River in East China. Chemosphere 68 (5), 838–847.