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Occurrence of pesticide residues in fish from south American rainfed agroecosystems

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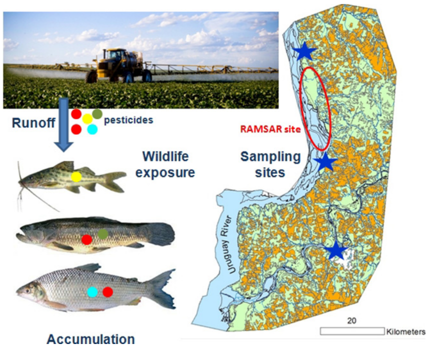
HIGHLIGHTS

- Multiple substances approved for agriculture detected at sublethal concentrations.
- Spatial differences are observed for non-migratory fishes depending on land use.
- Bioaccumulation positively associated to log Kow and trendy active ingredients.
- Trifloxystrobin exhibited higher occurrence in fish in spite of its reduced persistence.

GRAPHICAL ABSTRACT



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ABSTRACT

Environmental sustainability of South American rainfed agroecosystems is of current concern. In this work, we evaluate the occurrence of multiple pesticide residues in muscle tissue of wild fish species from two large rivers in South America (Uruguay and Negro Rivers). Two sampling campaigns (representing summer and winter crops) were performed during 2015 targeting a wide biodiversity of fish species used for human consumption (ranging from migratory to non-migratory and from detritivorous to top-predators). Three different localities associated to rainfed agriculture were assessed, two of them enclosed to a RAMSAR site (National Park "Esteros de Farrapos e Islas del Rio Uruguay"). Pesticide residues occurred in muscle tissue of 143 from 149 sampled fishes (96%). Thirty different pesticides were detected at concentrations from <1 to 194 $\mu g\ kg^{-1}$. Incidence of pesticides in fish were tightly related to: i) features of the contaminant: (Kow, environmental persistence and mobility) and ii) intensity of use of particular pesticides and land dedicated to rainfed agriculture. Trifloxystrobin, metolachlor and pyraclostrobin showed the highest rates of occurrence. Of great concern is that strobirulins have highest toxicity to fish from those detected compounds. From the pattern of pesticides occurring for non-migratory fish species it was possible to trend important spatial differences related to the intensity of rainfed agriculture. Results suggest a regular exposition of aquatic wild biota to sublethal concentrations of multiple semi-polar pesticides.

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1. Introduction

The intensification of agriculture in Southern South America has caused remarkable changes in the use of land by highly technified agricultural techniques. Particularly in Argentina, Brazil, Paraguay and Uruguay, an intensive and specialized cropping system under rainfed conditions has been adopted. Nowadays, soybean itself represents 13% of the total income of Uruguayan exportations (Uruguay XXI, 2015). Agricultural practices shifted from a crop-pasture rotation (CPR) technique to a continuous annual cropping under no-till (CCNT) (Franzluebbers et al., 2014; Wingeyer et al., 2015). More than a decade of increasing crop area has been accompanied by changes in land tenancy, tillage practices, reduced crop diversity and greater overall productivity. For more than a decade CCNT became the predominant technique in rainfed agriculture (García-Préchac et al., 2004; Franzluebbers et al., 2014; Wingeyer et al., 2015). Uruguayan rainfed crops increased from 278,000 ha in 2004 to 1334 million ha in 2015 (DIEA, 2015; Harriet et al., 2017).

In Uruguay, typical rainfed summer crop under CCNT include the sowing of soybean (*Glicine* max, rounding 50% of land area), sorghum (*Sorghum spp.*) and maize (*Zea mays*) during October – November period. Harvest is typically performed during March – April. Generally, after 1 month of fallow, a rainfed winter crop of wheat (*Triticum aestivum*) or barley (*Hordeum vulgare*) is sown in June – July to harvest prior to the next summer crop. In such scenario, a rapid increase in the amount of pesticides applied was particularly registered for Uruguay as well as in other South American countries (Schreinemachers and Tipraqsa, 2012). These new, highly technified procedures are based in the massive use of pesticides to maximize yields (Novelli et al., 2017; Wingeyer et al., 2015). Demand of herbicides, fungicides and insecticides has been continuously growing as higher portions of land are dedicated to rainfed agriculture (DGSA, 2017).

However, although Good Agricultural Practices (GAPs) are generally followed, it is well known that pesticide residues may diffuse over different environmental compartments and reach non-target biota (Andreu and Picó, 2012; Belenguer et al., 2014; Ccanccapa et al., 2015; Ginebreda et al., 2016; Masiá et al., 2015; Masiá et al., 2013; Niell et al., 2015; Niell et al., 2017). Fish muscle tissue has been habitually selected for monitoring several non-polar organic contaminants (Kalachova et al., 2011; Molina-Ruiz et al., 2015; Munaretto et al., 2013; Rose et al., 2015; Sapozhnikova and Lehotay, 2013; Sapozhnikova and Lehotay, 2015).

Despite the wide range of substances currently approved for use in agriculture, their effects and circulation among environmental compartments and particularly on wildlife are not studied or clearly recorded (Köhler and Triebskorn, 2013). The study of the diffuse contamination due to pesticides is a difficult and broad task as the agrochemicals range from low to relatively high solubility, toxicity and persistence. Therefore, wildlife organisms living in streams or rivers whose basin is subjected to rainfed CCNT practices are potentially exposed to pesticides. Their occurrence in local species would be able to depict spatial variation of exposure to pollutants. Aquatic biomonitoring strategy is useful in the identification of risk and prioritization approaches (Chalar et al., 2013).

Moreover, fish are important components structuring aquatic communities, with a great range of body sizes, habitats and trophic positions (Daufresne and Boët, 2007). They are consumed by humans and provide a source of protein to artisanal fishermen and local people. In order to understand the consequences of particular agricultural practices, wild fish species with different and similar feeding habits and migratory behavior should be examined.

The aim of this study is to show main findings of pesticide residues occurring in fish muscle tissue of selected South American fish species collected in fishing campaigns accomplished in 2015 at different locations of rainfed agroecosystems in Uruguay.

This study particularly focuses on different fish species living into the RAMSAR convention site National Park “Esteros de Farrapos e Islas del Rio Uruguay” (RAMSAR, 2017). Moreover, we show spatial distribution of occurring pesticides and its positive relationship with land dedicated to rainfed agriculture. Finally, we discuss the association with physicochemical properties, soil degradation and amount of pesticides that foster accumulation in fish tissues.

2. Materials and methods

2.1. Site description

Uruguay River and Negro River are the most important freshwater courses in Uruguay. Uruguay River divides Uruguay and Argentina whereas Negro River divides southern to northern territories of Uruguay. This study was carried out with fish samples from 4 sampling sites. San Javier (SJ) (32°41′00″S 58°08′00″O) and Nuevo Berlin (NB) towns (32°58′45″S 58°03′10″O) are two sites located at margins of Uruguay River. Both locations are adjacent to the National Park “Esteros de Farrapos e Islas del Río Uruguay” – a RAMSAR Convention site. The Uruguayan National System of Protected Areas (SNAP) administrates this Park. This area constitutes a system of river wetlands, islands and islets that are permanently or temporarily flooded as a result of floods of the River. Wetlands, natural field and scrubland ecosystem predominates. However, neighboring areas of NB are particularly dedicated to intensive CCNT plantations of soybean, maize, barley, wheat, sorghum. On the other hand, SJ neighboring areas range from intensive CCNT crop to natural pasture. In this area, secondary water streams that cross the rainfed fields fall into the main rivers (Uruguay River or Negro River). Forestry of exotic species (mainly *Eucalyptus spp.*) for cellulose pulp production is located between both localities. Fig. 1 shows the sites under study and land use.

The city of Mercedes (MC) (33°15′00″S 58°01′55″O) lies on the margin of the Negro River, 50 km upstream where the Negro River falls into the Uruguay River (Fig. 1C). Particularly, the surrounding areas of MC and NB developed the most intensive CCNT agriculture. Rainfed agriculture is performed at southern and northern margins of the Negro River. Fig. 1B shows land use in NB, SJ and MC. In MC land use is predominantly dedicated to soybean – wheat annual CCNT.

San Gregorio de Polanco (SGP) (32°37′00″S 55°55′00″O) is also located at margin of Negro River but >200 km upstream from MC and separated by two hydroelectric dams. Most land use is particularly associated to grassland and forestry although there is incipient rainfed agriculture. Natural herbaceous field dominates in this area as seen on Fig. 1D. SGP was selected as a CCNT negative site.

Intensity of land dedicated to rainfed agriculture is mostly associated to MC and NB. On the other hand, SJ has less land use dedicated to rainfed agriculture and some amount of protected area surrounding. Finally, SGP was considered as a negative control site of rainfed agriculture.

2.2. Sampling

Two sampling campaigns were developed in SJ, NB and MC at the beginning of April 2015 (fall season, after summer crop) and at the end of September 2015 (spring season, after winter crop). Sampling dates were selected in order to have two contrasting scenarios of agricultural production at the end of each crop. For SGP sampling campaign was performed at September 2015.

In all sites, fish samples were obtained from local fishermen. Individuals were size measured and taxonomically identified according to morphometric characters and visual inspection (Teixeira de Mello et al., 2011).

A total of eight fish species were acquired from these four sampling sites. Five non-migratory species (*Hoplias malabaricus*, *Rhamdia quelen*, *Pimelodus maculatus*, *Paraloricaria vetula*, *Hypostomus commersonni*) and three migratory ones (*Salminus brasiliensis*, *Megaleporinus obtusidens*, *Prochilodus lineatus*). Feeding habits of these species range

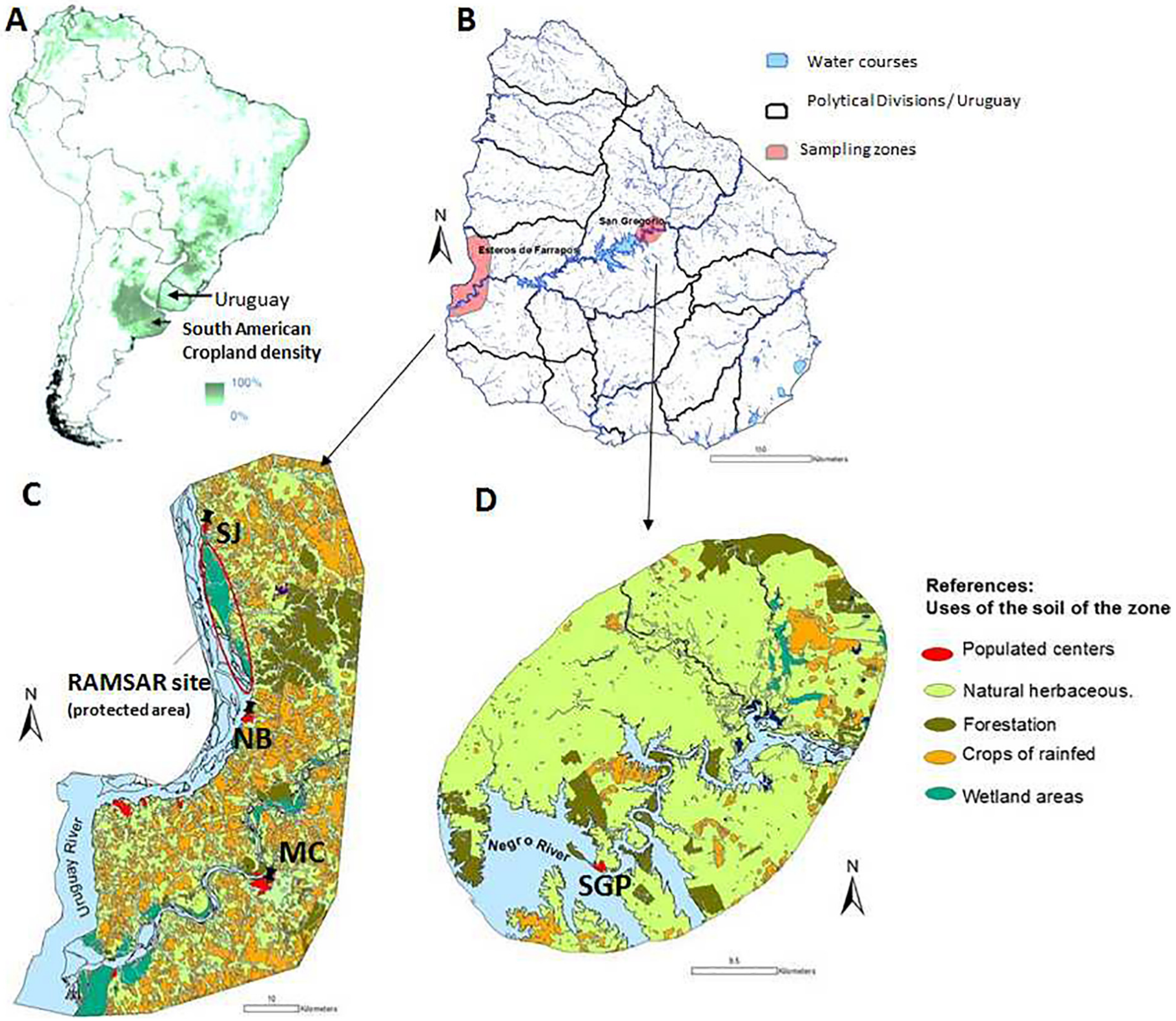

Fig. 1. Sites under study.

from predatory, omnivorous to detritivorous (see Table 1). Humans use all these species as food resource and are the main target of fishermen at studied sites. Therefore fisheries offer are particularly focused on these species and this reflects the natural distribution of species at each site. The minimum size of capture is determined according to the size at which the animal has been reproduced at least once. Therefore, all samples correspond to adult individuals of at least 2 years old. For SGP, only non-migratory species occur given that two hydroelectric dams prevent the presence of migratory fishes. Dorso-lateral muscle tissue was scaled and 50-100 g of muscle were dissected, placed in a polyethylene bag, coded and transported to the laboratory under ice. In this study each sample was obtained from adult individuals. At laboratory, samples were frozen until sample preparation step. To compare land use effect among localities, we selected only non-migratory organisms to avoid potential cross-contamination among sites caused by migration (Table 1).

2.3. Chemicals and reagents

Analytical standards were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). The purity of all the standards was >98%. HPLC-grade acetonitrile (MeCN) and methanol (MeOH) were supplied by

Table 1
Summary of samples, species and distribution among sites (n = 149).

Scientific name	Common name	Feeding habit	Migratory behavior	San Javier (SJ)	Nuevo Berlín (NB)	Mercedes (MC)	San Gregorio de Polanco (SGP)
Hoplias malabaricus	Tararira	Predatory	Non-migratory	5	9	2	5
Rhamdia quelen	Bagre negro	Omnivorous	Non-migratory	2	0	1	5
Pimelodus maculatus	Bagre amarillo	Omnivorous	Non-migratory	1	0	2	5
Paraloricaria vetula	Vieja cola de látigo	Detritivorous	Non-migratory	0	6	1	0
Hypostomus commersonni	Vieja del agua	Detritivorous	Non-migratory	0	4	8	0
Salminus brasiliensis	Dorado	Predatory	Migratory	3	13	0	0
Megaleporinus obtusidens	Boga	Omnivorous	Migratory	3	26	5	0
Prochilodus lineatus	Sábalo	Detritivorous	Migratory6	6	27	10	0
Summary of samples				20	85	29	15

J.T. Baker (Darmstadt, Germany). Water used for LC-MS/MS analysis was obtained from a Direct-Q3 Ultrapure Water System from Millipore (Billerica, MA, USA). Formic acid (FA) was from Sigma Aldrich (Steinheim, Germany).

Individual stock standard solutions of the target compounds were prepared, based on the solubility properties of each compound, in pure methanol or acetonitrile (ACN) or ethyl acetate (EtAc) and stored at −18 °C. Stock solutions were prepared from the standard substances at 2000 mg L^{-1} in a proper solvent. Working solutions were prepared by appropriate dilution of the stock solutions in ACN for liquid chromatography (LC) and EtAc for gas chromatography (GC) amenable compounds. Bulk anhydrous $MgSO_4 > 98\%$ purity, dispersive solid phase extraction (d-SPE) grade C18 (ocadecyl silica) and PSA (primary secondary amine) 40–60 μm were purchased from Scharlab (Barcelona, Spain). Triphenyl phospate (TPP) and bromophos methyl from Dr. Ehrenstorfer GmbH (Augsburg, Germany) were used as surrogate compound (SC) and internal standard (ISTD), respectively. Both were prepared in EtAc.

2.4. Scope of pesticides

A target list of 72 compounds was develop based on surveys of application and annual statistical volumes of used active ingredient according to the Uruguayan Ministry of Agriculture, Livestock and Fishery (DGSA, 2017). Agrochemicals approved for use in current pest management was based on updated cropping guidelines (Guía SATA, 2017). Agrochemicals recommended for use in rainfed (non-irrigated) farming (soybean, maize, barley, wheat, sorghum, sugarcane) according to agrochemicals sales were particularly included as well as those used on extensive irrigated production (particularly rice), animal breeding and forestation.

2.5. Sample preparation

Frozen samples of scaled fish muscle (skin included) were rapidly milled and homogenized with high-speed blender to avoid defrosting of the sample. Representative samples of each fish species were used as blank and matrix matched calibration purposes. Blank samples of fish muscle were selected in a former analysis to corroborate for non-containing the studied pesticides. QuEChERS (quick, easy, cheap, effective, rugged, and safe) method was used according to an in-house method validation and former reports on multiclass, multiresidue analysis of semi-polar pesticides (Belenguer et al., 2014; Masiá et al., 2015; Masiá et al., 2013; Rawn et al., 2010; Sapozhnikova and Lehotay, 2013). Frozen fish homogenate (10 g) were weighed into a 50 mL polypropylene centrifuge tube. A 10 μL aliquot of TPP 10 μg mL^{-1} solution was added to each tube as SC and let stand by 1 min. 10 mL of ACN was added afterwards. The tube was shaken vigorously by hand during 1 min. Then, 1.5 g of NaCl and 4.0 g of $MgSO_4$ were added and the resulting mixture was shaken vigorously by hand for 4 min. Each tube was centrifuged at 3500rcf for 5 min. Clean up was performed using dSPE. A 7 mL aliquot of the organic layer was transferred into a 15 mL polypropylene tube containing 350 mg PSA, 180 mg C18 and 1000 mg $MgSO_4$. The tube was vortexed for 1 min and centrifuged at 3500rcf for 5 min. A 1 mL aliquot of extract was transferred into a 2 mL screw cap autosampler vial and directly injected in LC-MS/MS. On the other hand, a 4 mL aliquot of extract was transferred into a conic glass test tube and driven to dryness under N_2 stream in the evaporation equipment. Finally, the extract was redissolved in 1.0 mL of 100 μg mL^{-1} bromophos methyl ISTD solution. The equivalent tissue concentration per sample extract was 1 g mL^{-1}.

2.6. Liquid chromatography - tandem mass spectrometry

An AB Sciex™ 4000 QTRAP (Concord, Canada) Quadrupole - Linear ion trap was operated in triple quadrupole MS/MS mode coupled to Agilent 1200 LC system (Agilent Technologies, Palo Alto, USA). An Agilent Technologies Zorbax Eclipse XDB-C18 (150 mm × 4.6 mm, 5 μm) analytical column was used. Column oven temperature was set at 20 °C. The mobile phase consisted of (A): 0.1% formic acid in water and (B) MeCN and the following elution program was used: It was run at 0.6 mL min^{-1} starting with 70% component A at injection time and stable for 3 min, gradually changing to 0% A (100% B) over 22 min and stable for 5 min, then to 30% A (70% B) over 5 min. This eluent composition was kept for 5 min and kept there until 40 min after injection. Tandem MS detection was performed using the multiple reaction monitoring (MRM) mode. The optimal MRM conditions for each analyte were optimized using direct infusion in the ESI+ mode. Source temperature was 500, the ionization voltage was 5000 V, curtain gas (CUR) was nitrogen at 20 psi and the nebulizer gas (GS1) was nitrogen at 50 psi. Scheduled multiple reaction monitoring was used with a setting of a 90s detection window covering the expected retention time of each analyte and the target scan time was 2 s for all pesticides. Analyst 1.5 software from AB Sciex™ was used for instrument control and data processing. Optimized conditions and settings (selected transitions, collision energies etc.) used in this study are listed in Table S1a.

2.7. Gas chromatography - mass spectrometry

A Shimadzu GC-QP2010 Ultra (Kyoto, Japan) equipped with Thermo Scientific (Waltham, MA, USA) TRACE™ TR-5MS (5% phenyl polysilphenylene-siloxane) bonded fused-silica capillary column (30 m × 0.25 mm i.d. × 0.25 mm film thickness). Electron ionization (EI) mass spectra was obtained at 70 eV and monitored from 50 to 550 m/z for full scan mode analysis. MS system was programmed in selected ion monitoring (SIM) mode. The working parameters were: injector temperature 280 °C; interface temperature 280 °C; carrier gas He at 1 mL min^{-1}. Oven conditions; from 120 °C initial (5 min hold), increased to 190 °C at a rate of 10 °C/min (1 min hold), then to 250 at 5 °C min (5 min hold), finally to 300 °C at 5 °C/min (5 min hold). Injection mode: splitless; injection volume: 1.0 μL. The identification of the compounds was confirmed by injection of solvent and matrix matched standards and comparison of their retention index and relevant MS ratios. Optimized conditions and settings for the compounds used in this study are listed in Table S1b. GC-MS Solution version 4.11 SU2 with MS libraries was used for instrument control and data processing.

2.8. Analytical quality assurance and quality control (QA/QC)

Identification of the compounds was assessed through those requirements established at SANTE guideline for different MS based techniques (European Commission, 2015). In case of LC-MS/MS operated under MRM mode, identification was based on retention time matching (±0.1 min); two MRM product ions plus the ion ratios within ±30% relative tolerance to those calibration standards from the analytical same sequence. In case of GC–MS operated in SIM mode, identification was based on retention time matching (±0.1 min); 3 m/z ions plus the ion ratios within ±30% relative tolerance to those calibration standards from the analytical same sequence. The limits of detection (LODs) and quantification (LOQs) were determined according to the criteria established at SANTE. As recommended, LODs were estimated as the signal to noise ratio (S/N) ≥3 for the qualifier product ion transition in LC-MS/MS or to the lower m/z ion for GC–MS. Instead, LOQs are based on the lowest concentration with acceptable accuracy on recovery experiments. Recovery study was assessed with blank fish matrix of *Megaleporinus obtusidens*. Total lipid content of blank fish was measured as $15 \pm 2\%$, $n = 5$ (Ramalhosa et al., 2012). Recoveries at two spiking levels are shown at Table S2 for LC and GC amenable compounds. We have noticed inherent variability on the color of the extracts (from pale to deep yellow) even for individuals from the same species. The lipid content of different individuals of the same species and fishing date has proved to change. This variability among samples was evaluated through within-laboratory reproducibility (RSD_{WR}) for the SC. A quality control chart of TPP was created for all the samples subjected to analysis. Precautions were particularly taken to avoid cross contamination during sample preparation and instrumental steps. Carryover was checked in the instrumental analytical sequences by incorporating periodical washing runs and blank samples among calibration standards and real samples. Routine recovery checks were also performed. Quantification was done via matrix matched calibration. LC-MS quantitation was performed through external calibration whereas GC-MS quantitation was performed by internal standard calibration via response factor relative to bromophos methyl.

2.9. Statistical analysis

Average number of detected pesticides per fish was estimated. A histogram showing the number of pesticides per fish was constructed. We estimated the frequency of occurrence (FO) for each pesticide as the number of times it was registered over the total number of fish analyzed in each locality. In order to correct for the different number of fish sampled in each locality, a sampling procedure without replacement taking the minimum number of fish sampled in any locality ($n = 8$) was used to estimate a confidence region for the estimated average FO. An Olmstead-Tukey diagram (Fisher, 1983) was constructed to classify pesticide residues substances in the space of average concentration per fish and frequency of occurrence (FO; %) using the whole sample. Substances found above the median values of average concentration and FO were classified as “dominant”; those with average concentration above the median but FO lower than the median value as “occasional”. Substances with high FO but low average concentration were considered as “frequent”, and substances with low average concentration and low FO as “rare”.

The effect of land use on pesticide occurrence in fish was evaluated with non-migratory (resident) species, which are expected to be exposed to substances used in the local basin and thus serve as local bioindicator. A gradient of intensity of land use dedicated to rainfed agriculture can be defined with MC and NB as the sites with higher portion of land dedicated to these practices. SJ site is lesser impacted by these practices as it is closely surrounded by a protected area with natural wetlands. Finally, SGP has a reduced fraction of area dedicated to rainfed agriculture and was considered as a negative control site. A generalized linear model with a Poisson distribution and log link was fitted on the number of pesticides per fish and the four localities as explanatory factors (Zuur et al., 2009) The model was built in R software (R Core Team, 2015).

Aiming to compare the whole pesticide community structure among localities, we first investigated the effect of sampling effort (number of non-migratory fish per locality) on the pseudo multivariate dissimilarity-based standard error (*MULTse*; *Anderson and Santana-Garcon, 2015*) using the functions and R codes provided in *Anderson and Santana-Garcon (2015)*. Standard error reaching an asymptote is indicative of an adequate sampling effort. After checking the *MULTse* was comparable among localities (i.e. similar dispersion) we tested for differences in the position in the multivariate space (Anderson and Walsh, 2013) using *adonis* and *betadisper* function from package Vegan (Oksanen et al., 2009). The distance metric used was Jaccard and based on the presence/absence of pesticide residues. Only MC presented a higher variability in *MULTse* and thus was not included in the comparison. It is worth mentioning that MC showed a high proportion of fishes with pesticides residues and with high intensity.

2.10. Information on agrochemicals

Information on logarithmic octanol-water partition coefficient (log Kow), soil (DT_{50}, aerobic), water phase (DT_{50}), water/sediment degradation (DT_{50}) and ecotoxicology for fish (Acute dose 96 h LC_{50} (mg L^{-1}) were obtained from website databases (IUPAC Footprint, 2017). Annual statistics of used agrochemicals were accessed via websites of Ministry of Agriculture (DGSA, 2017).

3. Theory

Bioaccumulation of organic pollutants in aquatic wildlife is related with Kow and environmental persistence of the compounds. Non-migratory (local) fish species can be used as local (spatial dimension) and integrative (temporal dimension) bioindicators of pesticide residue contamination.

4. Results and discussion

Residues of 30 pesticides were detected in 96% of individuals ($n = 149$). Results and discussion section is divided into four sections. First, some analytical issues of the monitoring strategy are described. Then the findings of pesticides in fish samples are presented. Finally, spatial differences related to land use and relationships with physicochemical properties, soil persistence, acute toxicity and intensity of the detected active ingredients are discussed.

4.1. Analytical issues

Recent publications aimed the use of QuEChERS extraction with dSPE cleanup for the large scale multiresidue analysis of non-polar, semi-polar and polar pesticide residues is fish muscle tissue and other fatty matrices (Baduel et al., 2015; Belenguer et al., 2014; Chatterjee et al., 2016b; Kaczyński et al., 2017; Lazartigues et al., 2011; Masiá et al., 2015; Munaretto et al., 2013; Munaretto et al., 2016; Sapozhnikova and Lehotay, 2013; Yao et al., 2016). Recent reports have introduced LC amenable pesticides determination in fish via LC-MS/MS (Kaczyński et al., 2017; Munaretto et al., 2016; Yao et al., 2016). Table S2 on the supplementary material shows analytical figures of merit for the scope of pesticides. On both equipment (GC/LC), linearity was assayed from LOQ to 250 μg kg^{-1} levels. Recoveries for those compounds were assessed at two levels as traditional levels expected on food matrices (~10 μg kg^{-1}) and an extra one. On GC–MS determination the selected level was 50 μg kg^{-1} whereas for LC-MS/MS the 1 μg kg^{-1} level was selected. The inherent higher selectivity and sensitivity anticipated on tandem MS techniques led to an imbalance between the levels detected between compounds according to their instrumental technique. As seen, LODs for most LC amenable compounds were at sub-μg kg^{-1} level while LOQs were at rounding 1 μg kg^{-1}. Only boscalid and imazalil had an LOD > 1 μg kg^{-1} in our instrumental determination system. Therefore, we established a sensitive analytical technique for pesticide detection and quantification of LC amenable compounds. GC–MS compounds had LODs ranging from 3 to 74 μg kg^{-1}. Therefore not all compounds were detected at the lowest spiking level (see synthetic pyrethroids, fenthion and bromopropilate shown on Table S2). LOQs of GC amenable compounds ranged from 11 to 85 μg kg^{-1}. Matrix effect (ME (%)) values are also shown. Most compounds showed middle to high ME (%) so matrix matched calibration is particularly recommended for quantification of residues.

Applicability of the method was assured on a combination of different QA/QC strategies. Within laboratory recovery for SC (TPP) was 100.6% with RSD_{WR} of 16.5% ($n = 149$). As demonstrated through this on-going approach the methodology was robust and fit for the purpose.

4.2. Occurrence of pesticides in fish samples

We evaluated 149 fish samples based on their natural abundance at the localities. On this basis, 30 pesticides were detected in the set of samples. Pesticide residues were detected in 96% ($n = 143$) of the samples. From those identified compounds, 7 pesticides were detected only once (acetamiprid, boscalid, imazalil, malathion, methidathion, pendimethalin and thiabendazole) in the whole dataset. An average and a mode of ca. 4 pesticides per fish were estimated for all dataset with a maximum of 21 pesticides detected in a single fish captured in Nuevo Berlin (NB) (the migratory *Salminus brasiliensis*; Fig. 2). This

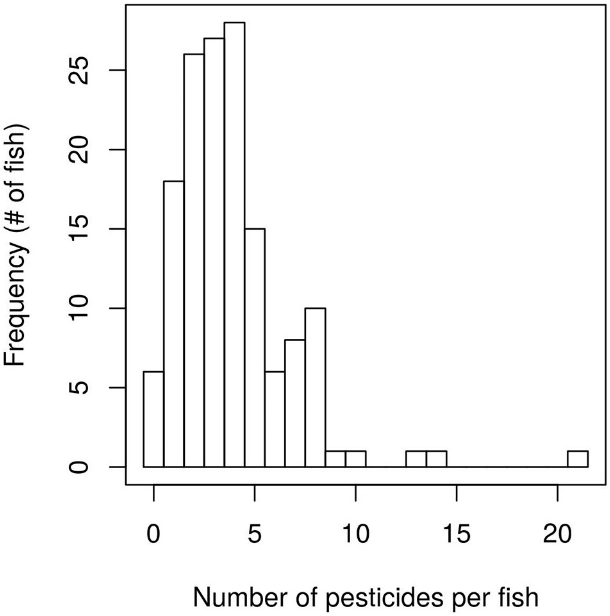

Fig. 2. Frequency of detection of pesticides per fish in the whole dataset ($n = 149$).

implies most fishes have incorporated multiple pesticides residues into their tissues. Authors stated a significant increase of deleterious effects on fish health caused by a combination of pesticides at sublethal doses (Bacchetta et al., 2014).

Considering migratory and non-migratory species the numbers of substances detected were as follow: 11; 11; 8 and 5 in MC, NB, SJ and SGP, respectively. Bootstrap estimates of the FO provide similar results to the observed values (see Fig. 3). The prevailing appearance of trifloxystrobin and metholaclor is particularly evidenced for all localities. MC and NB showed a complex pattern of occurring pesticides. Pyraclostrobin detection was particularly associated to MC, NB and SJ. That means that two fungicides and an herbicide are the most prevailing compounds. As can be seen from the scope of analytes, we prioritize a group of pesticides associated to the exposition level. We selected sampling sites at the two main rivers in Uruguay. This enabled us to select big freshwater species of fish including some with migratory behavior. The semi-polar nature of these occurring pesticides justify the concern for monitoring non-classical pesticides in freshwater fish.

Considering the whole dataset, those compounds with frequency of occurrence (FO (%)) above 10% were in the following order: trifloxystrobin, metolachlor, pyraclostrobin, carbendazim, tebuconazole, metalaxyl, pirimiphos methyl, azoxystrobin, pirimicarb, atrazine, difenoconazole and epoxiconazole. Highest occurrence rates were found for the fungicide trifloxystrobin (FO = 84%), the herbicide metolachlor (FO = 56%) and the fungicide pyraclostrobin (FO = 51%). As seen, two of these three compounds belong to the strobirulin family of fungicides. The occurrence of strobilurins in bees throughout different spatial scenarios in Uruguay has been recently pointed out (Niell et al., 2015; Niell et al., 2017). The results stress the importance of studying the fate and impact these fungicides in the environment.

A group of 5 pesticides were detected but always remained below LOQ (acetamiprid, amitraz, imazalil, pirimicarb and tricyclazole). Compounds with frequency of occurrence for quantified values (FO_Q (%)) above 5% were in the following order: carbendazim, atrazine,

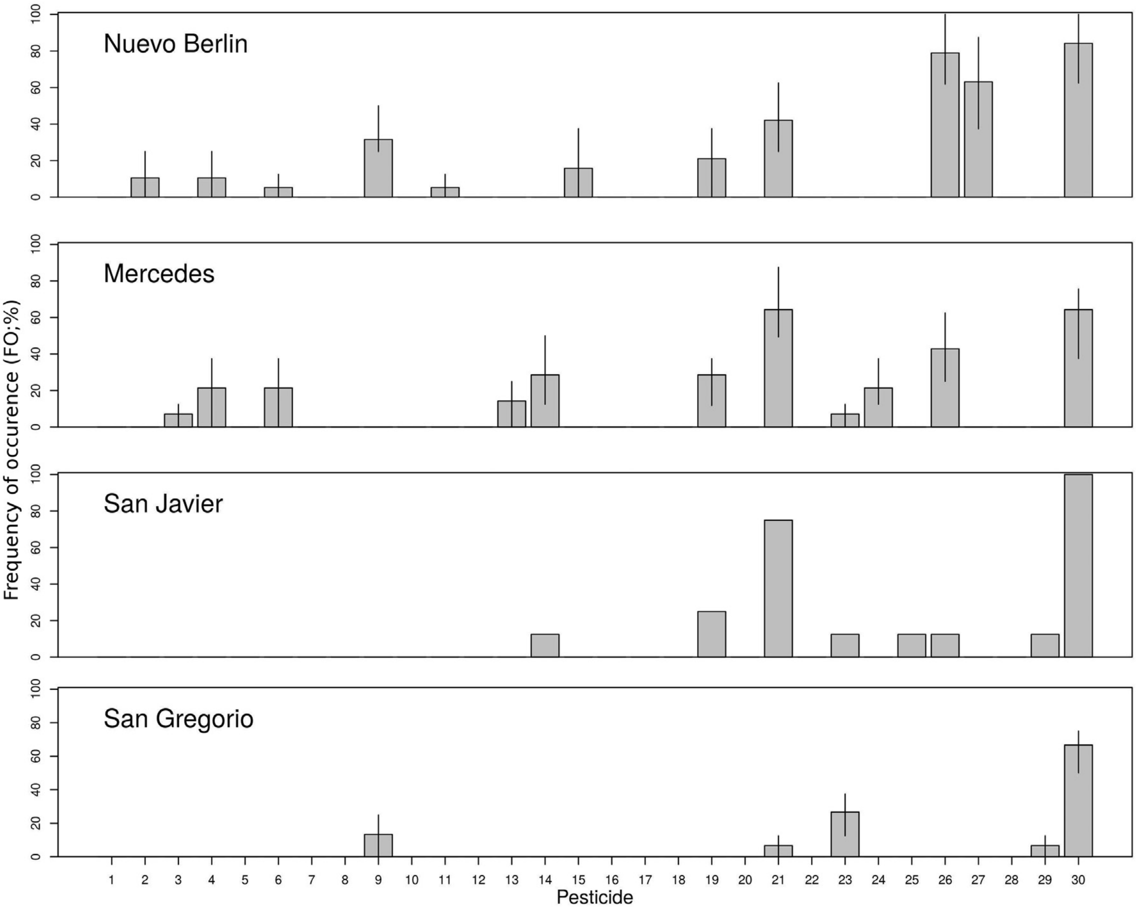

Fig. 3. Frequency of detection of thirty pesticides in non-migratory species in the four sampling sites. Vertical lines are the bootstrap estimate ($n = 8$) of the FO for the sites with >8 cases.

tebuconazole, difenoconazole, azoxystrobin. Carbendazim and atrazine presented quantifiable concentration levels (>LOQ) in 20% and 13% of the samples, respectively (see Table 2 for details).

It should be noted that most detected compounds are LC amenable pesticides. From the scope of GC amenable pesticides only the insecticide chlorpyrifos was detected. Chlorpyrifos residues were detected in 5 samples but only one individual had quantifiable residues. However, a maximum value of 194 μg kg^{-1} for chlorpyrifos was identified in a *Prochilodus lineatus* (a detritivourous and migratory specie) individual sampled at MC. Typical quantified residue concentrations have a mean of 10 μg kg^{-1}, a median of 2 μg kg^{-1} and ranged from 1 to 194 μg kg^{-1}. Similarly to other reports in fish from freshwater courses, pesticide residues occurred at low μg kg^{-1} levels. Chlorpyrifos, ethion and diazinon residues were found in fish species from Jucar River in Spain (Belenguer et al., 2014). Chlorpyrifos, etefenoprox, fipronil, malathion, parathion methyl and quinalphos residues were found from low to high μg kg^{-1} levels in pangasius fish (*Pangasianodon hypophthalmus*) from India (Chatterjee et al., 2016a).

The Olmsted-Tukey diagram allows observing the relative contribution of different substances (see Fig. 4). Five compounds that have not been quantified because their concentration always remained below LOQ were not classified (NC; Table 2).

Pesticides were classified into four categories: dominant, frequent, occasional and rare. Dominant ones are a group with both high FO and average concentration. The fungicides carbendazim, epoxiconazole, pyraclostrobin and trifloxystrobin were classified as dominant. While atrazine, azoxystrobin, difenoconazole, metalaxyl, metolachlor, pirimiphos methyl and tebuconazole were classified as frequent. Different agricultural uses are found for this category including fungicides, herbicides and an insecticide. Occasional substances that are infrequent but had higher concentrations included boscalid, cyproconazole, clomazone, chlorpyrifos, flusilazole, methidathion, pendimethalin and propiconazole (Table 2; Fig. 4). The rest of compounds were named as rare to occur.

To our knowledge this is the first study reporting the combined presence of pesticides approved for agriculture in fish muscle of South American rainfed agroecosystems pointing out a regular exposition to multiple substances at sublethal concentrations. Fungicides were the most detected class of compounds. A total of 15 fungicides, 10 insecticides and 5 herbicides were detected. The data gathered from migratory fish species is a good indicator of the overall presence of pesticide residues in the rivers under study.

As demonstrated, migratory fish species accumulated pesticides as well as non-migratory ones. Migratory species used in this work are potamodromous. That means they migrate up rivers to spawn and eggs are carried down the river and individuals recruit in the flooded areas. Then adults migrate back upstream to spawn (Reis et al., 2016). These species can migrate from 20 to 43 km a day (Teixeira de Mello et al., 2011). Considering that analogous agricultural techniques are performed at River Plate basin scale, we support the idea of exposure with pesticides at basin scale for migratory species during its lifetime. Therefore, exposition to pesticides might be hypothesized in a different spatio-temporal exposure for migratory species. Bioaccumulated residues could potentially belong to both, a recent or a long-standing exposure. Uptake kinetics of organic contaminants in fish has been studied for few fish species but particularly for highly lipophylic or banned compounds (Kobayashi et al., 2013; Sancho et al., 1998; Xu et al., 2014; Zhu et al., 2015). Trifloxystrobin uptake was studied on *Gobiocypris rarus* embryos (Zhu et al., 2015). These authors reported progressive accumulation with no evidence of elimination until 6 days of continuous exposure in water at sub μg L^{-1} levels. However, elimination rate for this pesticide in fishes has not been reported.

Table 2

FO: Frequency of occurrence; FO$_Q$(%): frequency of occurrence for quantified values; Mean$_Q$: average concentration when quantified; NC: not classified; NQ: not quantified. The classification into four classes was done following the Olmsted-Tukey diagram (see Fig. 3). Physicochemical properties, environmental fate and ecotoxicological data obtained from IUPAC Footprint. *Reference fish species: *Oncorhynchus mykiss*. S:soybean; SG: sugarcane; SGH: sorghum; AB: Animal breeding; R:rice; W:wheat; B:Barley; F: Forestry; M:Maize; NR: Not Registered. **Annual statistics of kg of active ingredient for season 2014 (DGSA, 2017).

						Pesticide data			Pesticide use in Uruguay	
Code	Pesticide	FO(%)	FO$_Q$ (%)	Mean$_Q$	Classification	log Kow	Soil degradation (days,.erobic) DT$_{50}$	Fish* Acute 96 h (LC50 mg L^{-1})	Approved for use in:	kg of active ingredient (2014 season)
1	Acetamiprid	0.7	0	NQ	NC	0.8	1.6	100	S	1100
2	Ametryn	2.7	2.7	1.1	RARE	2.63	37	5	SG/R	34,346
3	Amitraz	2	0	NQ	NC	5.5	0.2	0.74	AB	no data
4	Atrazine	16.1	12.8	1.6	FREQUENT	2.7	174	4.5	M/SGH/SG	312,385
5	Azinphos_methyl	1.3	0.7	1.9	RARE	2.96	10	0.02	NR	8820
6	Azoxystrobin	16.8	4.7	1.7	FREQUENT	2.5	78	0.47	S/R/W/B/F	259,233
7	Boscalid	0.7	0.7	5.5	OCCASIONAL	2.96	200	2.7	S/W/B	968
8	Carbaryl	5.4	1.3	1.2	RARE	2.36	16	2.6	S/R/W/B/F	4798
9	Carbendazim	20.1	19.5	4.2	DOMINANT	1.48	40	0.19	S/R/W/B	93,454
10	Carbofuran	1.3	0.7	1.1	RARE	1.8	29	0.18	S/R/W/B/F	1560
11	Cyproconazole	1.3	0.7	2.1	OCCASIONAL	3.09	142	19	S/R/W/B	178,086
12	Clomazone	5.4	4.7	2.2	OCCASIONAL	2.54	83	15.5	R	128,130
13	Chlorpyrifos	5.4	0.7	193.8	OCCASIONAL	4.7	50	0.0013	M/SGH/S/W/B	181,738
14	Difenoconazole	15.4	5.4	1.9	FREQUENT	4.36	130	1.1	S/R/W/B	26,181
15	Epoxiconazole	12.1	0.7	2.9	DOMINANT	3.3	354	3.14	S/R/W/B	16,857
16	Flusilazole	4.7	0.7	3.1	OCCASIONAL	3.87	300	1.2	S/W/B	no data
17	Imazalil	0.7	0	NQ	NC	2.56	76.3	1.48	W/B	1084
18	Malathion	0.7	0.7	1.9	RARE	2.75	0.17	18	M/SGH	2160
19	Metalaxyl	17.4	0.7	1.3	FREQUENT	1.65	36	100	S/W/B	11,847
20	Methidathion	0.7	0.7	2.5	OCCASIONAL	2.57	10	0.01	S	no data
21	Metolachlor	56.4	1.3	1.2	FREQUENT	3.4	90	3.9	S/M/SGH	98,880
22	Pendimethalin	0.7	0.7	4.3	OCCASIONAL	5.4	182.3	0.196	S/R/W/B	660
23	Pirimicarb	16.8	0	NQ	NC	1.7	86	100	SG/W/B	500
24	Pirimiphos_methyl	17.4	0.7	2	FREQUENT	3.9	39	0.404	AB	1386
25	Propiconazole	2	1.3	4	OCCASIONAL	3.72	71.8	2.6	S/R/W/B	350
26	Pyraclostrobin	51	4	3.6	DOMINANT	3.99	32	0.006	W/B/S	33,556
27	Tebuconazole	20.1	9.4	1.4	FREQUENT	3.7	63	4.4	W/B/S/R/F/SG	103,359
28	Thiabendazole	0.7	0.7	1.2	RARE	2.39	500	0.55	R/W/B	900
29	Tricyclazole	7.4	0	NQ	NC	1.4	450	7.3	R/W/B	121,570
30	Trifloxystrobin	83.9	3.4	2.1	DOMINANT	4.5	1.69	0.022	W/B/S/R	199,525

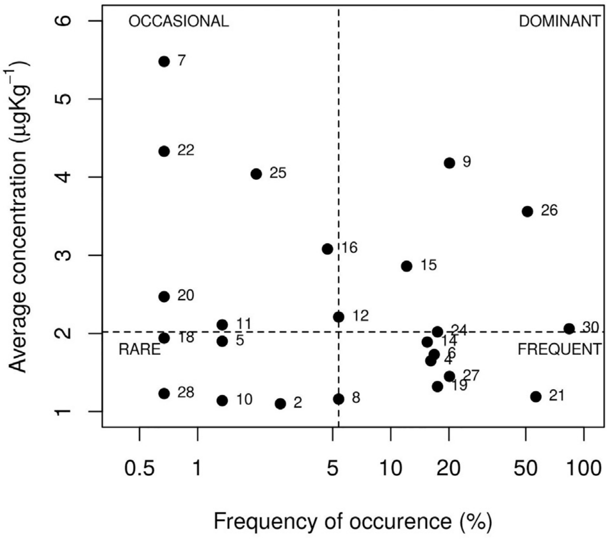

Fig. 4. Olmstead-Tukey diagram for the pesticides found at sampling sites. FO (%) vs the average concentration of the quantified pesticides (μg kg^{-1}) for the whole data set. Notice that clorpyrifos was removed for the diagram for easier interpretation because it presented an extreme average concentration (193.8 μg kg-1). Dashed lines represent the median value of FO (%) and concentration and delimited four quadrants to classify substances into Dominant, Frequent, Rare and Occasional classes.

Then, studies on in-vivo dynamics of pesticides in migratory and non-migratory aquatic wildlife with different feeding habits should be addressed for better understanding of contamination causes and consequences.

4.3. Land use effect on pesticides occurring in fish

A dataset of 54 non-migratory fish samples was analyzed including 14 fish from MC, 19 from NB, 8 from SJ and 15 from SGP.

Pesticide residues were detected in all localities while the average numbers of pesticides per fish were similar to the overall mean (ca. 4) in the most impacted sites 3.2, 3.7, 2.7 for MC, NB, SJ and lower (1.2) in SGP, respectively.

Similar incidence was found considering migratory and non-migratory individuals. Fig. 5 shows the distribution of detected pesticides from different sites where a dot means the number of pesticides detected from non-migratory individuals per sites. The average number of pesticides per fish were significantly lower in SGP as indicated by a significant negative coefficient (GLM; Family Poisson; link = identity; $p < 0.01$; coef = −2.0) with respect to the average of the other localities (Fig. 5).

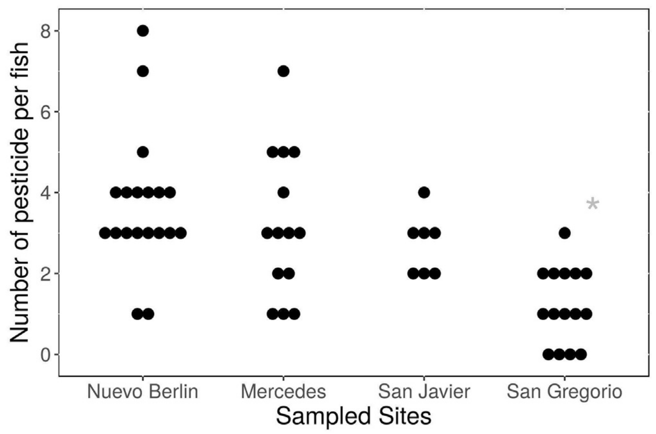

Fig. 5. Number of pesticides per fish in localities for non-migratory species. Each dot represents a non-migratory fish sampled in each locality and they are shifted horizontally for visual purposes. The gray asterisk denotes significant differences among SGP and the rest of the localities (GLM, Poisson model; see main text for details).

The amount of effort (measured in number of non-migratory fishes) exerted to analyze the diversity of pesticide residues showed that the multivariate standard error decreased with sample size as expected (See Fig. S1). Ideally the pattern should stabilize at a given variability. The result suggests a value reached after sampling ca. 12 fishes for MC and NB sites. MC showed a significantly larger variability than the other sampling sites whereas SJ does not seem to stabilize in the decrease of the multivariate error but the pattern closely follows the other sites (see Fig. S1). This data suggest that a 10–15 fish samples is enough to characterize the diversity of pesticide residues in those environments and further effort does not reduce the observed variability. It is a relevant point when planning and adjusting monitoring campaigns aiming to save resources (materials and time) efficiently.

The ordination method showed differences in the centroid among sites in high dimensional space (Fig. 6). MC showed a higher dispersion as observed previously. The variability among the remaining three sites was not different (mANOVA; SSsites = 0.002, dfsites = 2; SSresiduals = 1.79, dfresiduals = 34; F = 0.02; $p = 0.98$) (Fig. 5) (Fig. 5). However, there was a significant difference in centroid position among the remaining three localities (ANOVA; SSsites = 2.64, df = 2; SSresidual = 3.77, df = 34; F = 11.9; $p < 0.001$). The ordination diagram showed that SGP and SJ are close in the reduced dimensional space than NB (Fig. 6, Fig. S2).

Our results confirm that non-migratory wildlife present a close association with the land use, the nature of the pesticides and the intensity of pesticides used per area of land. Recent studies performed statistical analysis of pesticide residues in water samples matching dominant agricultural practices and intensity (Pascual Aguilar et al., 2017). To sum up, places with land use profusely dedicated to rainfed agriculture had the highest rates of occurrence and multiplicity of pesticides. This evidence should be addressed in the context of a RAMSAR site surrounded by in an intensive agroecosystem. New insights of considerations of environment at landscape scale are interesting alternatives to consider by regulatory systems of production and safety (Milner and Boyd, 2017).

4.4. Physicochemical properties, environmental fate and associated agriculture

The vast majority of the detected pesticides (except amitraz and chlorpyrifos) are classified as non-volatile/semi-volatile according to

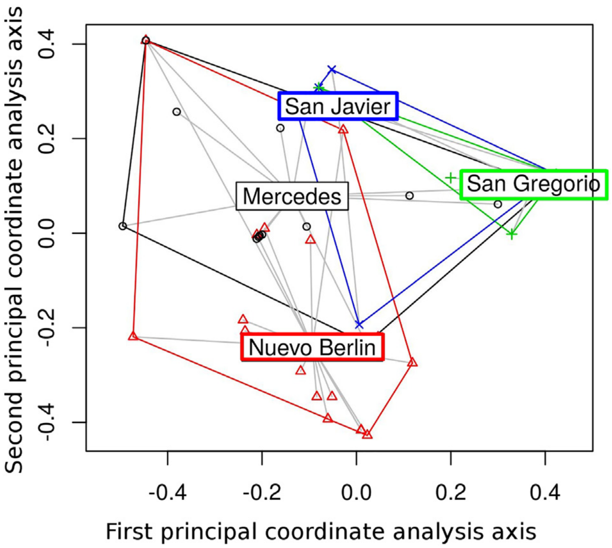

Fig. 6. Ordination in reduced dimensional space for sampling sites in rainfed agroecosystems (MC, NB, SJ) and control site (SGP).

Henry's law constant (H, Table S3). Therefore, the compounds that are expected to be found are those that remain in water and do not partition towards the atmosphere. Such observation suggests main transport of pesticides through runoff in the fields to water courses.

Major concern in literature was given to pollutants with high tendency to bioaccumulate on living organisms. The octanol–water partition coefficient (Kow) is widely used as a measure of lipophylia and accumulation on living organisms. On the other hand, persistence is another property of primary concern. In fact, pollutants with higher Kow values and greater persistence are considered priority pollutants. As a result, banned organochlorine pesticides are still matter of interest in fish (Colombo et al., 2011; Chatterjee et al., 2016b; Jabeen et al., 2015; Kalachova et al., 2013; Li et al., 2017; Lo et al., 2016; Masiá et al., 2013; Molina-Ruiz et al., 2015; Rawn et al., 2010; Rose et al., 2015; Sapozhnikova and Lehotay, 2013; Sapozhnikova and Lehotay, 2015). Pesticides currently approved for agriculture are semi-polar or polar with vast diversity of functional groups responsible to increase target selectivity but also to reduce their persistence in the environment. As a general rule, pesticides have the potential to accumulate on aquatic biota if their Kow is >1000 (it means log Kow > 3) and soil half-life (DT_{50} in soil) >30 days (Andreu and Picó, 2012; USGD, 2009).

Concerning our results, all compounds classified as rare presented lower log Kow values than 3. The remaining three classes presented log Kow values from 5.4 to 1.48 without clear pattern. Simultaneously, soil DT_{50} values ranged from 1.69 to 354 days which means non-persistent to persistent pesticides according to database criteria (IUPAC Footprint, 2017). It should be mentioned that from the detected pesticides, trifloxystrobin simultaneously has the highest log Kow (4.5) and the fastest soil degradation measured in DT_{50} (1.69, days). However, excepting trifloxystrobin all dominant and frequent compounds have soil degradation >30 days (see Table S3 for details).

The majority of these groups of compounds are classified as moderately persistent in soil (IUPAC Footprint, 2017). Although trifloxystrobin is considered not persistent in soil the high volumes used of active ingredient could explain its regular appearance through a habitual exposition. Fig. 7 shows a relationship between properties of these pesticides and their classification.

Interestingly, dominant compounds (carbendazim, epoxiconazole, pyraclostrobin and trifloxystrobin) had log Kow ranging from 3.3 to 4.5. The dominant class of compounds had the highest values of log Kow among the occurring pesticides. As seen, there is an agreement with the obtained FO (%) and predicted accumulation of pesticides in biota (Andreu and Picó, 2012; USGD, 2009). Median values of log Kow are consistent with the observed accumulation for dominant, frequent, occasional and rare classes (Fig. 7).

Regarding Freundlich constant (Kf) (see Table S3) used to categorize soil adsorption and mobility of pesticides, the dominant compounds are classified as slightly or moderately mobile. That means that only a fraction of pesticides are transported to the aquatic environment through runoff.

Aquatic half-life (DT_{50}) of pesticides in water and water-sediment phases are shown in Table S3. Detected compounds range from slow to fast degradation in both medias. Metholaclor shows to be moderately persistent in soil but also stable at aqueous or sedimentary phases during months. That means that metolachlor residues are available for a long-term exposure once they reach to the aquatic environment. Interestingly, some compounds have fast or moderately fast degradation at water or sedimentary phases. For example, trifloxystrobin and

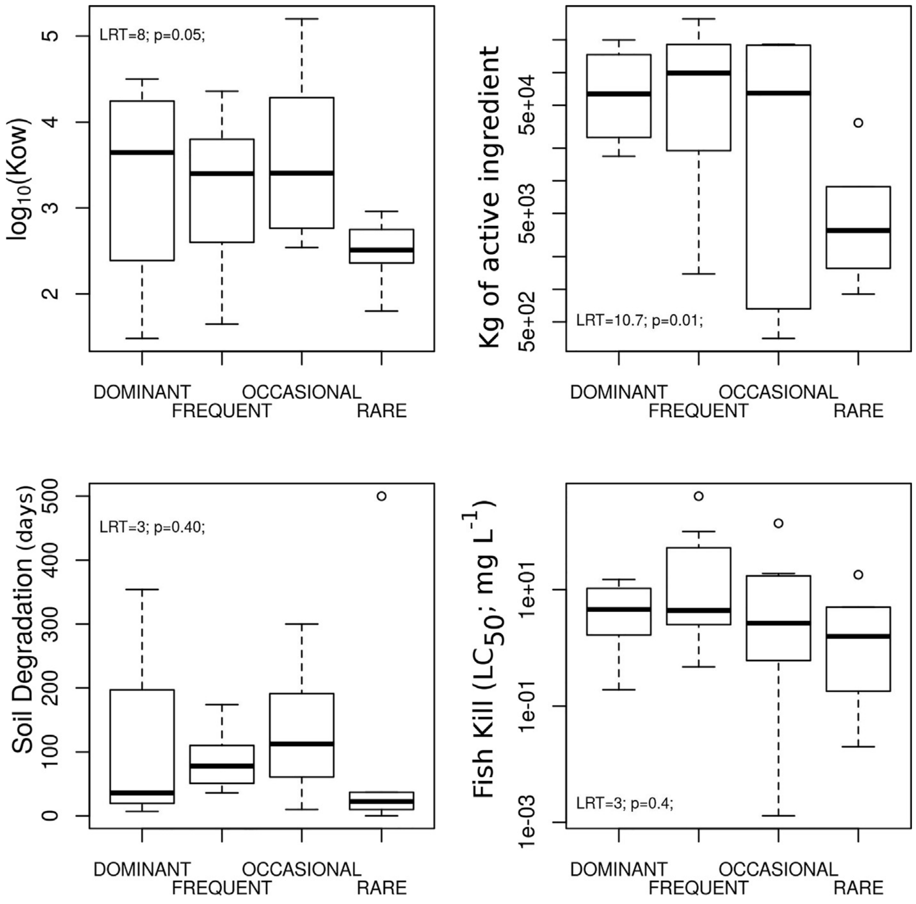

Fig. 7. Pesticides classes in relation to log Kow, soil degradation (DT_{50} in days, aerobic), Acute toxicity (LD_{50}, mg L^{-1}) and annual volumes of imported active ingredient (in kg) in Uruguay. LRT refers to the likelihood ratio test statistic and the *p*-value of comparing among the four groups in chemical properties. $P < 0.05$ implies a significant differences in average value among the four groups.

pyraclostrobin half-degradation in aquatic environment is in the order of days. That means that their residues are probably available for a short-term exposure to be accumulated. On the other hand, note that water-sediment DT_{50} for triazole fungicides (cyproconazole, difenoconazole, epoxiconazole, flusilazole, propizonazole, tebuconazole thiabendazole and tricyclazole) denotes slow degradation in periods of months to years.

Additionally, most compounds classified as occasional and rare have log Kow values ranging from higher to lower than 3. They are also classified from very persistent (i.e. tricyclazole) to non-persistent (malathion) pesticides in soil but also as slightly or moderately mobile (IUPAC Footprint, 2017). Interestingly, compounds not classified such as acetamiprid (log Kow = 0.8) were also detected in fish muscle. Regarding ecotoxicity, note that pyraclostrobin and trifloxystrobin are classified as highly toxic for fish with LC_{50} acute dose in water of 0.022 and 0.006 mg L^{-1} (IUPAC Footprint, 2017). Therefore, these compounds have to be considered as priority pesticides for agricultural management and conservation of aquatic wildlife.

Reports described that strobilurin fugicides inhibit growth of fish species at early-life stages (Liu et al., 2013; Zhu et al., 2015). Trifloxystrobin showed developmental toxicity to *Gobiocypris rarus* embryos by increasing malformation, decreasing body length and heart rate and affecting spontaneous movement and swimming speed (Zhu et al., 2015).

As seen from Fig. 7, no clear pattern is observed for acute toxicity for fish and soil persistence between classes. However, dominant, frequent and occasional compounds are clearly applied at higher levels in the field than rare ones. It should be mentioned that data supplied on volumes of active ingredient are indicative at national scale of rainfed agriculture rather than consumption on the sites under study.

Notice that 28 from 30 detected compounds are currently approved for rainfed agriculture, mostly in extensive barley, sorghum, soybean and wheat production (see Table 2). On the other hand, pirimiphos methyl and clomazone are used as veterinary drug and rice cropping, respectively.

Transport of pesticides from minor activities such as animal breeding or rice cropping through the Uruguay River is demonstrated. Close to the site under study, rice cropping is evidenced on the Argentinian side of the Uruguay River. If considering annual statistics of use of pesticides, those classified as dominant and frequent ones were also highly consumed active ingredients in comparison to occasional and rare ones. In view of the annual mass in kg of active ingredient imported in Uruguay in 2014 with >10 tons had the following order: atrazine, azoxystrobin, trifloxystrobin, chlorpyrifos, cyproconazole, clomazone, tricyclazole, tebuconazole, metolachlor, carbendazim, ametryn, pyraclostrobin, difenoconazole, epoxiconazole and metalaxyl (see Table 2 and Fig. 7). Atrazine has been recently banned in Uruguay (DGSA, 2017). Changes at temporal scale should be studied in the future.

In our opinion, a combination of reasons could explain the obtained pattern of occurring pesticides in fishes. Pesticides are prone to accumulate in biota due to particular physicochemical and persistence properties but also if they are extensively used in agriculture. In consequence, the short-term exposition is associated to the occurrence of these pesticides in fish muscle tissue. As demonstrated, although pesticides such as trifloxystrobin that are considered non-persistent in soil and/or slightly mobile, they show ability to reach the aquatic environment and accumulate in wildlife at sub-lethal concentrations.

Future studies focusing on the temporal incidence of pesticides during fish lifetime and risk assessment are needed.

5. Conclusions

It was demonstrated that migratory and non-migratory freshwater fish living in CCNT rainfed agroecosystems incorporate into their muscular tissues a wide variety of fungicides, insecticides and herbicides at μg kg^{-1} levels. Thirty pesticide residues were detected in selected South American wild fish species with a median of four compounds occurring per fish. Fungicides account for the half of pesticides occurring. Along with the herbicide metolachlor, the fungicides trifloxystrobin and pyraclostrobin had the highest rates of occurrence. However, these strobirulin compounds are the most toxic to fish from all occurring compounds. Incidence of pesticides in fish muscle tissue was related to: i) physicochemical properties that support accumulation, enable mobility and secure persistence and ii) inherent intensity of active ingredient used and land dedicated to agriculture. Both factors aim the reconsideration of GAPs based on potential impacts of type and amount of pesticides required for a given production. This evidence should be particularly addressed in the context of agricultural management for priority conservation areas such as RAMSAR sites.

The pattern of occurring pesticides in non-migratory species was able to trend significant spatial differences among sampling sites. Migratory fish species accumulated pesticides indicating regular exposition throughout its life in River Plate basin. However, the chronic effects on fish exposed to sublethal doses of multiple pesticides, the potential movement of these contaminants through the food web and the pressure exerted on the communities or early-life stages of the same species are open questions.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.02.320.

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Conflict of interest

Authors declare no conflict of interest.

References

Anderson, M.J., Santana-Garcon, J., 2015. Measures of precision for dissimilarity-based multivariate analysis of ecological communities. Ecol. Lett. 18, 66–73.

Anderson, M.J., Walsh, D., 2013. PERMANOVA, ANOSIM, and the mantel test in the face of heterogeneous dispersions: what null hypothesis are you testing? Ecol. Monogr. 83, 557–574.

Andreu, V., Picó, Y., 2012. Determination of currently used pesticides in biota. Anal. Bioanal. Chem. 404, 2659–2681.

Bacchetta, C., Rossi, A., Ale, A., Campana, M., Parma, M.J., Cazenave, J., 2014. Combined toxicological effects of pesticides: a fish multi-biomarker approach. Ecol. Indic. 36, 532–538.

Baduel, C., Mueller, J.F., Tsai, H., Gomez Ramos, M.J., 2015. Development of sample extraction and clean-up strategies for target and non-target analysis of environmental contaminants in biological matrices. J. Chromatogr. A 1426, 33–47.

Belenguer, V., Martinez-Capel, F., Masiá, A., Picó, Y., 2014. Patterns of presence and concentration of pesticides in fish and waters of the Júcar River (eastern Spain). J. Hazard. Mater. 265, 271–279.

Ccanccapa, A., Masiá, A., Picó, Y., Andreu, V., 2015. Spatio-temporal patterns of pesticide residues in the Turia and Júcar rivers (Spain). Sci. Total Environ. 540, 200–210.

Chalar, G., Delbene, L., González-Bergonzoni, I., Arocena, R., 2013. Fish assemblage changes along a trophic gradient induced by agricultural activities (Santa Lucía, Uruguay). Ecol. Indic. 24, 582–588.

Chatterjee, N.S., Banerjee, K., Utture, S., Kamble, N., Rao, B.M., Panda, S.K., et al., 2016a. Assessment of polyaromatic hydrocarbons and pesticide residues in domestic and imported pangasius (*Pangasianodon hypophthalmus*) fish in India. J. Sci. Food Agric. 96, 2373–2377.

Chatterjee, N.S., Utture, S., Banerjee, K., Ahammed Shabeer, T.P., Kamble, N., Mathew, S., et al., 2016b. Analytical methods: multiresidue analysis of multiclass pesticides and polyaromatic hydrocarbons in fatty fish by gas chromatography tandem mass spectrometry and evaluation of matrix effect. Food Chem. 196, 1–8.

Colombo, J.C., Cappelletti, N., Williamson, M., Migoya, M.C., Speranza, E., Sericano, J., et al., 2011. Risk ranking of multiple-POPs in detritivorous fish from the Río de la Plata. Chemosphere 83, 882–889.

Daufresne, M., Boët, P., 2007. Climate change impacts on structure and diversity of fish communities in rivers. Glob. Chang. Biol. 13, 2467–2478.

DGSA. Dirección de Servicios Agrícolas, 2017. Ministerio de Ganadería Agricultura y Pesca. Prohibición de atrazina, Resolución N°104 05–12-16 Diciembre de 2016. Available at. http://www.mgap.gub.uy/unidad-ejecutora/direccion-general-de-servicios-agricolas/normativa/resoluciones/resolucion, Accessed date: 4 October 2017.

DIEA, 2015. Estadísticas Agropecuarias. Ministerio de Ganadería Agricultura y Pesca, Regiones Agropecuarias del Uruguay Available at. http://www2.mgap.gub.uy/DieaAnterior/regiones/Regiones2015.pdf (Accessed September 10–26, 2017).

European Commission, 2015. SANTE/11945/2015 2015 Document. Guidance Document on Analytical Quality Control and Method Validation Procedures for Pesticides Residues Analysis in Food and Feed.Available at. https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_mrl_guidelines_wrkdoc_11945.pdf (Accessed September 1-26, 2017).

Fisher, Nicholas I., 1983. Graphical methods in nonparametric statistics: a review and annotated bibliography. Int. Stat. Rev. 51, 25–58.

Franzluebbers, A.J., Sawchik, J., Taboada, M.A., 2014. Agronomic and environmental impacts of pasture–crop rotations in temperate north and South America. Agric. Ecosyst. Environ. 190, 18–26.

García-Préchac, F., Ernst, O., Siri-Prieto, G., Terra, J.A., 2004. Integrating no-till into crop-pasture rotations in Uruguay. Soil Tillage Res. 77, 1–13.

Ginebreda, A., Pérez, S., Rivas, D., Kuzmanovic, M., Barceló, D., 2016. Pollutants of emerging concern in rivers of catalonia: occurrence, fate, and risk. In: Munné, A., Ginebreda, A., Prat, N. (Eds.), Experiences from Surface Water Quality Monitoring: The EU Water Framework Directive Implementation in the Catalan River Basin District (Part I). Springer International Publishing, Cham, pp. 283–320.

Guía SATA, 2017. Guía para la Protección y Nutrición Vegetal.Available at. http://www.laguiasata.com (Accessed September 1–26, 2017).

Harriet, J., Campá, J.P., Grajales, M., Lhéritier, C., Gómez Pajuelo, A., Mendoza-Spina, Y., Carrasco-Letelier, Y., 2017. Agricultural pesticides and veterinary substances in Uruguayan beeswax. Chemosphere 177, 77–83.

IUPAC Footprint, 2017. Pesticide Properties Database. Agriculture & Environment Research Unit (AERU), University of Hertfordshire Available at:. http://sitem.herts.ac.uk/aeru/iupac/index.htm, Accessed date: 3 January 2018.

Jabeen, F., Chaudhry, A.S., Manzoor, S., Shaheen, T., 2015. Examining pyrethroids, carbamates and neonicotenoids in fish, water and sediments from the Indus River for potential health risks. Environ. Monit. Assess. 187, 29.

Kaczyński, P., Łozowicka, B., Perkowski, M., Szabuńko, J., 2017. Multiclass pesticide residue analysis in fish muscle and liver on one-step extraction-cleanup strategy coupled with liquid chromatography tandem mass spectrometry. Ecotoxicol. Environ. Saf. 138, 179–189.

Kalachova, K., Pulkrabova, J., Drabova, L., Cajka, T., Kocourek, V., Hajslova, J., 2011. Simplified and rapid determination of polychlorinated biphenyls, polybrominated diphenyl ethers, and polycyclic aromatic hydrocarbons in fish and shrimps integrated into a single method. Anal. Chim. Acta 707, 84–91.

Kalachova, K., Cajka, T., Sandy, C., Hajslova, J., Pulkrabova, J., 2013. High throughput sample preparation in combination with gas chromatography coupled to triple quadrupole tandem mass spectrometry (GC–MS/MS): a smart procedure for (ultra)trace analysis of brominated flame retardants in fish. Talanta 105, 109–116.

Kobayashi, J., Sakurai, T., Mizukawa, K., Kinoshita, K., Ito, N., Hashimoto, S., et al., 2013. Respiratory uptake kinetics of neutral hydrophobic organic chemicals in a marine benthic fish, Pseudopleuronectes yokohamae. Chemosphere 93, 1479–1486.

Köhler, H.R., Triebskorn, R., 2013. Wildlife ecotoxicology of pesticides: can we track effects to the population level and beyond? Science 341, 759–765.

Lazartigues, A., Wiest, L., Baudot, R., Thomas, M., Feidt, C., Cren-Olivé, C., 2011. Multiresidue method to quantify pesticides in fish muscle by QuEChERS-based extraction and LC-MS/MS. Anal. Bioanal. Chem. 400, 2185–2193.

Li, W., Liu, D., Li, J., Gao, J., Zhang, C., Wang, P., et al., 2017. Matrix solid-phase dispersion combined with GC-MS/MS for the determination of organochlorine pesticides and polychlorinated biphenyls in marketed seafood. Chromatographia 80, 813–824.

Liu, L., Jiang, C., Wu, Z.-Q., Gong, Y.-X., Wang, G.-X., 2013. Toxic effects of three strobilurins (trifloxystrobin, azoxystrobin and kresoxim-methyl) on mRNA expression and antioxidant enzymes in grass carp (Ctenopharyngodon idella) juveniles. Ecotoxicol. Environ. Saf. 98, 297–302.

Lo, J.C., Letinski, D.J., Parkerton, T.F., Campbell, D.A., Gobas, F.A., 2016. In vivo biotransformation rates of organic Chemicals in Fish: relationship with bioconcentration and biomagnification factors. Environ. Sci. Technol. 50, 13299–13308.

Masiá, A., Campo, J., Vázquez-Roig, P., Blasco, C., Picó, Y., 2013. Screening of currently used pesticides in water, sediments and biota of the Guadalquivir River basin (Spain). J. Hazard. Mater. 263 (1), 95–104.

Masiá, A., Campo, J., Navarro-Ortega, A., Barceló, D., Picó, Y., 2015. Pesticide monitoring in the basin of Llobregat River (Catalonia, Spain) and comparison with historical data. Sci. Total Environ. 503–504, 58–68.

Milner, A.M., Boyd, I.L., 2017. Toward Pesticidovigilance. Science 357, 1232–1234.

Molina-Ruiz, J.M., Cieslik, E., Cieslik, I., Walkowska, I., 2015. Determination of pesticide residues in fish tissues by modified QuEChERS method and dual-d-SPE clean-up coupled to gas chromatography–mass spectrometry. Environ. Sci. Pollut. Res. 22, 369–378.

Munaretto, J.S., Ferronato, G., Ribeiro, L.C., Martins, M.L., Adaime, M.B., Zanella, R., 2013. Development of a multiresidue method for the determination of endocrine disrupters in fish fillet using gas chromatography-triple quadrupole tandem mass spectrometry. Talanta 116, 827–834.

Munaretto, J.S., May, M.M., Saibt, N., Zanella, R., 2016. Liquid chromatography with high resolution mass spectrometry for identification of organic contaminants in fish fillet: screening and quantification assessment using two scan modes for data acquisition. J. Chromatogr. A 1456, 205–216.

Niell, S., Jesús, F., Pérez, C., Mendoza, Y., Díaz, R., Franco, J., Cesio, V., Heinzen, H., 2015. QuEChERS adaptability for the analysis of pesticide residues in beehive products seeking the development of an agroecosystem sustainability monitor. J. Agric. Food Chem. 63, 4484–4492.

Niell, S., Jesús, F., Pérez, N., Pérez, C., Pareja, L., Abbate, S., Carrasco-Letelier, L., Díaz, S., Mendoza, Y., Cesio, V., Heinzen, H., 2017. Neonicotinoids transference from the field to the hive by honey bees: towards a pesticide residues biomonitor. Sci. Total Environ. 581, 25–31.

Novelli, L.E., Caviglia, O.P., Piñeiro, G., 2017. Increased cropping intensity improves crop residue inputs to the soil and aggregate-associated soil organic carbon stocks. Soil Tillage Res. 165, 128–136.

Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Simpson, G.L., Solymos, P., Stevens, M.H., Wagner, H., 2009. Vegan: Community Ecology Package. (Version 1.15-2). R-Software.

Pascual Aguilar, J.A., Andreu, V., Campo, J., Picó, Y., Masiá, A., 2017. Pesticide occurrence in the waters of Júcar River, Spain from different farming landscapes. Sci. Total Environ. 607-608, 752–760.

R Core Team, 2015. R Foundation for statistical computing. R: A Language and Environment for Statistical Computing. Vienna, Austria Available at. https://www.R-project.org/.

Ramalhosa, M.J., Paíga, P., Morais, S., Rui Alves, M., Delerue-Matos, C., Oliveira, M.B.P.P., 2012. Lipid content of frozen fish: comparison of different extraction methods and variability during freezing storage. Food Chem. 131, 328–336.

RAMSAR. Convention Site: Esteros de Farrapos e Islas del Río Uruguay. Available at: http://www.ramsar.org/news/uruguay-designates-its-second-ramsar-site-along-the-uruguay-river-uruguay-designa-su-segundo. (September 23), 2017.

Rawn, D.F.K., Judge, J., Roscoe, V., 2010. Application of the QuEChERS method for the analysis of pyrethrins and pyrethroids in fish tissues. Anal. Bioanal. Chem. 397, 2525–2531.

Reis, R.E., Albert, J.S., Di Dario, F., Mincarone, M.M., Petry, P., Rocha, L.A., 2016. Fish biodiversity and conservation in South America. J. Fish Biol. 89, 12–47.

Rose, M., Fernandes, A., Mortimer, D., Baskaran, C., 2015. Contamination of fish in UK fresh water systems: risk assessment for human consumption. Chemosphere 122, 183–189.

Sancho, E., Ferrando, M.D., Gamón, M., Andreu-Moliner, E., 1998. Uptake and elimination kinetics of a pesticide in the liver of the European EEL. J. Environ. Sci. Health B 33, 83–98.

Sapozhnikova, Y., Lehotay, S.J., 2013. Multi-class, multi-residue analysis of pesticides, polychlorinated biphenyls, polycyclic aromatic hydrocarbons, polybrominated diphenyl ethers and novel flame retardants in fish using fast, low-pressure gas chromatography-tandem mass spectrometry. Anal. Chim. Acta 758, 80–92.

Sapozhnikova, Y., Lehotay, S.J., 2015. Evaluation of different parameters in the extraction of incurred pesticides and environmental contaminants in fish. J. Agric. Food Chem. 63, 5163–5168.

Schreinemachers, P., Tipraqsa, P., 2012. Agricultural pesticides and land use intensification in high, middle and low income countries. Food Policy 37, 616–626.

Teixeira de Mello, F., González-Bergonzoni, I., Loureiro, M., 2011. Peces de Agua Dulce del Uruguay. PPR-MGAP, Montevideo, Uruguay Available at. http://www.mgap.gub.uy/sites/default/files/multimedia/10_-_peces_de_uruguay_2011.pdf, Accessed date: 26 September 2017.

Uruguay XXI, 2015. Informe Anual de Comercio Exterior. Available at. http://www.uruguayxxi.gub.uy/informacion/wp-content/uploads/sites/9/2016/01/Informe-Anual-Comercio-Exterior-2015.pdf, Accessed date: 26 September 2017.

USGD, 2009. Pesticides in stream sediment and aquatic biota. Current understanding of distribution and major influences. Fact Sheet 092-00. U.S. Geological Survey.

Wingeyer, A.B., Amado, T.J., Pérez-Bidegain, M., Studdert, G.A., Perdomo Varela, C.H., Garcia, F.O., et al., 2015. Soil quality impacts of current south American agricultural practices. Sustainability 7, 2213–2242.

Xu, J., Luo, J., Ruan, J., Zhu, F., Luan, T., Liu, H., et al., 2014. In vivo tracing uptake and elimination of organic pesticides in fish muscle. Environ. Sci. Technol. 48, 8012–8020.

Yao, L., Zhao, J.-L., Liu, Y.-S., Yang, Y.-Y., Liu, W.-R., Ying, G.-G., 2016. Simultaneous determination of 24 personal care products in fish muscle and liver tissues using QuEChERS extraction coupled with ultra-pressure liquid chromatography-tandem mass spectrometry and gas chromatography-mass spectrometer analyses. Anal. Bioanal. Chem. 408, 8177–8193.

Zhu, B., Liu, G.-L., Liu, L., Ling, F., Wang, G.-X., 2015. Assessment of trifloxystrobin◘ uptake kinetics, developmental toxicity and mRNA expression in rare minnow embryos. Chemosphere 120, 447–455.

Zuur, A., Ieno, E.N., Walker, N., Saveliev, A.A., Smith, G.M., 2009. Mixed effects models and extensions in ecology with R. Statistics for Biology and Health. Springer, New York, NY.