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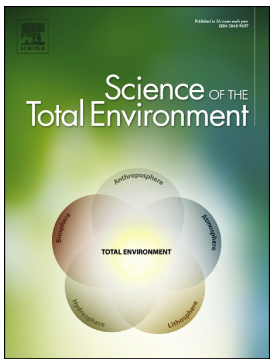

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PII: S0048-9697(18)33354-0
DOI: doi:10.1016/j.scitotenv.2018.08.383
Reference: STOTEN 28445

To appear in: *Science of the Total Environment*

Received date: 29 June 2018
Revised date: 24 August 2018
Accepted date: 26 August 2018

Please cite this article as: Paola M. Ondarza, Samuel P. Haddad, Esteban Avigliano, Karina S.B. Miglioranza, Bryan W. Brooks , Pharmaceuticals, illicit drugs and their metabolites in fish from Argentina: Implications for protected areas influenced by urbanization. Stoten (2018), doi:10.1016/j.scitotenv.2018.08.383

Pharmaceuticals, illicit drugs and their metabolites in fish from Argentina: implications for protected areas influenced by urbanization

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Abstract

Because an understanding of aquatic bioaccumulation of human pharmaceuticals in Latin America is limited, this area was recently identified as a priority environmental quality research need. We examined bioaccumulation of twenty-seven pharmaceuticals, illicit drugs and their metabolites in muscle, liver and gills of multiple fish species (*Rhamdia quelen*, *Hypostomus commersoni*, *Hoplias lacerdae*, *Prochilodus lineatus*) from an urban river receiving wastewater discharges (Paraná) and a lotic system (Acaraguá) without direct wastewater sources, which runs through a protected area. All samples were analyzed using isotope-dilution liquid chromatography-tandem mass spectrometry. Caffeine, which was detected up to 13 μg/kg, and antibiotics were consistently detected in all fish. Among antibiotics, erythromycin was ubiquitous (0.7-5.6 μg/kg) but its tissue concentrations were lower than levels of sulfamethoxazole, sulfathiazole and trimethoprim (0.9-5.5 μg/kg), which are used in human medicine, aquaculture and livestock. Erythromycin bioaccumulation in fish is reported here from Argentina for the first time, though levels of antibiotics in edible muscles of these species were lower than the maximum residue limits for human consumption. We observed norfluoxetine, the primary active metabolite of the antidepressant fluoxetine, ranging from 1.1-9.1 μg/kg in fish. We further identified benzoylecgonine, a primary metabolite of cocaine, in fish from both study systems, representing the first observation an illicit drug or associated metabolites bioaccumulation in aquatic life from Argentina. Interestingly, high pharmaceutical levels were observed in fish from the Acaraguá river suggesting their transport into the protected area, from the surrounding lands. Though fish from the Paraná river were sampled near WWTP discharges, pharmaceutical concentrations may have been reduced by hydrological and other environmental conditions, and biological differences among species. These findings, which observed

bioaccumulation of select pharmaceuticals, their metabolites and illicit drugs in wild fish sampled inside a protected area, highlight the importance of developing an advanced understanding of urban influences on inland protected watersheds.

Highlights

Fish from a protected area accumulated licit and illicit drugs and their metabolites

Caffeine and antibiotics were the major pharmaceuticals observed in fish

The macrolide erythromycin was ubiquitous in its first report in fish from Argentina

First identification of illicit drugs in fish from South America

Urban influences on aquatic contamination of protected areas require future attention

Keywords:

Emergent contaminants, pharmaceuticals, illicit drugs, fish, Argentina, protected areas.

Abbreviations

Acetaminophen: ACE, amitriptyline: AMTP, amlodipine: AML, aripiprazole: ARI, benzoylecgonine: BEN, buprenorphine: BUP, caffeine: CAF, carbamazepine: CBZ, desmethylsertraline: DES-Me-SER, diclofenac: DCF, diltiazem: DLZ, diphenhydramine: DIP, duloxetine: DUL, erythromycin: ERY, fluoxetine: FLX, ketamine: KET, methylphenidate: MPH, norfloxacin: NOR, norfluoxetine: NOR-FLX, promethazine: PROM, propranolol: PROP, sertraline: SER, sucralose: SUC, sulfadimethoxine: SFD, sulfamethoxazole: SUL, sulfathiazole: STZ, trimethoprim: TMP.

1. Introduction

Growing urbanization and frequent disposal of untreated sewage and treated effluent to water resources causes widespread contamination of freshwater and drinking supplies with contaminants of emerging concern (CECs) (Daughton, 2013). Among them, human and veterinary pharmaceuticals have raised an increasing concern due to their extensive use, physico-chemical characteristics, and continuous discharge to the aquatic environment (Evgenidou et al., 2015). Illicit drugs have been described as new and unexpected CECs with potent bioactive properties and unknown effects to organisms (Evgenidou et al., 2015). After consumption, pharmaceuticals and their metabolites are discharged into aquatic ecosystems via mainly wastewater effluents, landfill leaching, and manufacture, among others (Daughton, 2013). Since pharmaceuticals are highly water soluble, fish and other aquatic organisms accumulate these CECs primarily from water and also from food (Brooks et al., 2005; Du et al., 2014; Grabicova et al., 2015). Numerous studies worldwide have confirmed the occurrence of pharmaceuticals in a wide variety of environmental compartments showing that conventional wastewater treatment plants (WWTPs) are not effective to remove these CECs and current WWTP technologies vary among countries (Kookana et al., 2014).

Globally, ~80% of sewage production is released to the environment untreated (WWAP 2017). These facts raise concern about the potential for bioaccumulation and associated adverse effects to aquatic wildlife (Daughton and Brooks, 2011), which was previously identified as a key research need to define risks of pharmaceuticals in the environment (Boxall et al., 2012; Rudd et al., 2014). Unfortunately, few studies have been focused on bioaccumulation of CECs in wastewater receiving water bodies or with biota under controlled laboratory conditions from Argentina (Cazenave et al., 2014; Dorelle et al., 2017; Elorriaga et al., 2013 a, b; Pérez et al., 2018; Valdes et al., 2014; 2015; 2016). In

fact, Furley et al. (2018) recently identified better understanding relationships among pharmaceutical bioaccumulation, adverse effects and management as a priority research need for Latin America.

For this reason, the primary objective of the present study was to improve the knowledge about the occurrence of pharmaceuticals and illicit drugs, and their metabolites, in fish from the Atlantic Rain Forest rivers in the Misiones province, Argentina. We examined fish that were sampled in rivers commonly used for drinking and domestic purposes (>1,200,000 residents) but at the same time, receives untreated wastewater from diverse settlements. This research specifically examined bioaccumulation of twenty-seven selected pharmaceuticals, illicit drugs and their metabolites in three tissues of four fish species from the Paraná river, an urban system that receives wastewater discharge, and the Acaraguá river, which runs through a protected area without direct wastewater sources, in the Misiones province of Argentina. Potential influences of these human activities into the protected area were also considered.

2. Material and methods

2.1. Study area and sampling sites

Misiones province of Argentina has the largest remaining area of Atlantic Rain Forest (>10,000 km^2), which is one of the International Conservation Hotspots and one of the Global 200 World Wildlife Fund ecoregions (Izquierdo et al., 2008). However, this area has experienced rapid population growth and extensive deforestation during the last 40 years (Izquierdo et al., 2008). The region has nearly 1.7 million inhabitants (native and non-native population) with poor demographic and health conditions where sewers, wastewater service and improved drinking water supplies, are absent. Consequently,

communities commonly use rivers for both disposing wastewater and direct consumption and domestic use. Moreover, various anthropogenic activities, such as towns, agriculture, deforestation, livestock, aquaculture and industries depend on and release contaminants to the water resources in this area (Avigliano and Schenone, 2015).

As introduced above, fish were collected inside at the protected area called "Antonia Ramos Research Center" (CIAR) in the Acaraguá river (27º26´S 54º56´W), and in the Paraná river (27º22´S 55º53´W) close to Posadas city, an area with over 360,000 inhabitants (Figure 1). No WWTP effluents discharges are found in the protected area, which includes more than 750 ha of native rain forest, promoting the biodiversity conservation. The Acaraguá river is a higher gradient mountain river running through the protected area; however, upstream of our sampling site, this river receives runoff from agriculture and sewage from small towns, with extremely vulnerable socioeconomic conditions. In contrast, the Paraná river is influenced by several human activities, such as industries, agriculture, livestock and urbanization. In the present study, fish were sampled close to the discharge of two WWTPs, with secondary treatment, in Posadas (Figure 1). This sampling site was specifically located upstream from the Yacyretá impoundment.

2.2 Fish collection

Fish species were selected because they are representative of neotropical communities with ecological relevance and economic importance to the local residents. *Rhamdia quelen* (Heptapteridae) is a bottom dwelling catfish that feeds on detritus, invertebrates and small fish. *Hypostomus commersoni* (Loricariidae) is an armored bottom-dwelling detritivorous/periphytivorous catfish. *Hoplias lacerdae* (Erythrinidae) or thararira, is a

higher trophic level piscivorous predators. *Prochilodus lineatus* (Prochilodontidae) or streaked prochilod is a strict detritivor, previously identified as a useful bioindicator of aquatic pollution (Colombo et al., 2011; Pérez et al., 2018; Santos-Silva et al., 2018).

Sampling of the two study systems occurred at midday and samples from each river were collected on consecutive days in May 2016. General water conditions, including pH, water temperature, turbidity (NTU), dissolved oxygen (mg/L), water conductivity (μS/cm) and total suspended solids (TSS, mg/L), were measured at each sampling site at midday using a calibrated multiparametric probe Horiba U-52 (Kyoto, Japan). All fish were then collected following standard fishing procedures with multi filament gillnet and transported frozen to the laboratory. Muscle, liver and gills tissues were dissected, wrapped in pre-cleaned aluminum foil and stored at -20 °C until analyses. A total of thirty (n=30) samples from the four study species were analyzed. Detailed information regarding biological characteristics of these fish is provided in the electronic supplementary material (Table S1).

2.3 Chemicals

All chemicals and their corresponding isotopically-labeled analogs were obtained from various vendors. Acetaminophen, acetaminophen-*d*4, amitriptyline, amitriptyline-*d*3, aripiprazole, aripiprazole-*d*8, benzoylecgonine, benzoylecgonine-*d*3, buprenorphine, buprenorphine-*d*4, caffeine, carbamazepine, carbamazepine-*d*10, diltiazem, diphenhydramine, diphenhydramine-*d*3, duloxetine, duloxetine-*d*3, fluoxetine, fluoxetine-*d*6, ketamine, ketamine-*d3*, methylphenidate, methylphenidate-*d*9, norfluoxetine, norfluoxetine-*d*6, promethazine, promethazine-*d*3, and sertraline were purchased as certified analytical standards from Cerilliant (Round Rock, TX, USA).

Amlodipine, amlodipine-*d*4, caffeine-*d*9, desmethylsertraline, desmethylsertraline-*d*4, diclofenac, diclofenac-*d*4, diltiazem-*d*3, erythromycin-*d3*, propranolol, propranolol-*d7*, sertraline-*d*3, sulfadimethoxine-*d4*, sulfathiazole-*d4*, sulfamethoxazole, sulfamethoxazole-*d4*, trimethoprim, and trimethoprim-*d9* were purchased from Toronto Research Chemicals (Toronto, ON, CA). Erythromycin, norfloxacin, norfloxacin-*d5*, sulfadimethoxine, sulfathiazole and sucralose were purchased from Sigma-Aldrich (St. Louis, MO, USA) and sucralose-*d*6 was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). All chemicals were reagent grade and used as received. HPLC grade methanol (MeOH) and methyl tert-butyl ether (MTBE) were obtained from Fisher Scientific (Fair Lawn, NJ, USA), formic acid was purchased from Sigma-Aldrich (St. Louis, MO, USA), and a Thermo Barnstead™ Nanopure™ (Dubuque, IA, USA) Diamond UV water purification system was used throughout sample analysis to provide 18 MΩ water.

2.4. Fish tissue extraction

Tissue samples were extracted following previously reported methods (Du et al., 2012; Ramirez et al., 2009) with some modification. Briefly, muscle, liver and gills homogenates, using 1 g wet weight, were prepared and placed in a 20 mL borosilicate glass vial (Wheaton; VWRScientific, Rockwood, TN, USA). Next, 50µL of 2000 µg/LISS was spiked in each sample. Then, 4 mL of MeOH and 4 mL of aqueous 0.1 M acetic acid (pH 4.0) were added to the sample vials. Vials were inverted by hand for 30 seconds to mix the contents prior to placement on a rotating table at 15 rpm for 25 minutes. After mixing, samples were centrifuged at 7500 rpm for 45 minutes. Following centrifugation, supernatant was collected and blown to dryness under a gentle stream of nitrogen in a Turbovap (Zynmark, Hopkinton, MA, USA) set of 45°C, then

reconstituted to 1 mL of mobile phase, with 5:95 (v/v) MeOH:aqueous 0.1% formic acid. All reconstituted samples were syringe filtered using a BD 1 mL TB syringe (BD, Franklin Lakes, NJ, USA) and Acrodisc® hydrophobic Teflon Supermembrane syringe filters (13 mm diameter, 0.2 μm pore size, Pall Corporation, Port Washington, NY, USA) and placed in 2 mL analytical vials (Agilent Technologies, Santa Clara, CA, USA) for analysis.

2.5 Instrumental analysis

Samples were analyzed using isotope-dilution liquid chromatography-tandem mass spectrometry (LC-MS/MS), with an Agilent Infinity 1260 autosampler/quaternary pumping system Agilent jet stream thermal gradient electrospray ionization source (ESI), and model 6420 triple quadrupole mass analyzer (Agilent Technologies, Santa Clara, CA, USA). A binary gradient method consisting of aqueous 0.1 % formic acid as solvent A, and MeOH as solvent B, was used (Table S2). Separation was performed using a 10 cm x 2.1 mm Poroshell 120 SB-AQ column (120Å, 2.7μm, Agilent Technologies, SantaClara, CA, USA) preceded by a 5 mm x 2.1 mm Poroshell 120 SB-C18 attachable guardcolumn (120Å, 2.7 μm, Agilent Technologies, SantaClara, CA, USA). The flow rate was held constant at 0.5 mL/min with a column temperature maintained at 60 °C. The injection volume was 10 μL. Cycle time was adjusted to 500 ms for acquisition of data. Multiple reaction monitoring (MRM) transitions for target analytes and associated instrument parameters can be found elsewhere (Bean et al., 2018; Du et al., 2012; Ramirez et al., 2009).

Quantitation was performed using an isotope dilution calibration method. Calibration standards, containing mixture of internal standards and variable concentrations of target compounds, were prepared in 95:5 0.1% (v/v) aqueous formic acid-methanol. The linear

range for each analyte (0.1-500 ng/mL) was confirmed from plots of sensitivity (i.e., response factor; RF) versus analyte concentration. Our criterion for linearity required that the relative standard deviation of RFs for standards spanning the noted range was ≤ 15%. Internal standard calibration curves were constructed for each analyte using eight standards that were within the corresponding linear range. Calibration data were fit to a linear regression, and correlation coefficients (r^2) for all analytes were ≥ 0.995. Quality assurance and quality control measures included running a continued calibration verification (CCV) sample every five samples to check calibration validity during the run. A criterion of ± 20% of CCV concentration was held to be acceptable for all analytes. One blank, one field blank and duplicate matrix spikes were included in each analytical sample batch. Method detection limits (MDLs) represented the lowest concentration of an analyte reported with 99% confidence that the concentration is different from zero in a given matrix. The EPA guideline (40 CFR Part 136, Appendix B) for generating method detection limits was followed to generate MDLs in fish (Table S2). In the present study <MDL is defined as analytes that were detected in the matrices, but below corresponding MDLs (Table S3).

2.6. Statistical analyses

Concentrations were expressed as μg/kg wet weight (μg/kg wwt). Spearman correlation coefficient was done between sulfonamides and TMP concentrations. Since normality and homocedasticity assumptions were not fulfilled, Wilcoxon test wass used to compare pharmaceutical concentrations between fish sampled in both studied rivers, with significance determined at $p<0.05$. Considering the small size of fish sample, comparisons between species or tissues in each river, were not done. Statistical analyses were performed using the Infostat Software Package (Di Rienzo et al., 2008).

3. Results and discussion

3.1 Water quality parameters

Several parameters (temperature, pH, dissolved oxygen, conductivity) in the Paraná river were higher than measures in the Acaraguá (Table 1). Though TSS was slightly elevated in the Paraná, turbidity was markedly higher in the Acaraguá. Such influences of these suspended materials on sorption and bioavailability of study compounds requires further attention. As noted above, the Acaraguá is located in a mountainous area and its stream temperature was 8 °C lower than the Paraná. Whether this difference increased microbial biotransformation of pharmaceuticals (Eggen and Vogelsang, 2015) in the Paraná river is not known. It is also important to note that, aquatic bioavailability of ionizable contaminants, including the weakly basic pharmaceuticals examined in the present study, is influenced by surface water pH and the dissociation constant (pKa) of each compound (Daughton and Brooks 2011). In our study, surface water of the Acaraguá river was more neutral (pH=7.3) while a slightly alkaline environment was found in the Paraná (pH=8.4, Table 1). These pH values inherently influence differential uptake, bioconcentration, and toxicity of these chemicals in fish (Nakamura et al., 2008; Valenti et al., 2009). For example, Nakamura et al. (2008) observed higher bioconcentration factors of weakly basic fluoxetine and norfluoxetine at pH 9 than pH 7, due to the increase of hydrophobic nonionized species, in Japanese medaka (*Oryzias latipes*). More recent data from our research team expanded earlier uptake modeling with weak acids by Erickson et al. (2006) to identify influences of environmentally relevant pH gradients on inhalational uptake of the weak base diphenhydramine in adult fathead minnows (*Pimephales promeleas*; Nichols et al., 2015). Thus, observed differences in pharmaceutical accumulation between these systems, which are discussed

below, were likely influenced by surface water pH because inhalational uptake is more important than diet in fish for these ionizable bases (Du et al., 2014; Haddad et al., 2018).

3.2 Pharmaceuticals accumulation

As a consequence of the continue discharge of pharmaceuticals into aquatic environments, the organisms are chronically exposed to these pollutants. Fish absorb them via gills, skin and food, while in liver happened biotransformation reactions and the metabolites formed are excreted to intestine via bile (Connors et al., 2013; Nakamura et al., 2008).

To our knowledge, these data represent the first report of these pharmaceuticals, illicit drugs and their metabolites in fish from Argentina, including a protected area identified for its ecological importance in the Misiones province. Of 27 target analytes examined, none were detected in blank samples, while seven were detected in fish tissues (Table 2, Figure 3). The distribution pattern of these CECs presented several differences between both studied rivers. Fish from the Acaraguá river showed higher total pharmaceutical levels than those from the Paraná river, near an urban area (Posadas) (Figure 2). These results suggest that pharmaceutical residues would be released and transported into the ecologically protected area, from the surrounding areas. Although fish from the Paraná river were sampled close to WWTP discharges (14.6 m^3/s), this exposure may have been reduced by higher stream dilution. Interestingly, such observations were made despite lower surface water pH, which decreases bioavailability of bases examined here, in the Acaraguá than the Paraná. Future studies are need to determine whether bioaccumulation differences among organisms and targeted CECs could be also influenced by physiology, habitat preferences, migratory behavior and dietary exposure

differences among species. Previous studies have shown that *P. lineatus* migrates up to 400 km to feed and reproduce in the upper section of the Paraná river, between Yaciretá and Itaipú dams, where these fish were sampled in this work (Bonetto et al., 1981; Sverlij et al., 1993). On the other hand, the species sampled in the Acaraguá river, *Rhamdia quelen* and *Hoplias* spp are resident species (Pouilly et al., 2015; Schulz and Leuchtenberger, 2006), while *H. commersoni* could make short displacements (Alves et al., 2012).

3.3 Caffeine

Caffeine was consistently detected in all species from Acaraguá and Paraná rivers. In fish from the Acaraguá river, caffeine represented up to 91% of the total measured pharmaceutical concentrations, ranging from 1.2 to 13 μg/kg wwt (Table 2, Figure 3). The highest caffeine levels were found in liver (mean: 8.1 μg/kg wwt) followed by gills and muscle (means: 5.7 and 2.2 μg/kg wwt, respectively). Nevertheless, caffeine was mainly identified in muscle of all streaked prochilods from the Paraná river (0.8-6 μg/kg wwt), with the exception of female livers (0.6 μg/kg wwt, Table 2). These caffeine concentrations presented in streaked prochilod were several times lower than fish from the Acaraguá river. These results, similar to previously mentioned for total pharmaceutical levels, would be explained by biological differences among species or environmental conditions. Caffeine is one of the most consumed stimulants around the world including in foods, drinks and medicines, with a global consumption between 80 and 400 mg/person/day, but the mean intake in South America was estimated at 210 mg/person/day (Erikson et al., 2015; Verster and Koenig, 2017). In the environment, caffeine is relatively stable and not completely metabolized, with half-lives between 100

and 240 days (Hillebrand et al., 2012). Therefore, this compound has been used as a tracer of anthropogenic pollution. In Argentina, caffeine is one of the most consumed pharmaceuticals, according to the National Health Service (Elorriaga et al., 2013a). Widespread sources of caffeine in Argentina can be attributed to excretions of medicines, food, energy drinks and soft beverages, coffee and tea, by local consumers. In addition, the yerba mate (*Ilex paraguariensis*) dry leaves are used to prepare the beverage called "mate", which is largely consumed in South America. Yerba mate is a caffeine-rich tree (0.5-0.8 %) with known stimulant properties, which is mainly produced in Misiones province as well as in the southern of Brazil and Paraguay (>300,000 tons/year) (Pérez-Parada et al., 2010). The predominance of caffeine found in all fish sampled provides a representative indication of effluent discharges, and clearly showed its uptake and bioaccumulation in fish from the Misiones province, in agreement with previous studies conducted in our country (Elorriaga et al., 2013 a, b). These previous authors reported the predominance of caffeine (2.6-13 μg/L) in surface water and sewers discharges from different urban areas in the Pampas region in Argentina. Furthermore, Santos-Silva and colleagues (2018) found the effect of caffeine on biotransformation processes in juveniles of streaked prochilod (*P. lineatus*) exposed to 0.3-30 μg/L. It is important to consider that these concentrations are in the same range as those found in other surface water of Argentina (Elorriaga et al., 2013 a, b) and elsewhere (Rodriguez-Gil et al., 2018), and exceed ecotoxicological thresholds (Rodriguez-Gil et al., 2018), indicating that caffeine represents an important current risk to these aquatic ecosystems.

3.4 Antibiotics

In addition to caffeine, several target antibiotics were also detected in fish. Total antibiotic concentrations in fish from the Acaraguá river range from 1.3 to 13.4 μg/kg wwt, with the highest levels in muscle (mean: 9.7 μg/k wwt), followed by gills and liver without significant differences (mean: 2.9 and 3.3 μg/kg wwt, respectively). On the other hand, streaked prochilods from the Paraná river presented total antibiotic levels between 0.7 and 8.1 μg/kg wwt. Specially, higher concentrations were observed in livers of females and mature individuals in which sex could not be determined compared to males, which showed the highest levels in muscle followed by liver and gills (Table 2). These levels were higher than those reported by Kim et al. (2017) in muscle of fish cultured in Korea. The comparison of the total antibiotic concentrations between rivers showed a different tissue distribution which would be attributed to hydrological conditions and specie-specific differences (physiology, trophic position, migration, etc.). Thus, muscle of fish from the Acaraguá river and, liver and gills of streaked prochilods from Paraná river acummulated highest of antibiotics, excluding male prochilods.

In the present study, the total antibiotic concentrations found in fish may be attributed to their widespread administration, with or without prescription. The World Health Organization (2011) reported an urgent need for regulation the medicine use, especially for antibiotics. In Argentina, information about antibiotic use is really scarce but consumption includes over the counter access to antibiotics and self medication, including potential overuse. However, it is important to consider other inputs of antibiotics to the environment, including discharge from production facilities, improper disposal of medicines, effluents, and leachate from landfills. For example, Chung et al. (2018) recently observed several antibiotics to exceed predicted no effect concentrations for the development of antibiotic resistance in leachates from active and closed landfills

in Hong Kong. Further, livestock and aquaculture could be other sources to the spread of antibiotics for aquatic ecosystem. In Misiones province, the aquaculture has increased significantly in the last 5 years, with an average production of 50 T/year (http://www.agro.misiones.gov.ar). Erythromycin, sulfonamides and trimethoprim, which are recommended for use in human medicine, aquaculture and livestock, were the main antibiotics found in fish tissues from both rivers in Misiones province (Figure 3). The US Food and Drug Administration estimates that tons of antibiotics sold for animal use were 4 times higher than those sold for human care (Kupusamy et al., 2018). Considering all these data, our antibiotic profiles suggest an association between the land use (urbanization and production) and the observed aquatic pollution. An acceptable level of antibiotics in fish fillet of <100 μg/kg has been proposed by the European Union Council Regulation (Directive, 2010). In the present study, antibiotic concentrations in muscle not exceed these limits.

3.4.1 Erythromycin

Erythromycin (ERY) was ubiquitous (100% of detection frequency) in all samples from both studied rivers in Misiones province (Figure 3), but its concentrations (0.7-5.6 μg/kg wwt) were lower than sulfonamides and trimethoprim (1.9-4.8 and 2.2-8.8 μg/kg wwt, respectively, Table 2). For fish from Acaraguá river, ERY concentrations ranged from 1 to 2 μg/kg wwt in thararira, between 1.3 and 2.9 in catfish, and from 0.7 to 5.6 in armored catfish (Table 2). Livers of catfish and armored catfish showed the highest ERY levels (1.3 and 5.6 μg/kg wwt, Table 2), which were from 2 until 8 times higher than gills and muscle, respectively. ERY profiles in streaked prochilods were similar among individuals, following the general pattern of muscle>liver>gills across a concentration range of 0.7 to 1.9 μg/kg wwt (Table 2). Similar tissue distribution in

crucian carp (*Carassius auratus*) was previously reported by Liu et al. (2014). Among the two rivers studied, the major differences in ERY concentrations were displayed by liver and gills of armored catfish and streaked prochilods (5 and 2.4 fold majors, respectively). Though, ERY is a common over-the-counter medication, its accumulation in fish from Argentina has not been analyzed in previous studies. ERY concentrations were considerably higher in freshwater fish from more populated areas in China (Gao et al., 2012; Liu et al., 2014). Du and colleagues (2016) also reported that ERY was frequently detected in several fish species from Buffalo Bayou, Texas, USA. Thus, ERY concentration found in the armored catfish *Hypostomus plecostomus* in the Texas study were in the same order of magnitude than those found in *Hypostomus commersoni* sampled inside the protected area in our study.

As a result of the wide occurrence of ERY and concerns for antibiotic resistance it was included in the Drinking Water Contaminant Candidate List (CCL3, US-EPA) and was identified as a "priority monitoring substance" by the European Union (Barbosa et al., 2016). In Latin America, there is not current legislation with ERY limits to protect ecosystems or human health, therefore, more data is necessary, particularly given limited information for this substance in Latin America (Schafhauser et al., 2018).

3.4.2 Sulfonamides and trimethoprim

Sulfonamide antibiotics are increasingly prescribed for human and veterinary infections, alone or in combination with trimethoprim (TMP). Sulfonamides and TMP are broad spectrum antibiotics that block the folic acid metabolism and produce synergistic antibacterial activity (Bedor et al., 2008). In fish from studied rivers, sulfonamide and TMP concentrations (1.9-12.7 μg/kg wwt) were higher than ERY (Table 2). In fish from the Acaraguá river, the combined concentrations of sulfamethoxazole (SUL) and

sulfathiazole (STZ) was 3.9 μg/kg wwt, while TMP was only found in muscle of catfish and armored catfish (Table 2). Sulfonamides concentrations were similar among tissues in females and males of streaked prochilod from the Paraná river, while liver of mature individuals of indeterminate sex contained elevated sulfas levels compared to gills. However, TMP residues (range 2.2-5.5 μg/kg wwt) were found in liver of females and only observed in muscle of males. Interestingly, gills of streaked prochilods presented TMP levels 2.5 higher than its liver concentrations (Table 2). Similar to sulfonamides, TMP levels in fish from Misiones province were higher than or similar to those reported in liver and/or muscle of freshwater wild fish and marine cultured fish from China (Chen et al., 2015; Zhao et al., 2015).

Sulfonamides and TMP are frequently coadministered in human medicine. Sulfonamides +TMP were only observed in muscle of catfish and armored catfish from the protected area, reaching up to 12.7 μg/kg wwt in muscle (94.9% of the total antibiotics levels). Compared to the protected area, sulfonamides+TMP were the principal antibiotics found in streaked prochilods compared to ERY (Figure 3), with the highest levels in liver and gills of mature indeterminate sex individuals and in muscle of males. However, the major contribution of total antibiotics were found in gills (92%).In the present study, we found a positive relationship between SUL and TMP concentrations in fish ($r=0.82$, $p<0.05$), which could be attributed to their prescription together in human care. Here again, the spread of antibiotic resistance genes (ARG) in microorganisms makes the presence of sulfonamides, trimethoprim and other antibiotics, an issue of environmental concern (Kümmerer, 2009), particularly in Latin America and other rapidly growing regions with limited wastewater infrastructure (Schafhauser et al., 2018, Kelly and Brooks 2018). Present results clearly pointed out

the ubiquitous presence of antibiotics and the need of further studies in order to establish their transport, fate and potential adverse effects.

3.5 Antidepressants

The primary antidepressant or metabolite observed in fish was norfluoxetine (NOR-FLX) (Figure 3), which is the primary metabolite of fluoxetine (FLX), and is more lipophilic, more readily crosses the blood brain barrier and more potent at blocking serotonin reuptake transporters than the parent compound (Hiemke and Härtter, 2000). In fish from the Acaraguá river, NOR-FLX concentrations ranged between 1.1 and 9.1 μg/kg wwt (Figure 3). Armored catfish was the only species that accumulated NOR-FLX residues in all tissues, accounting for 7%, 48% and 35% of total pharmaceutical levels in muscle, liver and gills, respectively, while catfish accumulated NOR-FLX in muscle and liver (Table 2, Figure 3). In both species, the highest NOR-FLX concentrations were found in liver which were 8 and 2.4 fold higher than those found in muscle, respectively. Conversely, NOR-FLX residues were mainly observed in muscle of all streaked prochilods from the Paraná river (0.9-3.9 μg/kg w wt, Table 2). Among the four species studied, NOR-FLX concentrations in liver were higher compared to muscle, with the exception of female´ streaked prochilod. These results suggest slow depuration of NOR-FLX in fish (Brooks et al., 2005; Connors et al., 2013). Paterson and Metcalfe (2008) showed the transformation of FLX to NOR-FLX in fish, until 30 days after exposure. In addition, limited biotransformation of FLX to NOR-FLX in vitro studies with rainbow trout suggested elevated potential for bioaccumulation of these substances by fish (Connors et al., 2013).

A number of neuroactive medicines (e.g., diazepam, fluoxetine, sertraline, carbamazepine) have been detected in waters, sediments and fish from Argentina,

Brazil, Spain, China and USA (Beretta et al., 2014; Brooks, 2014; da Silva et al., 2011; Du et al., 2014; Kim et al., 2017; Valdes et al., 2014). Compared to black rockfish muscle from a farm in China (0.8 μg/kg wwt, Kim et al., 2017), our results showed NOR-FLX concentrations 2 and 3 times higher. Fish from Pecan Creek, an effluent-dependent river in Texas, USA, accumulated NOR-FLX in liver and muscle to concentrations similar to our study (Brooks et al., 2005).In Argentina, antidepressants are widely consumed by modern society, sometimes without prescription or co-administrated with other medications. Nevertheless, information regarding the occurrence and fate of antidepressant in non-target organisms is limited. A laboratory study carried out with the freshwater fish *Cichlasoma dimerus* exposed to FLX suggested that this drug affects the reproductive physiology as a mild endocrine disrupting compound (Dorelle et al., 2017). Additional research by our team and others has identified behavioral consequences associated with exposure to selective serotonin reuptake inhibitors (Brooks, 2014). Such observations highlight the importance of understanding environmental risks of antidepressants, particularly in Latin America and other urbanization regions.

3.6 Illicit drugs

Despite the ubiquitous occurrence of pharmaceuticals in Argentinean surface waters, assessment of illicit drugs and their metabolites are very limited. To the best of our knowledge, this is the first report of these compounds in Argentina rivers. Since 2010, cocaine use has been rising in South America, where Argentina represents the main cocaine transit country of this region (UNODC, 2015, 2016). Our results showed the occurrence of benzoylecgonine (BEN), a primary cocaine metabolite, in muscle of thararira and gills of catfish from the Acaraguá river, as well as in liver and gills of

females of streaked prochilod from the Paraná river (Table 2, Figure 3). Cocaine and its metabolites are commonly found in effluents, because these compounds are not completely removed in WWTP (Archer et al., 2017; Mastroianni et al., 2017; McCall et al., 2016; Rosi-Marshall et al., 2015). Parolini et al. (2018) reported modification in protein profile, lipid and energy metabolism and expression of vitellogenin in zebrafish (*Danio rerio*) embryos exposed to environmental concentrations of cocaine and its metabolites. Though we did not compare results from bioaccumulation in wild fish from Argentina to worldwide reports due to the lack of data, it is important to note the need to develop an advanced understanding of environmental risks in future studies.

4. Conclusions

The bioaccumulation of pharmaceuticals in fish from Argentina and other parts of Latin America is poorly understood. The current study fills an information gap in this region. We observed select pharmaceuticals and their biologically active metabolites, including antibiotics and caffeine, in wild fish sampled inside a protected area. In Argentina, only 50% of the population has wastewater treatment infrastructure, including mainly primary and secondary treatment capacity, which results in considerable discharge of raw-sewage to water resources. Taking this into account, together with the increased population growth, urbanization and associated use of pharmaceuticals, it is important that environmental management of expanded sewage systems be implemented. Bioaccumulation data should be incorporated in monitoring programs and national legislation to the protection of non-target organisms and human health. Moreover, adequate actions for conservation of protected areas are needed. Additional studies are warranted to evaluate the broader extent of environmental pollution and biological adverse effects of pharmaceutical mixtures in surface waters of Latin America.

Acknowledgements

This study was done by grants of CONICET PIP Nº 0596/2013 and ANPCyT PICT Nº 2160/2015. This study has been partly supported by Baylor University. Authors wish to thank the Fundación Bosques Nativos Argentinos para la Biodiversidad (N RS 1538), Ministerio de Ecología y Recursos Renovables de la provincia de Misiones, for logistical support.

References

Alves, A.L., de Borba, R.S., Oliveira, C., Nirchio, M., Granado, A., Foresti, F., 2012. Karyotypic diversity and evolutionary trends in the neotropical catfish genus *Hypostomus lacépède*, 1803 (Teleostei, Siluriformes, Loricariidae). Comp. Cytogenet. http://dx.doi.org/10.3897/CompCytogen.v6i4.4028.

Archer, E., Petrie, B., Kasprzyk-Hordern, B., Wolfaardt, G., 2017. The fate of pharmaceuticals and personal care products (PPCPs), endocrine disrupting contaminants (EDCs), metabolites and illicit drugs in a WWTW and environmental waters. Chemosphere 174, 437-446.

Avigliano, E., Schenone, N., 2015. Human health risk assessment and environmental distribution of trace elements, glyphosate, fecal coliform and total coliform in Atlantic Rain forest mountain rivers (South America). Microchem. J. 122, 149-158.

Barbosa, M., Moreira, N., Ribeiro, A., Pereira, M., Silva, A., 2016. Occurrence and removal of organic micropollutants: An overview of the watch list of EU Decision 2015/495. Water Res. 94, 257-279.

Bean, T.G., Rattner, B.A., Lazarus, R.S., Day, D.D., Burket, S.R., Brooks, B.W., et al., 2018. Pharmaceuticals in water, fish and osprey nestlings in Delaware River and Bay. Environ. Pollut. 232, 533-545.

Bedor, D.C.G., Gonçalves, T.M., Ferreira, M.L.L., de Sousa, C.E.M., Menezes, A.L., Oliveira, E.J., et al., 2008. Simultaneous determination of sulfamethoxazole and trimethoprim in biological fluids for high-throughput analysis: Comparison of HPLC with ultraviolet and tandem mass spectrometric detection. J. Chromatogr. Part B 863, 46-54

Beretta, M., Britto, V., Tavares, T., Teixeira, S., Pletsch, A., 2014. Occurrence of pharmaceutical and personal care products (PPCPs) in marine sediments in the Todos os Santos Bay and the north coast of Salvador, Bahia, Brazil. J. Soils Sediments 14, 1278-1286.

Bonetto, A.A., Canon Veron, M., Roldán, D., 1981. Nuevos aportes al conocimiento de las migraciones de peces en el río Paraná. Ecosur 8, 29-40.

Boxall, A.B.A., Rudd, M., Brooks, B.W., Caldwell, D., Choi, K., Hickmann, S., et al., 2012. Pharmaceuticals and personal care products in the environment: What are the big questions? Environ. Health Perspec. 120, 1221-1229.

Brooks, B.W., 2014. Fish on Prozac (and Zoloft): Ten years later. Aq. Toxicol. 151, 61-67.

Brooks, B.W., Chambliss, C., Stanley, J., Ramirez, A., Banks, K., Johson, R., et al., 2005. Determination of select antidepressants in fish from an effluent-dominated stream. Environ. Toxicol. Chem. 24, 464-469.

Cazenave, J., Bacchetta, C., Rossi, A., Ale, A., Campana, M., Parma, M.J., 2014. Deleterious effects of wastewater on the health status of fish: A field caging study. Ecol. Indic. 38, 104-112.

Chen, H., Liu, S., Xu, X., Liu, S., Zhou, G., Sun, K., et al., 2015. Antibiotics in typical marine aquaculture farms surrounding Hailing Island, South China: Occurrence, bioaccumulation and human dietary exposure. Mar. Pollut. Bull. 90, 181-187.

Chung, S.S., Zheng, J.S., Burket, S.R., Brooks, B.W., 2018. Select antibiotics in leachate from closed and active landfills exceed thresholds for antibiotic resistance development. Environ. Int. 115, 89-96.

Colombo, J.C., Cappelletti, N., Williamson, M., Migoya, M., 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.

Connors, K., Du, B., Fitzsimmons, P., Hoffman, A., Chambliss, K., Nichols, J., et al., 2013. Comparative pharmaceutical metabolism by rainbow trout (*Oncorhynchus mykiss*) liver S9 fractions. Environ. Toxicol. Chem. 32, 1810-1818.

da Silva, B.F., Jelic, A., López-Serna, R., Mozeto, A., Petrovic, M., Barceló, D., 2011. Occurrence and distribution of pharmaceuticals in surface water, suspended solids and sediments of the Ebro river basin, Spain. Chemosphere 85, 1331-1339.

Daughton, C.G., 2013. Pharmaceuticals in the environment: sources and their management. In: Petrovic, M., Barcelo, D., Pérez, S. (Eds.), Analysis, removal, effects and risk of pharmaceuticals in the water cycle. Comprehensive Analytical Chemistry. Elsevier, New York, pp. 37-69.

Daughton, C.G., Brooks, B.W., 2011. Active pharmaceuticals ingredients and aquatic organisms. In: Meador, J., Beyer, N. (Eds.), Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations. 2nd Ed. Taylor and Francis, pp. 281-341.

Di Rienzo, J., Casanoves, F., Balzarini, M., Gonzalez, L., Tablada, M., Robledo, C., 2008. InfoStat versión 2008, Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina.

Directive, 2010. Directive 037/10/EEC of the European Union Council Regulation. Off. J. Eur. Union, L15, 1-80.

Dorelle, L., Da Cuña, R., Rey Vazquez, G., Höcht, C., Shimizu, A., Genovese, G., et al., 2017. The SSRI fluoxetine exhibits mild effects on the reproductive axis in the cichlid fish *Cichlasoma dimerus* (Teleostei, Cichliformes). Chemosphere 171, 370-378.

Du, B., Perez-Hurtado, P., Brooks, B.W., Chambliss, C.K., 2012. Evaluation of an isotope dilution liquid chromatography tandem mass spectrometry method for pharmaceuticals in fish. J. Chromatogr. Part A 1253, 177-183.

Du, B., Haddad, S., Luek, A., Scott, W., Saari, G., Kristifco, L., et al., 2014. Bioaccumulation and trophic dilution of human pharmaceuticals across trophic positions of an effluent-dependent wadeable stream. Phill. Trans. R Soc. B 369, 20140058

Du, B., Haddad, S., Luek, A., Scott, W., Saari, G., Burket, B., et al., 2016. Bioaccumulation of human pharmaceuticals in fish across habitats of a tidally influenced urban Bayou. Environ. Toxicol. Chem. 35, 966-974.

Eggen, T., Vogelsang, C., 2015. Occurrence and fate of pharmaceuticals and personal care products in wastewater. Comp. Analyt. Chem. 67, 245-297.

Elorriaga, Y., Marino, D.J., Carriquiriborde, P., Ronco, A.E., 2013a. Screening of pharmaceuticals in surface water bodies of the Pampas region of Argentina. Int. J. Environ. Health. 6, 330-339.

Elorriaga, Y., Marino, D.J., Carriquiriborde, P., Ronco, A.E., 2013b. Human pharmaceuticals in wastewaters from urbanized areas of Argentina. Bull. Environ. Contam. Toxicol. 90, 397-400.

Environmental Protection Agency (EPA). Drinking Water Contaminant Candidate List 3. Federal Register 74(194):51850-62.

Erickson, R.J., McKim, J.M., Lien, G.J., Hoffman, A.D., Batterman, S.L., 2006. Uptake and elimination of ionizable organic chemicals at fish gills: I. Model formulation, parameterization, and behavior. Environ. Toxicol. Chem. 25, 1512-1521.

Erikson, Q., Kulikov, S., Garner-O'Neale, L., 2015. Caffeine in surface and wastewaters in Barbados, West Indies. Springer Plus 4, 57-68.

Evgenidou, E., Konstantinou, I., Lambropoulou, D., 2015. Occurrence and removal of transformation products of PPCPs and illicit drugs in wastewaters: A review. Sci. Total Environ. 505, 905-926.

Furley, T.H., Brodeur, J.C,. Silva de Assis, H.C., Carriquiriborde, P., Chagas, K.R., Corrales, J., et al., 2018. Towards Sustainable Environmental Quality: Identifying Priority Research Questions for Latin America. Integr. Environ. Assess. Manag. 14, 344-357.

Gao, L., Shi, Y., Li, W., Liu, J., Cai, Y., 2012. Occurrence, distribution and bioaccumulation of antibiotics in the Haihe River in China. J. Environ. Monit. 14, 1248-1255.

Grabicova, K., Grabic, R., Blaha, M., Kumar, V., Cerveny, D., Fedorova, G., et al., 2015. Presence of pharmaceuticals in benthic fauna living in a small stream affected by effluent from a municipal sewage treatment plant. Water Res. 72, 145-153.

Haddad, S.P., Luek, A., Scott, W.C., Saari, G.N., Burket, S.R., Kristofco, L.A., et al., 2018. Spatio-temporal bioaccumulation and trophic transfer of ionizable pharmaceuticals in a semi-arid urban river influenced by snowmelt. J. Hazard. Mater. 359, 231-240.

Hillebrand, O., Nödler, K., Licha, T., Sauter, M., Geyer, T., 2012. Caffeine as an indicator for the quantification of untreated wastewater in karts systems. Water Res.

46, 395-402. Hiemke, C., Härtter, S., 2000. Pharmacokinetics of selective serotonin reuptake inhibitors. Pharmacol. Ther. 85, 11-28.

http://www.agro.misiones.gov.ar Acceded May 21, 2018

Izquierdo, A., De Angelo, C., Aide, M.T., 2008. Thirty years of human demography and land-use change in the Atlantic Forest of Misiones, Argentina: an evaluation of the forest transition model. Ecol. Soc. 13, 3-20.

Kelly, K.R., Brooks, B.W., 2018. Global aquatic hazard assessment of ciprofloxacin: Exceedances of antibiotic resistance development and ecotoxicological thresholds. Prog. Mol. Biol. Transl. Sci. In press. https://doi.org/10.1016/bs.pmbts.2018.07.004

Kim, H., Lee, I., Oh, J., 2017. Human and veterinary pharmaceuticals in the marine environment including fish farms in Korea. Sci. Total Environ. 579, 940-949.

Kookana, R.S., Williams, M., Boxall, A.B., Larsson, D.J., Gaw, S., Choi, K., et al., 2014. Potential ecological footprints of active pharmaceutical ingredients: an examination of risk factors in low-, middle- and high- income countries. Phill. Trans. R Soc. Part B 369, 20130586.

Kümmerer, K., 2009. Antibiotics in the aquatic environment-A review-Part I. Chemosphere 75, 417-434.

Kupusamy, S., Kakarla, D., Venkateswarlu, K., Megharaj, M., Yoon, Y., Lee, Y., 2018. Veterinary antibiotics (VAs) contamination as a global agro-ecological issue: A critical view. Agric. Ecosyst. Environ. 257, 47-59.

Liu, J., Lu, G., Ding, J., Zhang, Z., Wang, Y., 2014. Tissue distribution, bioconcentration, metabolism, and effects of erythromycin in crucian carp (*Carassius auratus*). Sci. Total Environ. 490, 914-920.

Nakamura, Y., Yamamoto, H., Sekizawa, S., Kondo, T., Hirai, N., Tatarazako, N., 2008. The effects of pH on fluoxetine in Japanese medaka (*Oryzias latipes*): Acute toxicity in fish larvae and bioaccumulation in juvenile fish. Chemosphere 70, 865-873.

Nichols, J.W., Du, B., Berninger, J.P., Connors, K.A., Chambliss, C.K., Erickson, R., et al., 2015. Observed and modeled effects of pH on bioconcentration of diphenhydramine, a weakly basic pharmaceutical, in fathead minnows. Environ. Toxicol. Chem. 34, 1425-1435.

Mastroianni, N., López-García, E., Postigo, C., Barceló, D., López de Alda, M., 2017. Five-year monitoring of 19 illicit and legal substances of abuse at the inlet of a wastewater treatment plant in Barcelona (NE Spain) and estimation of drug consumption patterns and trends. Sci. Total Environ. 609, 916-926.

McCall, A.K., Bade, R., Kinyua, J., Lai, F.Y., Thai, P.K., Covaci, A., et al., 2016. Critical review on the stability of illicit drugs in sewers and wastewater samples. Water Res. 88, 933-947.

Parolini, M., Bini, L., Magni, S., Rizzo, A., Ghilardi, A., Landi, C., et al., 2018. Exposure to cocaine and its main metabolites altered the protein profile of zebrafish embryos. Environ. Pollut. 232, 603-614.

Paterson, G., Metclafe, C., 2008. Uptake and depuration of the anti-depressant fluoxetine by the Japanese medaka (*Oryzias latipes*). Chemosphere 74, 125-130.

Pérez, M.R., Rossi, A., Bacchetta, C., Elorriaga, Y., Carriquiriborde, P., Cazenave, J., 2018. In situ evaluation of the toxicological impact of a wastewater effluent on the fish *Prochilodus lineatus*: biochemical and histological assessment. Ecol. Indic. 84, 345-353.

Pérez-Parada, A., González, J., Pareja, L., Geis-Asteggiante, L., Colazzo, M., Niell, S., et al., 2010. Transfer of pesticides to the brew during mate drinking process and their relationship with physicochemical properties. J. Environ. Sci. Health B 45, 796-803.

Pouilly, M., Point, D., Sondag, F., Henry, M., Santos, R.V, 2014. Geographical origin of amazonian freshwater fishes fingerprinted by $^{(87)}Sr/^{(86)}Sr$ ratios on fish otoliths and scales. Environ. Sci. Technol. 48, 8980-8987.

Ramirez, A.J., Brain, R., Usenko, S., Mottaleb, M.A., O'Donnell, J., Stahl, L., et al., 2009. Occurrence of pharmaceuticals and personal care products in fish: results of a national pilot study in the United States. Environ. Toxicol. Chem. 28, 2587-2597.

Rodríguez-Gil, J., Cáceres, N., Dafouz, R., Valcárcel, Y., 2018. Caffeine and paraxanthine in aquatic systems: Global exposure distributions andprobabilistic risk assessment. Sci. Total Environ. 612, 1058-1071.

Rosi-Marshall, E., Snow, D., Bartelt-Hunt, S., Paspalof, A., Tank, J., 2015. A review of ecological effects and environmental fate of illicit drugs in aquatic ecosystems. J. Hazard Mater. 282, 18-25.

Rudd, M.A., Ankley, G.T., Boxall, A.B.A., Brooks, B.W., 2014. International scientists' research priorities for pharmaceuticals and personal care products in the environment. Integr. Environ. Assess. Manag. 10, 576-587.

Santos-Silva, T., Montagner, C., Martinez, C.B., 2018. Evaluation of caffeine effects on biochemical and genotoxic biomarkers in the neotropical freshwater teleost *Prochilodus lineatus*. Environ. Toxicol. Pharmacol. 58, 237-242.

Schafhauser, B., Kristofco, L., de Oliveira, C., Brooks, B.W., 2018. Global review and analysis of erythromycin in the environment: Occurrence, bioaccumulation and antibiotic resistance hazards. Environ. Pollut. 238, 440-451.

Schulz, U.H., Leuchtenberger, C., 2006. Activity patterns of South American silver catfish (*Rhamdia quelen*). Braz. J. Biol.

Sverlij, S.B., Espinach Ros, A., Orti, G., 1993. Sinopsis de los datos biológicos y pesqueros del sábalo *Prochilodus lineatus* (Valenciennes, 1847). Organización de las Naciones Unidas para la Agricultura y Alimentación. FAO Sinopsis sobre la Pesca N°154. FIR/S 154. SAST-Sábalo 1,38(01)001,15.

Valdés, M.E., Amé, M.V., Bistoni, M.A., Wunderlin, D.A., 2014. Occurrence and bioaccumulation of pharmaceuticals in a fish species inhabiting the Suquía River basin (Córdoba, Argentina). Sci. Total Environ. 472, 389-396.

Valdés, M.E., Marino, D.J., Wunderlin, D.A., Somoza, G., Ronco, A., Carriquiriborde, P., 2015. Screening concentration of E1, E2 and EE2 in sewage effluents and surface waters of the ‘‘Pampas’’ region and the ‘‘Río de la Plata’’ Estuary (Argentina). Bull. Environ. Contam. Toxicol. 94, 29-33.

Valdés, M.E., Huerta, B., Wunderlin, D.A., Bistoni, M.A., Barceló, D., Rodriguez-Mozaz, S., 2016. Bioaccumulation and bioconcentration of carbamazepine and other pharmaceuticals in fish under field and controlled laboratory experiments. Evidences of carbamazepine metabolization by fish. Sci. Total Environ. 557-558, 58-67.

Valenti, T.W., Perez Hurtado, P., Chambliss, C.K., Brooks, B.W., 2009. Aquatic toxicity of sertraline to Pimephalespromelas at environmentally relevant surface water pH. Environ. Toxicol. Chem. 28, 2685-2694.

Verster, J.C., Koenig, J., 2017. Caffeine intake and its sources: A review of national representative studies. Crit. Rev. Food Sci. Nutr.

UNODC, 2015. United Nations Office on Drugs and Crime World Drug Report. http://www.unodc.org/documents/wdr2015/World_Drug_Report_2015.pdf e-ISBN 978-92-1-057300-9.

UNODC, 2016. United Nations Office on Drugs and Crime World Drug Report. http://www.unodc.org/doc/wdr2016/WORLD_DRUG_REPORT_2016_web.pdf

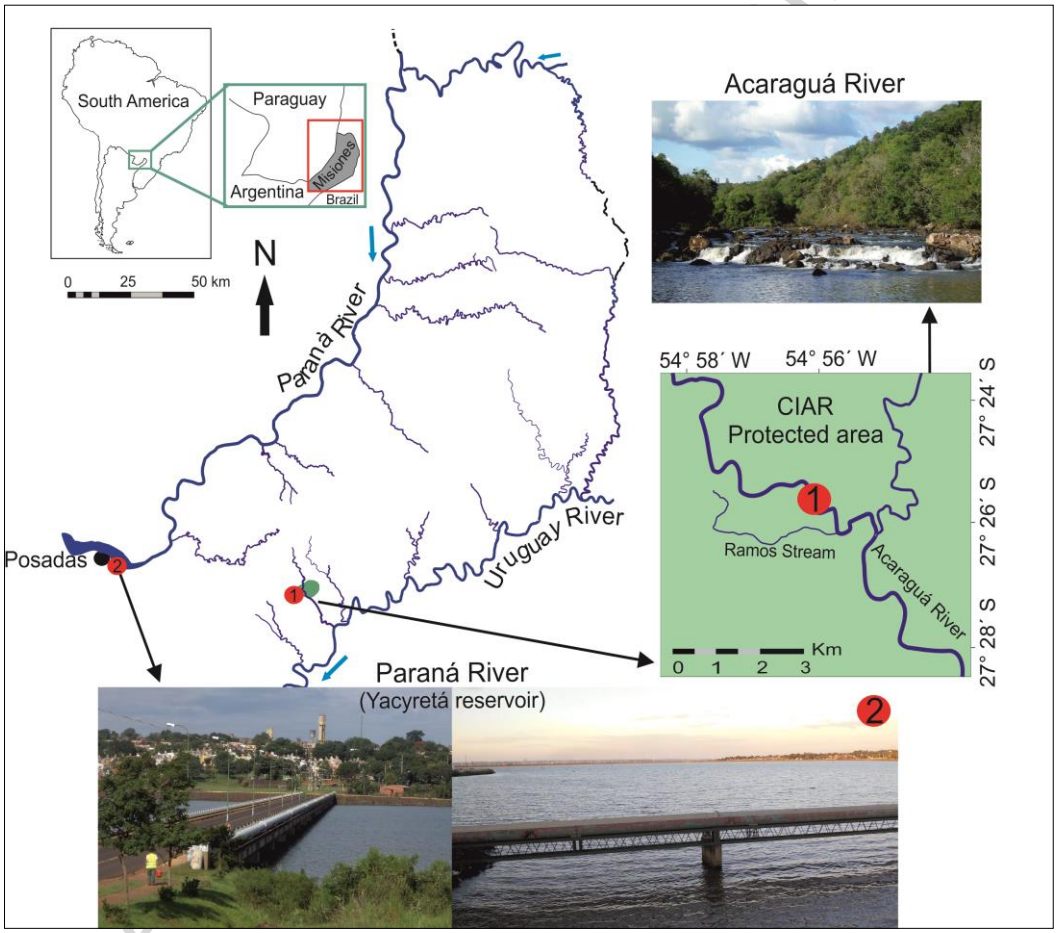
World Health Organization, 2011. Combat drug resistance: no action today means no cure tomorrow. http://www.who.int/mediacentre/news/statements/2011/whd_20110407/en/ Acceded May 21, 2018

WWAP (United Nations World Water Assessment Programme). 2017. The United Nations World Water Development Report 2017: Wastewater, The Untapped Resource. Paris, UNESCO.

Zhao, J., Liu, Y., Liu, W., Jiang, Y., Su, H., Zhang, Q., et al., 2015. Tissue-specific bioaccumulation of human and veterinary antibiotics in bile, plasma, liver and muscle tissues of wild fish from a highly urbanized region. Environ. Pollut. 198, 15-24.

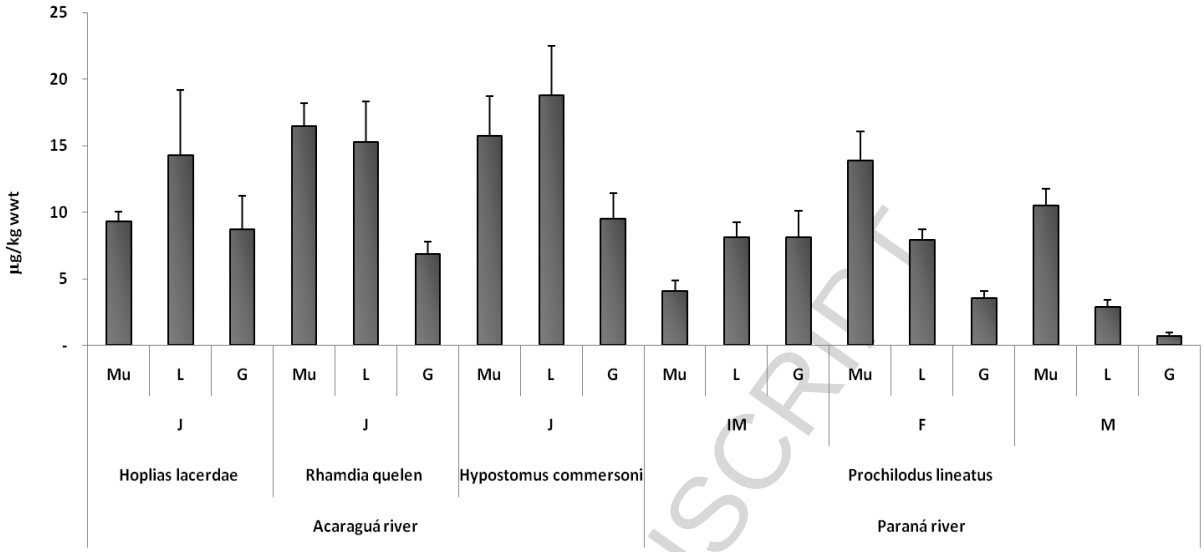
Figures

Figure 1. Map of the study area in the Misiones province of Argentina.



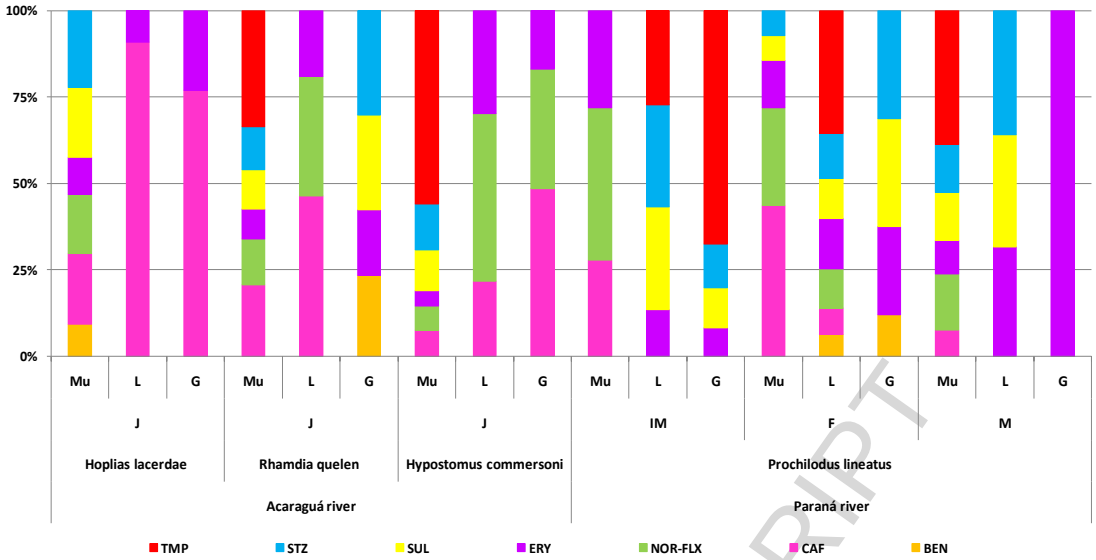
(1) Acaraguá river and (2) Paraná river. CIAR: Protected area "Antonia Ramos Research Center". Posadas: Posadas city.

Figure 2. Total pharmaceutical concentrations in different tissues of four fish species from two rivers in the Misiones province of Argentina (mean ± SD, μg/kg wet weight).



Mu: muscle. L: liver. G: gills. J: juvenile. IM: mature indeterminate. F: female. M: male. Total pharmaceutical levels= Σ(benzoylecgonine, caffeine, norfluoxetine, erythromycin, sulfathiazole, sulfamethoxazole and trimethoprim).

Figure 3. Percentage distribution of pharmaceuticals different tissues of four fish species from two rivers in the Misiones province of Argentina (mean ± SD, μg/kg wet weight).



Mu: muscle. L: liver. G: gills. J: juvenile. IM: mature indeterminate. F: female. M: male. TMP: trimethoprim. STZ: sulfathiazole.SUL: sulfamethoxazole. ERY: erythromycin. NOR-FLX: norfluoxetine. CAF: caffeine. BEN: benzoylecgonine.

Table 1. Water quality parameters in the Acaraguá and Paraná rivers of Argentina during May 2016.

Parameters	Acaraguá River	Paraná River
pH	7.3	8.4
Temperature (ºC)	14.5	22.9
Turbidity (NTU)	11.3	3.9
Dissolved oxygen (g/L)	8.3	10.06
Conductivity (μS/cm)	0.034	0.064
Total suspended solids (mg/L)	0.02	0.042

Table 2. Concentration of pharmaceuticals and their metabolites in different tissues of four fish species from two rivers in the Misiones province of Argentina (mean ± SD, μg/kg wet weight).

				Human tracer/ Stimulant	Psychoactive drugs		Antibiotics				
					Stimulant	Antidepressant					
	Species name	Sex	Tissue	CAF	BEN[#]	NOR-FLX[#]	ERY	SUL	STZ	TMP	Total
Acaraguá river	Thararira (*Hoplias lacerdae*)	J	Muscle	1.90	0.87	1.60	1.00	1.87	2.07	<MDL	4.94±0.95
			Liver	13.00	<MDL	<MDL	1.30	<MDL	<MDL	<MDL	1.30±0.65
			Gills	6.70	<MDL	<MDL	2.00	<MDL	<MDL	<MDL	2.00±1.00
	Catfish (*Rhamdia quelen*)	J	Muscle	3.40	<MDL	2.20	1.40	1.87	2.07	5.50	10.84±1.88
			Liver	7.10	<MDL	5.30	2.90	<MDL	<MDL	<MDL	2.90±1.45
			Gills	<MDL	1.60	<MDL	1.30	1.87	2.07	<MDL	5.24±0.93
	Armored catfish (*Hypostomus commersoni*)	J	Muscle	1.20	<MDL	1.10	0.69	1.87	2.07	8.80	13.43±3.68
			Liver	4.10	<MDL	9.10	5.60	<MDL	<MDL	<MDL	5.60±2.80
			Gills	4.60	<MDL	3.30	1.60	<MDL	<MDL	<MDL	1.60±0.80
Paraná river	Streaked prochilod (*Prochilodus lineatus*)	IM	Muscle	1.14±0.52	<MDL	1.80±0.14	1.15±0.07	<MDL	<MDL	<MDL	1.15±0.58
			Liver	<MDL	<MDL	<MDL	1.10±0.28	2.40±3.39	2.43±3.29	2.20±3.11	8.10±0.62
			Gills	<MDL	<MDL	<MDL	0.68±0.32	0.94±1.32	1.14±1.45	5.50±7.78	8.15±2.31
		F	Muscle	6.05±7.00	<MDL	3.90±2.97	1.90±1.41	1.00±1.41	1.00±1.11	<MDL	3.90±0.78
			Liver	0.60±0.85	0.50±0.71	0.90±1.27	1.15±0.35	0.94±0.35	1.00±1.40	2.80±3.86	5.92±0.88

			Gills	<MDL	0.42±0.59	<MDL	0.90±0.01	1.10±0.01	1.10±1.56	<MDL	3.10±0.53	
		M	Muscle	0.80±0.17	<MDL	1.70±0.28	1.02±0.11	1.45±2.01	1.45±2.05	4.05±3.61	7.97±1.39	
			Liver	<MDL	<MDL	<MDL	0.92±0.26	0.94±1.32	1.04±1.46	<MDL	2.89±0.48	
			Gills	<MDL	<MDL	<MDL	0.71±0.27	<MDL	<MDL	<MDL	0.71±0.27	

J: juvenile. IM: mature indeterminate. F: female. M: male.BEN: benzoylecgonine. CAF: caffeine, NOR-FLX: norfluoxetine, ERY: erythromycin, SUL: sulfamethoxazole, STZ: sulfathiazole,TMP: trimethoprim.Total antibiotics= Σ(ERY, SUL, STZ, TMP). #Metabolites.MDL: method detection limit.

Highlights

Fish from a protected area accumulated licit and illicit drugs and their metabolites

Caffeine and antibiotics were the major pharmaceuticals observed in fish

The macrolide erythromycin was ubiquitous in its first report in fish from Argentina

First identification of illicit drugs in fish from South America

Urban influences on aquatic contamination of protected areas require future attention

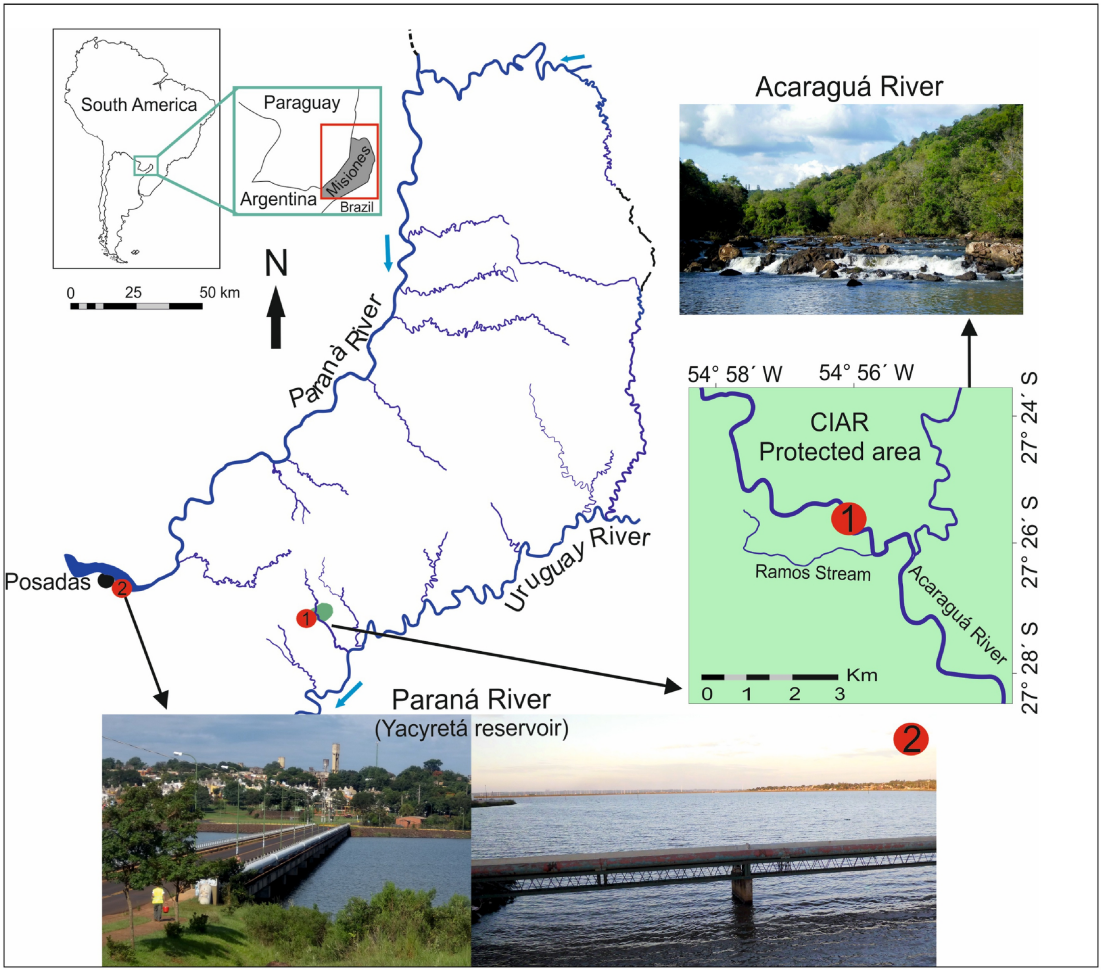

South America
Paraguay
Argentina
Brazil
Misiones
0 25 50 km
N
Paraná River
Uruguay River
Posadas
2
1
Acaraguá River
54° 58´ W
54° 56´ W
27° 24´ S
27° 26´ S
27° 28´ S
CIAR
Protected area
1
Ramos Stream
Acaraguá River
Km
0 1 2 3
Paraná River
(Yacyretá reservoir)
2

Figure 1

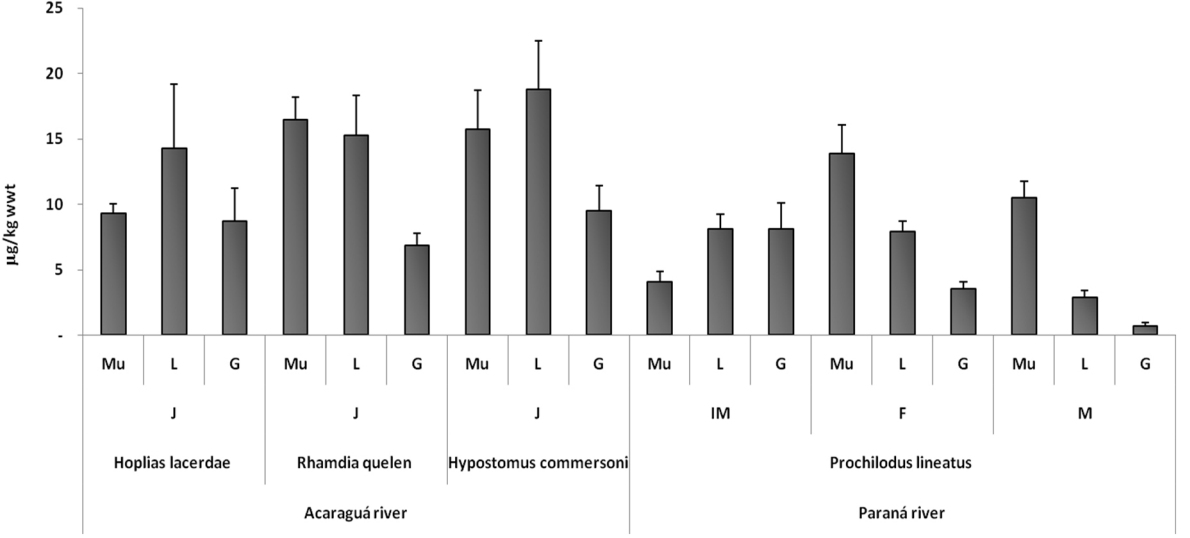

25
20
15
10
5
-
µg/kg wwt
Mu
L
G
J
IM
F
M
Hoplias lacerdae
Rhamdia quelen
Hypostomus commersoni
Prochilodus lineatus
Acaraguá river
Paraná river

Figure 2

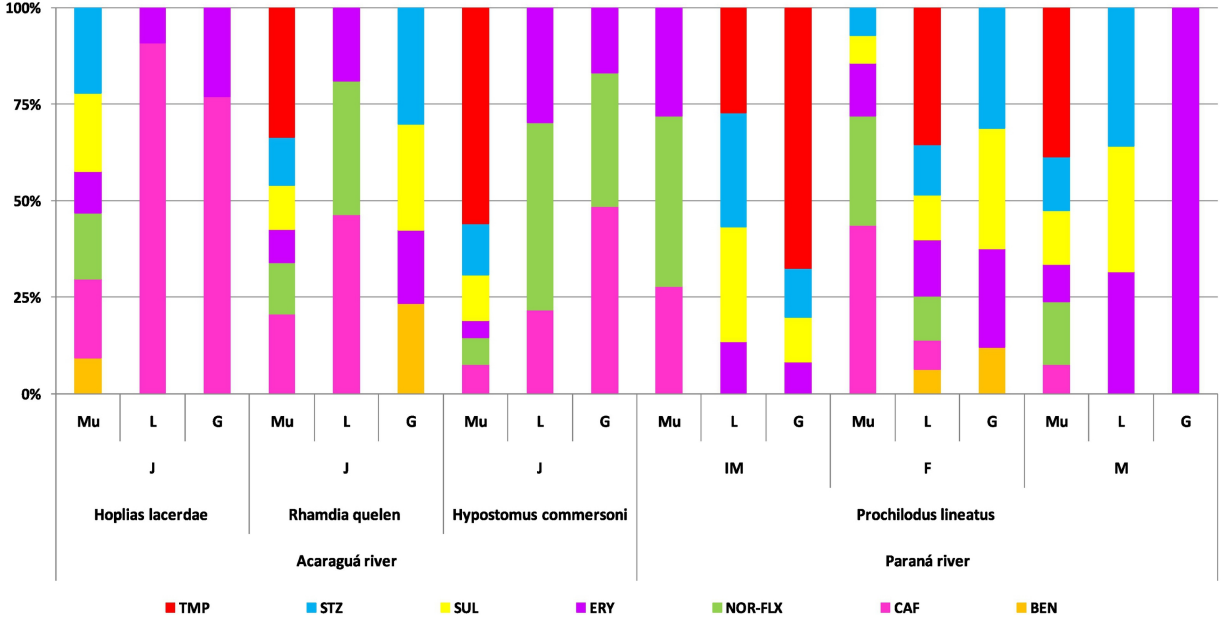

100%
75%
50%
25%
0%
Mu
L
G
J
IM
F
M
Hoplias lacerdae
Rhamdia quelen
Hypostomus commersoni
Prochilodus lineatus
Acaraguá river
Paraná river
TMP
STZ
SUL
ERY
NOR-FLX
CAF
BEN

Figure 3