Human pharmaceuticals in three major fish species from the Uruguay River (South America) with different feeding habits

Rojo M.[1], Álvarez-Muñoz D.[2,3], Dománico A.[4,5,6], Foti R.[4,7], Rodriguez-Mozaz S.[2], Barceló D. [2,3], Carriquiriborde, P.[1,4*]

[1] Centro de Investigaciones del Medio Ambiente (CIMA), Facultad de Ciencias Exactas, Universidad Nacional de La Plata – CONICET, Argentina

[2] Catalan Institute for Water Research (ICRA), Spain

[3] Department of Environmental Chemistry, IDAEA-CSIC, Spain

[4] Comisión Administradora del Río Uruguay (CARU)

[5] Dirección de Pesca Continental- Subsecretaría de Pesca y Acuicultura de la Nación, Argentina.

[6] Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CIC), Argentina

[7] Dirección Nacional de Recursos Acuáticos, Ministerio de Agricultura, Ganadería y Pesca del Uruguay, Constituyente 1497 - Montevideo, Uruguay.

Corresponding author: Dr. Pedro Carriquiriborde, Centro de Investigaciones del Medio Ambiente (CIMA), Facultad de Ciencias Exactas, Universidad Nacional de La Plata – CONICET, Argentina. Calle 47 y 115, s/n 1900 La Plata Buenos Aires, Argentina, Tel/fax: +54 221 4229329. E-mail: pcarriquiriborde@gmail.com

Abstract

The accumulation of 17 human pharmaceuticals (HPs) was investigated in the muscle of three fish species characteristic of the "Rio de la Plata Basin" with different feeding habits and of relevance for human consumption: *Megaleporinus obtusidens*, *Salminus brasiliensis,* and *Prochilodus lineatus*. Fish were sampled in fall and spring from 8 localities distributed along 500 Km of the Uruguay River. Atenolol and carbamazepine were the most frequently detected HPs (>50%), but at concentrations always below 1 µg/Kg wet weight (w/w). Hydrochlorothiazide, metoprolol, venlafaxine, propranolol, codeine, and the carbamazepine metabolite, 2-hydroxycarbamazepine, were accumulated at higher levels showing maximum concentrations between 1 and 10 µg/Kg (w/w), but infrequently (<50%). The other HPs were always below 1 µg/Kg (w/w) and at frequencies lower than 50%. Distinctive accumulation patterns were observed among species at different trophic levels. However, biomagnification trends were not identified for any compound. The highest number and concentration of HPs were found in *M. obtusidens* (omnivorous), followed by *P. lineatus* (detritivorous), and lastly *S. brasiliensis* (piscivorous). The most recurrent HPs (i.e. carbamazepine and atenolol) were present in all species, but others exclusively in one. Geographical variations were only found for carbamazepine and atenolol in *M. obtusidens* and *P. lineatus*, showing higher concentrations in localities closer to the Rio de la Plata estuary. Differences in the HPs concentrations among seasons were not identified. Acceptable daily intake and predicted no effect concentrations would indicate that measured muscle concentrations in fish from the Uruguay River do not pose a serious risk for human consumption nowadays. Further studies will be necessary for assessing the potential adverse effects on studied fish species.

Capsule: Human pharmaceuticals in native fish from the Uruguay River, Rio de la Plata Basin, South America.

Keywords: “emerging pollutants”, “Neotropical fish”, “Rio de la Plata Basin”, “biomagnification”, “health risk”

1. Introduction

Human pharmaceuticals (HPs) are bioactive substances designed to be used in the diagnosis, cure, mitigation, treatment, or prevention of disease, and have significantly contributed to the rise in quality of life and life expectancy. In addition, a significant amount of studies on animals and humans are provided by the pharmaceutical industry during the registration process to minimize potential adverse effects on human and environmental health. However, concern still exists about pharmaceuticals in the environment. Environmental fate of veterinary and human pharmaceuticals after fecal and urinary excretions are quite different. Veterinary drugs are more likely to directly contaminate soil and groundwater and eventually, reach surface waters indirectly through run-off. On the other hand, HPs (and their metabolites) are mainly discharged to the sewage, pass through wastewater treatment plants (when existing), and if they are not efficiently removed, directly discharged into surface waters (Khetan and Collins, 2007). Therefore, the environmental risks of HPs are mainly expected on the aquatic ecosystems. Moreover, due to their widespread use and continual input to the environment, HPs have been classified as "pseudo-persistent" pollutants (Daughton, 2003). Several studies all around the world have demonstrated that HPs are able to reach aquatic ecosystems, showing concentrations in surface waters usually ranging from 1 to 10,000 ng/L (Fent et al., 2006). In addition, important differences in the type and environmental concentrations of pharmaceuticals were observed across different regions of the world due to a different pattern of prescription and use, sewage connectivity and treatment, and receiving environment characteristics (Kookana et al., 2014). Previous studies performed in Argentina have identified that carbamazepine, atenolol, diclofenac, and ibuprofen are among the most prescribed HPs and they have been ubiquitously detected in wastewaters and surface waters (Elorriaga et al., 2013a; Elorriaga et al., 2013b; Valdés et al., 2014).

Since the first report of (Brooks et al. (2005)), several other studies have demonstrated that

HPs are also able to be uptake and accumulated by the aquatic biota (Huerta et al., 2012). Therefore, to understand the bioaccumulation patterns of pharmaceuticals was stated as a priority research question regarding the effects of pharmaceuticals on the ecosystem health (Boxall et al., 2012). In particular, fish is one of the most conspicuous communities in the aquatic ecosystems, presenting diverse living strategies and interplaying regulation roles. In addition, some species are of economic relevance as game fish or for commercial fisheries (Lynch et al., 2016). Bioaccumulation of pharmaceuticals in fish could not only affect the organisms that are directly exposed to the chemicals but also pose risks to their predators and even humans. Although most of the previous studies seem to indicate that biomagnification of most pharmaceuticals through the trophic web is not significant, selective accumulation was observed across species (Arnnok et al., 2017; Du et al., 2014). Within the Neotropical region, only a few studies have assessed the accumulation of pharmaceuticals in fish of some rivers of Argentina. A large number of HP's was identified in seasonal samplings of three small fish species along the Suquía River, in Cordoba Province (Valdés et al., 2016). In addition, not only HP's but also illicit drugs were found in fish collected in a grab sampling conducted simultaneously in the Paraná (close to Posadas City) and Acaraguá River, in Misiones Province (Ondarza et al., 2019). A good review was recently published by Llorca et al. (2017), indicating that information is still scarce for the region. In particular, regional scale assessment on HP's accumulation in fish along major South American rivers and its potential biomagnification through different trophic levels of the Neotropical fish communities is not currently available.

In comparison with North America and Europe, large rivers of South America still hold a highly diverse fish community. However, unplanned development and demographic growth together with deficient connectivity and wastewater treatment systems are threatening their biological integrity. The Uruguay River is a major river (after the Parana-Paraguay River) of

105 the Rio de la Plata Basin, the second largest drainage basin of South America (after the
106 Amazon basin). With a drainage basin area of 365,000 km^2 and a total length of 1838 km the
107 Uruguay River start in the south of Brazil, at the Serra do Mar, and empties into the Río de la
108 Plata at Punta Gorda. The River average discharge is 5500 m^3/s. The lower sector is part of
109 the international border between Argentina and Brazil, and Argentina and Uruguay. Ichthyo-
110 geographically, the Uruguay River belong to the Guayano-Brazilian Region, and more
111 specifically to the Parano-Platense province, and has a rich fish diversity with more than 150
112 described fish species (CARU, 1996). In particular, Sábalo (*Prochilodus lineatus*), Boga
113 (*Megaleporinus obtusidens*) and Dorado (*Salminus brasiliensis*) are three relevant species for
114 the River ecosystem, not only because of their biomass and biological role, but also their
115 relevance for commercial and game fishing. In addition, different tropic niches are occupied
116 by these species; while Sábalo is a detritivorous fish, Boga is omnivorous and Dorado
117 piscivorous (Burress et al., 2013). On the other hand, several medium-sized cities (population
118 higher than 10000 people) are quickly developing along the Uruguay River and its
119 wastewaters are still discharged untreated or poorly treated directly to the river. Therefore,
120 concern has risen in the Uruguay River Binational Commission (CARU) about the potential
121 impacts of emerging contaminants on the river ecosystem and living resources.
122 The aim of the present study was: i) to recognize the most frequently accumulated HPs in
123 Uruguay River fish species, ii) to analyze the accumulation patterns among species with
124 different feeding habits, looking for potential biomagnification processes, iii) to detect
125 changes in the accumulation of HPs in relation to geographical and seasonal variations and iv)
126 to assess the potential risk for human health, linked to fish consumption, and the risk for the
127 fish health.
128

129 **2. Materials and Methods**

130 *2.1. Selected HP's, area of study and fish sampling*

131 The 17 studied HP's were selected on the basis of the prescription information and the
132 occurrence in surface waters and fish, previously reported for the region (Elorriaga et al.,
133 2013b; Valdés et al., 2014; Valdés et al., 2016). In addition, accumulation of a similar list of
134 HP's was studied in fish from Europe and North America (Huerta et al., 2013; Huerta et al.,
135 2018), allowing intercontinental comparisons. Finally, the list was bounded by analytical
136 capabilities. The list of assessed HP's included six β-blockers: atenolol (ATE), carazolol
137 (CAR), metoprolol (MET), nadolol (NAD), propranolol (PRO) and sotalol (SOT); four
138 psychiatric drugs: diazepam (DIA), lorazepam (LOR), and carbamazepine (CBZ), including
139 two of its metabolites: 10,11-epoxycarbamazepine (EPO-CBZ) and 2-hydroxycarbamazepine
140 (OH-CBZ), and venlafaxine (VEN); one antiplatelet agent: clopidogrel (CLO); one drug to
141 treat asthma: salbutamol (SAL); two analgesics/anti-inflammatories: codeine (COD) and
142 diclofenac (DIC); and one diuretic: hydrochlorothiazide (HCT).

143 Fish sampling was conducted within the framework of the Fish and Fisheries Conservation
144 Program of the CARU. Two campaigns (fall and spring) were conducted in 2016 including 8
145 sampling localities distributed along approximately 500 Km of the Uruguay River, under the
146 CARU jurisdiction (Figure 1). Due to logistic limitations, sampling localities were not exactly
147 the same in the two campaign, but alternative sites were representative of the same sector of
148 the River. Localities sampled in the fall campaign were: Bella Union, Arapey, Puerto Yeruá,
149 San Salvador, and Villa Paranacito 1. During the spring campaign fish were collected from
150 Mocoretá, Gualeguaychú, Concepción del Uruguay and Villa Paranacito 2.

151 Three fish species of ecologic and economic relevance in the Uruguay River were selected for
152 the present study. All selected species belonged to the Characiformes systematic group, one
153 of the most representative phylogenetic orders of the River: i) Sabalo (*P. lineatus*,
154 Valenciennes, 1836) a detritivorous fish (TL_{max}: 40-60 cm, W_{max}: 5-6 kg) represents the

155 highest biomass of the River ecosystem and is the most important species for commercial
156 fisheries, ii) Boga (*M. obtusidens*, Valenciennes, 1847) is an omnivorous fish (TL_{max}: 40-100
157 cm, W_{max}: 9-10 kg), and also abundant species, very appreciated by local fishermen and
158 recreational fishing too, and iii) Dorado (*S. brasiliensis*, Cuvier, 1816) is a strictly piscivorous
159 fish (TL_{max}: 130 cm, W_{max}: 34 kg) highly valued as game fish. Fish were captured using
160 different fishing gears: gillnets, trawls, and long-lines. Gears were placed during the night and
161 collected at first light in the morning. Fish were held in ice until reach the camp and then
162 quickly processed. Dorsal muscles of both sides were dissected (removing the skin) with
163 stainless-steel instruments, wrapped separately in foil and labeled respectively. They were
164 finally placed in food-grade polyethylene bags and stored at -20 °C, then they were transferred
165 to the laboratory and kept at -80 °C until processing. A total of 94 fish were collected and
166 processed in the two campaigns and 8 sampling localities: *M. obtusidens* = 32, *S. brasiliensis*
167 = 32, and *P. lineatus* = 30 (Supplemental Table 1).
168
169 *2.2. Standards and reagents*
170 High purity grade (>95%) pharmaceutical standards were acquired from Sigma–Aldrich,
171 except VEN, purchased from the European Pharmacopeia (EP) and MET obtained from the
172 US Pharmacopeia (USP). Metabolites OH-CBZ and EPO-CBZ were purchased from Toronto
173 Research Chemicals (TRC). Isotopically labeled compounds, used as internal standards,
174 diazepam-d5, ronidazole-d3, and fluoxetine-d5 were acquired from Sigma–Aldrich. Atenolol-
175 d7, carbamazepine-d10, hydrochlorothiazide-d2, and citalopram-d4 were purchased from
176 CDN isotopes. Venlafaxine-d6 was from TRC. Sulfadoxine-d3 and ketoprofen-d3, used as
177 surrogate standards, were purchased from CDN isotopes. HPLC grade methanol, water,
178 dichloromethane, and acetonitrile were purchased from Merck (Darmstadt, Germany).
179

180 *2.3. Samples processing and chemical analysis*

181 Muscle sample from 3 to 5 fish of the same species, sampling site and season were pooled and
182 homogenized using a stainless-steel meat grinder at 12,000 rpm, obtaining a total of 27
183 composite samples, 9 per species (Supplemental Table 1). The *L. obtusidens* pooled sample
184 collected in Gualeguaychú during spring was accidentally lost during analytical preparation.
185 Following, 10 g of each composite sample were freeze-dried and kept at -20 °C until analysis.
186 Extraction, purification, and analysis of samples were conducted following analytical
187 methodology by (Huerta et al. (2013)). Briefly, three subsamples 1 gr dry weight (d.w.) were
188 spiked with the isotope-labeled pharmaceutical mixture at 20 µg/kg and a surrogate standard
189 mixture of sulfadoxine-d3 and ketoprofen-d3 at 1 mg/kg. Sample extraction was conducted
190 by pressurized liquid extraction (PLE) using an ASE 350® (Thermo Scientific Dionex).
191 Further sample purification was done by gel permeation chromatography (GPC) using an
192 Agilent 1260 Infinity high-pressure liquid chromatography (HPLC) system. Extracts were
193 analyzed in a Waters Acquity Ultra-Performance® Liquid Chromatography (UPLC) system
194 coupled to a SCIEX QTRAP® 5500 hybrid triple quadrupole-linear ion trap mass
195 spectrometer (Applied Biosystems). Chromatographic separation for analytes in positive
196 electrospray ionization mode (PI) was achieved in an Acquity HSS T3 column (50 mm x
197 2.1mm i.d., 1.8 µm particle size) using methanol and 10 Mm formic acid/ammonium formate
198 (pH 3.2) as mobile phase at a flow rate of 0.5 ml/min. On the other hand, an Acquity BEH
199 C18 column (50 mm x 2.1 mm i.d., 1.7 µm particle size) was used for analytes in negative
200 electrospray ionization mode (NI), using acetonitrile and 5 Mm ammonium acetate/ ammonia
201 (pH 8) at a flow rate of 0.6 ml/min. The volume of injection was 5 µl in both cases. Mass
202 spectrometer parameters under PI and NI were the set as described in Huerta et al. (2013).
203 Two SRM transitions were monitored for the target compounds; the first one was used for
204 quantification and the second for identity confirmation. The relative abundance of the two

SRM in the sample was also compared with those in the standards and must be within 20% of the two SRM ratios in the analytical (Gros et al., 2012). For laboratory quality assurance, recovery, matrix effect assessment and limit of detection and quantifications of the method calculations, spike samples with a mix of pharmaceuticals stable isotope labeled standards, extract addition with surrogate standards of sulfadoxine-d3 and ketoprofen-d3 were used (Huerta et al., 2013). Lipid content was determined gravimetrically after removed from the extract by gel permeation chromatography (Ondarza et al., 2011). Humidity percentage was calculated from the weight of each pooled sampled before and after freeze-drying, and used to correct the concentration values obtained from quantification of HPs in freeze-dried samples as follow: wet weight = dry weight/humidity % x 100. Concentrations are expressed as µg/kg (w/w) along with the whole document.

2.4. Data analysis

HPs were grouped in four classes: Class I (maximum concentrations >1 µg/kg (w/w) and detection frequencies >50%), Class II (maximum concentrations <1 µg/kg (w/w) and detection frequencies >50%), Class III (maximum concentrations >1 µg/kg (w/w) and detection frequencies <50%) and Class IV (maximum concentrations <1 µg/kg (w/w) and detection frequencies <50%). Data are presented as the mean ± standard error. In some cases, also de median is shown. When measurements were below the method detection limit (MDL) or within this and the method quantification limit (MQL) the "Simple Substitution Method" approach was followed (Helsel and Hirsch, 1992), using the proxies MDL/2 and (MQL-MDL)/2, respectively. The total HPs load (THP) was used as a comparative estimator of the degree of contamination of each sample. It was calculated as the sum of those measurements above the MDL only, substituting MQL by the proxy (MQL-MDL)/2. The one-way analysis of variance (ANOVA) and the Student's t-tests were used for testing the statistical differences

among species and seasons, respectively, after the log transformation of the concentrations for normalization. Normality and homoscedasticity were tested using the Kolmogorov-Smirnov and Levene's tests, respectively. *Post-hoc* comparisons were done using the Fisher's Least Significant Difference (LSD) test. In the scatterplot of *pKa* vs. accumulation, it was plotted the *pKa* value of each HP closer to the average River pH (7.2). Pearson's correlation was used to assess the relationship between HP's accumulation and $LogP_{ow}$ and *pKa*. In all cases, the critical *p*-value was 0.05. The moving average was used for describing the variation of the HPs concentration in fish muscle with the latitude of sampling locality.

3. Results and discussion

3.1. Method performance

The method proved to be suitable for analyzing 17 HPs in the muscle of the three studied Uruguay River fish species (Table 1). The performance was similar to the one reported by Huerta et al. (2013), but some slight differences were obtained for the recoveries and detection limits of some analytes compared with those obtained in the previous study for *Cyprinus carpio, Barbus graellsii* and *Silurus glanis*. Average recoveries were significantly higher in *P. lineatus* than in *M. obtusidens* and *S. brasiliensis*, indicating differences in the method performance among species. That was in agreement with results previously reported by Huerta et al. (2013). Measured lipid content was 4.2 ± 1.6 % for *M. obtusidens*, 2.6 ± 0.61 % for *P. lineatus*, and 1.0 ± 0.32 % for *S. brasiliensis*. It was not correlated with the recoveries obtained for each species, and therefore, other factors a part of lipid content would explain variations in the method performance. Due to variations obtained among the studied species, the recoveries for each analyte and the MDL and MQL were separately reported (Table 1).

3.2. Occurrence and concentrations of studied HPs

255 The overall occurrence (frequencies and concentrations) of detected HPs in the studied
256 Uruguay River fish species is summarized in Table 2. Average and maximum concentrations
257 ranged between 0.083-1.82 and 0.15-10.2 µg/kg, respectively. In both cases, sotalol and 2-
258 hydroxycarbamazepine were the lowest and upper ends. Frequencies for detected HPs ranged
259 between 4% for carazolol and 92% for carbamazepine. Diclofenac was not detected in any
260 sample. The four groups delimited according to the detection frequency and the maximum
261 concentration are easily visualized in Figure 2 A. No studied pharmaceuticals, or its
262 metabolites, were within Class I (right-up corner), and only a few were among Class III
263 (codeine, venlafaxine, propranolol, metoprolol, hydrochlorothiazide, and the metabolite of
264 carbamazepine, 2-hydroxycarbamazepine). Although this small group of HPs was
265 infrequently detected, they should be considered more prone to be accumulated in fish muscle,
266 since they were the only reaching concentrations above 1 µg/kg. In particular, maximum
267 concentrations above 1 µg/kg for venlafaxine, hydrochlorothiazide and the carbamazepine
268 metabolite were recently reported in fish from the USA (Huerta et al., 2018). On the other
269 hand, only carbamazepine and atenolol were comprised among the Class II, frequently
270 detected, but always at concentrations below 1 µg/kg. That group was in accordance with the
271 high occurrence of carbamazepine and atenolol found in wastewater and surface waters of
272 Argentina (Elorriaga et al., 2013a; Elorriaga et al., 2013b; Valdés et al., 2014). All the other
273 studied pharmaceuticals were among the Class IV, infrequently detected and presenting
274 maximum concentrations below 1 µg/kg.
275 Although a relatively broad range of octanol/water partition coefficient (log P_{ow}) and acid
276 dissociation constants (p*K*a) values were presented by the studied pharmaceuticals (from -0.58
277 to 4.3 and 2.9 to 19.7, respectively), no significant relationships were found between those
278 physicochemical parameters and the maximum concentrations measured in fish muscle
279 (Figure 2 B and C). Therefore, other factors than physicochemical properties alone would be

driving the accumulation of the studied HPs in Uruguay River fish. In addition, HP's occurrence observed in the present study was different from that reported in other studies where the same analytical methodology (and thus the same target compounds) was used. Namely in the case of small fish species gathered in the central region (Suquía River) of Argentina (Valdés et al., 2016) or fish species from Iberian rivers in Spain and in fish collected in rivers and stream from USA (Huerta et al., 2013; Huerta et al., 2018). The comparison with other studies conducted in Germany (Subedi et al., 2012) and United States (Arnnok et al., 2017; Ramirez et al., 2009) was more difficult since targeted HPs differ from one study to the other. However, important variations are expected among regions of the same or different countries. Factors explaining variability among regions of the same country or between different countries are complex and multicausal, but socio-economic factors (regulations, market, consumption-pattern, disposal, sewage treatment, etc.) would play a relevant role (Kookana et al., 2014).

3.3. Trophic preferences and HPs accumulation patterns

Differential accumulation patterns of the studied HPs were found among Uruguay River fish species. This was evidenced by the number, the frequencies and the mean and maximum concentrations for the targeted HPs in each species (Figure 3). The highest number of detected HPs was found in *M. obtusidens* (13/17), followed by *P. lineatus* (10/17) and *S. brasiliensis* (9/17). Some HPs were present in all species (ATE, CBZ, OH-CBZ, PRO, VEN and HCT), while others were only common in two (EPO-CBZ and NAD were shared between *M. obtusidens* and *P. lineatus*, while MET and SOT were shared between *M. obtusidens* and *S. brasiliensis*) or even exclusively found in only one (CLO, SOD, and SAL were only found in *M. obtusidens*, CAR and LOR in *P. lineatus*, and DIA in *S. brasiliensis*). Moreover, the relative contributions of the detected HPs to the total load of the targeted pharmaceuticals

(THPs) were also different among species (Figure 3). For the three studied species, more than half of the THP was only explained by the three more abundant HPs. Those were OH-CBZ, CBZ, and HCT in *P. lineatus*, MET, HCT and PRO in *M. obtusidens*, and MET, CBZ and VEN in *S. brasiliensis*. The contribution of the other measured was much less due to the lower concentrations at which they were detected.

When the average concentration of the most frequent pharmaceuticals (CBZ and ATE) and THPs were compared between species, it was found that the highest concentrations were clearly found in *M. obtusidens*, followed by *P. lineatus* and *S. brasiliensis* presenting the lowest (Figure 4). However, those differences were not statistically significant due to the big dispersion of the values among samples.

Characteristic accumulation patterns of HPs were recently described in the United States for fish with different feeding strategy (Arnnok et al., 2017; Huerta et al., 2018). In the present study, it was expected that the highest concentrations and greater number of HPs are found in *P. lineatus*, since it has been demonstrated this detritivorous fish is attracted by sewage for feeding and is able to accumulate several types of pollutants, such as hydrocarbons and PCBs, frequently found in wastewater discharges of Buenos Aires Metropolitan Area, Argentina (Colombo et al., 2007a; Colombo et al., 2007b). However, results have shown *M. obtusidens* as the most contaminated species regarding the number and concentration of HPs. This omnivorous fish feed on a broad number of items, but in the last decades, it has increasingly preyed on the exotic mussel, *Limnoperna fortunei*. This mussel has become an abundant feeding resource in the Uruguay River, since its accidental introduction in the La Plata River during the ’90s (Penchaszadeh et al., 2000). Recent studies have shown that trophic transfer of HPs can be substantial and that some of them are mostly accumulated by benthic organisms (Lagesson et al., 2016). In particular, it has been found that pharmaceuticals are particularly accumulated at higher levels by mussels (Álvarez-Muñoz et al., 2015). Therefore, the

benthonic food pathway through *L. fortunei* could help to explain the higher levels of HPs found in *M. obtusidens*.

HCT was detected in the three species, however, it was the highest HP accumulated in *M. obtusidens*, showing average concentrations up to 10 times higher than in the others species (Figure 3). According to the feeding habit of these species, uptake of HCT via the benthic pathway through *L. fortunei* could be an important route of exposure. MET was the highest accumulated in *S. brasiliensis*, but also important in *M. obtusidens*. Since *S. brasiliensis* is strictly piscivorous and *M. obtusidens* omnivorous, uptake via small fish and invertebrates could explain the exposure pathway for this HP. In a different way, the accumulation of CBZ and its metabolites were more relevant in *P. lineatus*. This species frequently feeds in areas located nearby wastewater discharges, and therefore, uptake of those compounds could occur more direct from the source. Particularly, CBZ and ATE have been evenly detected in the three fish species at high frequencies. Considering these HPs were reported mostly for the dissolved fraction of surface waters, receiving wastewater discharges, of different regions of Argentina (Elorriaga et al., 2013b), results would suggest they could be primarily accumulated directly from the water.

As mentioned above, the trophic transfer has been proposed as an important pathway explaining field exposure to HPs, and it could help to understand the accumulation pattern found for some HPs studied in fish species from the Uruguay River. However, biomagnification through the food web was not observed for any of the studied HPs, since concentrations in the top predator, *S. brasiliensis*, were always lower than in the omnivorous and detritivorous species. These findings agree with the lack of biomagnification reported in lakes of China (Xie et al., 2017), or even more, the trophic dilution observed in food webs of streams in the USA (Du et al., 2014; Haddad et al., 2018).

355 *3.4. Geographical and seasonal variations of HPs in Uruguay River fish species*

356 Concentrations of the most frequent HPs and the THPs load measured in the muscle of fish
357 gathered in different sampling localities along the Uruguay River are shown in Figure 5. Clear
358 differences were observed for *M. obtusidens* along with sampling sites, showing
359 concentrations of CBZ and ATE, as well as the THPs, markedly higher in the southern sector
360 of the river (downstream), where it discharges into the La Plata River estuary. The same
361 pattern was observed in *P. lineatus* regarding the concentrations of CBZ and ATE. In the case
362 of THPs, higher values were also found in the lower sector, but not in all sites. On the other
363 hand, the accumulation pattern in *S. brasiliensis* was the opposite, presenting the highest CBZ
364 and THPs concentrations up north. However, the geographic gradient was not as clear as the
365 one observed for *M. obtusidens* and *P. lineatus* down south.

366 Despite the southern sector of the Uruguay River is a relatively unpopulated area, the obtained
367 results were not unexpected. The three studied species are big migratory fish of the Río de la
368 Plata Basin, and it is known they seasonally move between the Uruguay River, La Plata River,
369 and Paraná River. A clear gradient in the accumulation of hydrocarbon, linear alkylbenzenes,
370 organochlorine pesticides and polychlorinated biphenyls in *P. lineatus* has been previously
371 described, decreasing from the Southern coast of inner La Plata River (higher concentrations)
372 to the Lower Paraná River (200 to 1000 Km from Buenos Aires) and to the Upper Paraná and
373 Paraguay Rivers (more than 1000 Km from Buenos Aires) (Speranza et al., 2012). Although
374 data about water concentrations of studied HPs are still unavailable for the lower Uruguay
375 River, higher HPs concentrations in *P. lineatus* an *M. obtusidens* in the southern sector could
376 be explained as fish migrating upstream coming from the southern coast of inner La Plata
377 River, a heavily polluted area, receiving raw sewage from most of the Buenos Aires
378 Metropolitan Area, and a place where *P. lineatus*, and probably other species, are attracted for

379 feeding. The different pattern observed in *S. brasiliensis* would indicate a different feeding or
380 migratory behavior in this big predatory fish.
381 Seasonal variations in the concentrations of studied HPs were not conclusive and it was not a
382 general factor affecting all species and sites in the same manner (Supplementary Table 5).
383 Although higher values of THPs were observed for *P. lineatus* and *S. brasiliensis* in the
384 springtime, differences were not statistically significant and a more detailed sampling
385 designed would be necessary for understanding seasonal changes.
386
387 *3.5. The risk for human consumption and fish health.*
388 All three studied species are commonly eaten by local people along the Uruguay River.
389 Moreover, the commercial fishery of *P. lineatus* is very important in the region and fish is
390 mainly exported to the international market. Therefore, concern has arisen about HP's
391 accumulation in fish and the risk for human consumption. Maximum residue limits (MRL) for
392 studied HPs in fish are established neither by international legislation nor by Argentina or
393 Uruguay regulations. Some HPs have been included as veterinary drugs by FAO-WHO
394 (CODEX), USDA or the European Union, but for farm animals (not including aquaculture
395 fish). For example, MRL for diclofenac in bovine and porcine muscle stated by the
396 Commission Regulation 37/2010 (EU) is 5 µg/Kg. Similarly, MRL for carazolol in porcine
397 muscle stated in the CODEX is also 5 µg/Kg. Alternatively, available acceptable daily intake
398 (ADI) values for human health risk assessments of some HPs have been reported by other
399 authors: being 0.4, 2.9, 0.3, 2.0 and 0.2 µg/Kg/day for ATE, CBZ, MET, COD and SAL,
400 respectively (Prosser and Sibley, 2015; Schwab et al., 2005). In addition, the coefficient (F)
401 to calculate the predicted no-effect concentrations (PNEC) linked to fish consumption for
402 children (worst scenario) was estimated using the values published by Schwab et al. (2005) as
403 7.1 x 10^2 day. Therefore, estimated PNEC values for ATE, CBZ, MET, COD, and SAL were

404 calculated as ADI x F: 284, 2059, 213, 1420 and 142 µg/Kg, respectively. All estimated PNEC
405 values were well above the concentrations measured in the muscle of the three Uruguay River
406 fish species, which were always lower than 10 µg/Kg. Therefore, measured HPs
407 concentrations in the muscle of the studied fish from the Uruguay River would not pose a risk
408 for human consumption. Although further studies collecting fish consumption information
409 from local people would be desirable to a more precise assessment, results agree with a recent
410 study conducted in Europe, where the human risk of pharmaceuticals compounds through
411 seafood consumption was considered negligible (Álvarez-Muñoz et al., 2018).
412 Muscle concentrations found in this study have evidenced that in the Uruguay River fish are
413 being exposed to HPs, at different levels depending on the compound. Despite skeletal muscle
414 is not the main target tissue of the studied HPs, it is known that tissue residues are a good
415 estimator of plasma levels, that in turn, are the best dose metric for assessing adverse effects
416 for these chemicals from WWTP (Meador et al., 2017). A significant correlation was found
417 between muscle and plasma concentrations in the fish *Pimephales promelas* waterborne-
418 exposed to the psychoactive drug oxazepam (Meador et al., 2017; Tanoue et al., 2015).
419 Unfortunately, almost nothing is known about internal concentrations and its relationship to
420 adverse effects of studied HPs on Uruguay River fish species. In a recent study, biochemical
421 and histological responses were assessed in *P. lineatus* exposed under field conditions to
422 surface waters receiving a wastewater discharge (Pérez et al., 2018). Although in that study,
423 concentrations ATE and CBZ were detected in the water, they were not assessed in plasma or
424 tissue. Therefore, exposure levels to HPs evidenced in the Uruguay River could be a useful
425 supply of information for further laboratory studies directed to investigate how close, or far,
426 are those muscle concentrations from the effective concentrations triggering adverse health
427 effects on studied fish species.
428

4. Conclusions

The occurrence of HPs was first-time reported in the muscle of three major fish species of the "Río de la Plata" basin collected along the 500 Km of the Uruguay River shared by Argentina and Uruguay. CBZ and ATE were the only HPs showing frequencies of detection above 50%, but concentrations always below 1 μg/Kg. In addition, maximum concentrations for any of the assessed HPs were higher than 10 μg/Kg. Relationships between tissue concentration and selected physicochemical properties of HPs (i.e. Log P_{ow}, pK_a) were not identified, indicating that accumulation would be mainly driven by other factors.

Although some HPs were ubiquitous (e.g. CBZ and ATE) others were characteristically accumulated among species, suggesting that biological habits of fish (i.e. feeding, migratory) would be important factors explaining species-specific accumulation patterns. Biomagnification of none of studied HPs was supported by the accumulation patterns observed among species, since the number of detected HPs and concentrations were always lower in the piscivorous fish (*S. brasiliensis*) and higher in the omnivorous fish (*M. obtusidens*).

The accumulation of HPs in *M. obtusidens* and *P. lineatus* was higher in localities closer to the La Plata River estuary, probably as a consequence of fish moving into the Uruguay River from the Buenos Aires Metropolitan Area. On the other hand, no clear geographical variations were found for *S. brasiliensis*, suggesting a different feeding or migratory behavior. Seasonal changes in the concentrations were not detected along the River for any species or HP.

Concentrations of the HPs measured in the muscle of studied fish species from the Uruguay River were not identified as a risk for human consumption nowadays. On the other hand, little information is available on the sensitivity of Uruguay River fish species to toxic effects of HPs, and therefore, further studies are required for a better understanding of the potential risks of these HPs to these fish and wild organisms inhabiting the River.

Acknowledgments

Macarena Rojo, the sampling and partially the sample analysis were granted by the Fish and Fisheries Program of the Administrative Commission of the Uruguay River (CARU). The study was also granted by CONICET (PIP2012-2014-0090) and ANPCyT (PICT2014-1690). The scientific facilities of ICRA have been co-financed by the Spanish Ministry of Economy and Competitiveness (MINECO) and by the European Regional Development Fund (ERDF) in the frame of the Catalonia ERDF Operative programme 2007-2013. This work was partly supported by the Generalitat de Catalunya (Consolidated Research Group: Catalan Institute for Water Research 2014 SGR 291). S. Rodriguez-Mozaz also acknowledges the Ramon y Cajal program (RYC-2014-16707) and. Diana Álvarez-Muñoz the support of the project XENOMETABOLOMIC (CTM2015-73179-JIN) (AEI/FEDER/UE).

References

Álvarez-Muñoz D., Rodríguez-Mozaz S., Jacobs S., Serra-Compte A., Cáceres N., Sioen I., et al., 2018. Pharmaceuticals and endocrine disruptors in raw and cooked seafood from European market: Concentrations and human exposure levels. Environ. Int.; 119: 570-581.

Álvarez-Muñoz D., Rodríguez-Mozaz S., Maulvault A.L., Tediosi A., Fernández-Tejedor M., Van den Heuvel F., et al., 2015. Occurrence of pharmaceuticals and endocrine disrupting compounds in macroalgaes, bivalves, and fish from coastal areas in Europe. Environmental Research; 143: 56-64.

Arnnok P., Singh R.R., Burakham R., Pérez-Fuentetaja A., Aga D.S., 2017. Selective Uptake and Bioaccumulation of Antidepressants in Fish from Effluent-Impacted Niagara River. Environ. Sci. Technol.; 51: 10652-10662.

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

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

Burress E.D., Duarte A., Gangloff M.M., Siefferman L., 2013. Isotopic trophic guild structure of a diverse subtropical South American fish community. Ecology of Freshwater Fish; 22: 66-72.

CARU, 1996. Peces del Río Uruguay - Guía ilustrada de las especies más comunes del Río Uruguay inferior y el embalse de Salto Grande. Comisión Administradora del Río Uruguay (CARU), www.caru.org.uy/web/pdfs_publicaciones/Peces-del-Rio-Uruguay.pdf.

489 Colombo J.C., Cappelletti N., Migoya M.C., Speranza E., 2007a. Bioaccumulation of
490 anthropogenic contaminants by detritivorous fish in the Río de la Plata estuary: 1-Aliphatic
491 hydrocarbons. Chemosphere; 68: 2128-2135.
492 Colombo J.C., Cappelletti N., Migoya M.C., Speranza E., 2007b. Bioaccumulation of
493 anthropogenic contaminants by detritivorous fish in the Río de la Plata estuary: 2-
494 Polychlorinated biphenyls. Chemosphere; 69: 1253-1260.
495 Daughton C.G., 2003. Cradle-to-cradle stewardship of drugs for minimizing their
496 environmental disposition while promoting human health. I. Rational for and avenues
497 toward a green pharmacy. Environ. Health Perspect.; 111: 757-774.
498 Du B., Haddad S.P., Luek A., Scott W.C., Saari G.N., Kristofco L.A., et al., 2014.
499 Bioaccumulation and trophic dilution of human pharmaceuticals across trophic positions of
500 an effluent-dependent wadeable stream. Philos. Trans. R. Soc. Lond. B Biol. Sci.; 369.
501 Elorriaga Y., Marino D.J., Carriquiriborde P., Ronco A.E., 2013a. Human Pharmaceuticals
502 in Wastewaters from Urbanized Areas of Argentina. Bull. Environ. Contam. Toxicol.; 90:
503 397-400.
504 Elorriaga Y., Marino D.J., Carriquiriborde P., Ronco A.E., 2013b. Screening of
505 pharmaceuticals in surface water bodies of the Pampas region of Argentina. Int. J. Environ.
506 Health; 6: 330-339.
507 Fent K., Weston A.A., Caminada D., 2006. Ecotoxicology of human pharmaceuticals.
508 Aquat. Toxicol.; 76: 122-159.
509 Gros M., Rodríguez-Mozaz S., Barceló D., 2012. Fast and comprehensive multi-residue
510 analysis of a broad range of human and veterinary pharmaceuticals and some of their
511 metabolites in surface and treated waters by ultra-high-performance liquid chromatography
512 coupled to quadrupole-linear ion trap tandem mass spectrometry. J. Chromatogr. A; 1248:
513 104-121.

514 Haddad S.P., Luek A., Scott W.C., Saari G.N., Burket S.R., Kristofco L.A., et al., 2018.
515 Spatio-temporal bioaccumulation and trophic transfer of ionizable pharmaceuticals in a
516 semi-arid urban river influenced by snowmelt. J Hazard Mater; 359: 231-240.
517 Helsel D.R., Hirsch R.M., 1992. Statistical methods in water resources. Statistical methods
518 in water resources.
519 Huerta B., Jakimska A., Gros M., Rodríguez-Mozaz S., Barceló D., 2013. Analysis of multi-
520 class pharmaceuticals in fish tissues by ultra-high-performance liquid chromatography
521 tandem mass spectrometry. J. Chromatogr. A; 1288: 63-72.
522 Huerta B., Rodríguez-Mozaz S., Barceló D., 2012. Pharmaceuticals in biota in the aquatic
523 environment: Analytical methods and environmental implications. Analytical and
524 Bioanalytical Chemistry; 404: 2611-2624.
525 Huerta B., Rodriguez-Mozaz S., Lazorchak J., Barcelo D., Batt A., Wathen J., et al., 2018.
526 Presence of pharmaceuticals in fish collected from urban rivers in the U.S. EPA 2008–
527 2009 National Rivers and Streams Assessment. Sci. Total Environ.; 634: 542-549.
528 Khetan S.K., Collins T.J., 2007. Human Pharmaceuticals in the Aquatic Environment: A
529 Challenge to Green Chemistry. Chemical Reviews; 107: 2319-2364.
530 Kookana R.S., Williams M., Boxall A.B.A., Larsson D.G.J., Gaw S., Choi K., et al., 2014.
531 Potential ecological footprints of active pharmaceutical ingredients: An examination of risk
532 factors in low-, middle- and high-income countries. Philos. Trans. R. Soc. Lond. B Biol.
533 Sci.; 369.
534 Lagesson A., Fahlman J., Brodin T., Fick J., Jonsson M., Byström P., et al., 2016.
535 Bioaccumulation of five pharmaceuticals at multiple trophic levels in an aquatic food web -
536 Insights from a field experiment. Sci. Total Environ.; 568: 208-215.

537 Llorca M., Farré M., Eljarrat E., Díaz-Cruz S., Rodríguez-Mozaz S., Wunderlin D., et al.,
538 2017. Review of emerging contaminants in aquatic biota from Latin America: 2002–2016.
539 Environ. Toxicol. Chem.; 36: 1716-1727.
540 Lynch A.J., Cooke S.J., Deines A.M., Bower S.D., Bunnell D.B., Cowx I.G., et al., 2016.
541 The social, economic, and environmental importance of inland fish and fisheries.
542 Environmental Reviews; 24: 115-121.
543 Meador J.P., Yeh A., Gallagher E.P., 2017. Determining potential adverse effects in marine
544 fish exposed to pharmaceuticals and personal care products with the fish plasma model and
545 whole-body tissue concentrations. Environ. Pollut.; 230: 1018-1029.
546 Ondarza P.M., Gonzalez M., Fillmann G., Miglioranza K.S.B., 2011. Polybrominated
547 diphenyl ethers and organochlorine compound levels in brown trout (*Salmo trutta*) from
548 Andean Patagonia, Argentina. Chemosphere; 83: 1597-1602.
549 Ondarza P.M., Haddad S.P., Avigliano E., Miglioranza K.S.B., Brooks B.W., 2019.
550 Pharmaceuticals, illicit drugs and their metabolites in fish from Argentina: Implications for
551 protected areas influenced by urbanization. Sci. Total Environ.; 649: 1029-1037.
552 Penchaszadeh P.E., Darrigran G., Angulo C., Averbuj A., Brögger M., Dogliotti A., et al.,
553 2000. Predation of the invasive freshwater mussel *Limnoperna fortunei* (Dunker, 1857)
554 (Mytilidae) by the fish *Leporinus obtusidens* Valenciennes, 1846 (Anostomidae) in the Rio
555 de la Plata, Argentina. Journal of Shellfish Research; 19: 229-231.
556 Pérez M.R., Rossi A.S., Bacchetta C., Elorriaga Y., Carriquiriborde P., Cazenave J., 2018. In
557 situ evaluation of the toxicological impact of a wastewater effluent on the fish *Prochilodus*
558 *lineatus*: biochemical and histological assessment. Ecological Indicators; 84: 345-353.
559 Prosser R.S., Sibley P.K., 2015. Human health risk assessment of pharmaceuticals and
560 personal care products in plant tissue due to biosolids and manure amendments, and
561 wastewater irrigation. Environ. Int.; 75: 223-233.

562 Ramirez A.J., Brain R.A., Usenko S., Mottaleb M.A., O'Donnell J.G., Stahl L.L., et al.,
563 2009. Occurrence of pharmaceuticals and personal care products in fish: Results of a
564 national pilot study in the United States. Environ. Toxicol. Chem.; 28: 2587-2597.
565 Schwab B.W., Hayes E.P., Fiori J.M., Mastrocco F.J., Roden N.M., Cragin D., et al., 2005.
566 Human pharmaceuticals in US surface waters: A human health risk assessment. Regulatory
567 Toxicology and Pharmacology; 42: 296-312.
568 Speranza E.D., Cappelletti N., Migoya M.C., Tatone L.M., Colombo J.C., 2012. Migratory
569 behaviour of a dominant detritivorous fish *Prochilodus lineatus* evaluated by multivariate
570 biochemical and pollutant data. Journal of Fish Biology; 81: 848-865.
571 Subedi B., Du B., Chambliss C.K., Koschorreck J., Rüdel H., Quack M., et al., 2012.
572 Occurrence of pharmaceuticals and personal care products in German fish tissue: A
573 national study. Environ. Sci. Technol.; 46: 9047-9054.
574 Tanoue R., Nomiyama K., Nakamura H., Kim J.W., Isobe T., Shinohara R., et al., 2015.
575 Uptake and Tissue Distribution of Pharmaceuticals and Personal Care Products in Wild
576 Fish from Treated-Wastewater-Impacted Streams. Environ. Sci. Technol.; 49: 11649-
577 11658.
578 Valdés M.E., Amé M.V., Bistoni M.D.L.A., Wunderlin D.A., 2014. Occurrence and
579 bioaccumulation of pharmaceuticals in a fish species inhabiting the Suquía River basin
580 (Córdoba, Argentina). Sci. Total Environ.; 472: 389-396.
581 Valdés M.E., Huerta B., Wunderlin D.A., Bistoni M.A., Barceló D., Rodriguez-Mozaz S.,
582 2016. Bioaccumulation and bioconcentration of carbamazepine and other pharmaceuticals
583 in fish under field and controlled laboratory experiments. Evidences of carbamazepine
584 metabolization by fish. Sci. Total Environ.; 557-558: 58-67.

585 Xie Z., Lu G., Yan Z., Liu J., Wang P., Wang Y., 2017. Bioaccumulation and trophic
586 transfer of pharmaceuticals in food webs from a large freshwater lake. Environ. Pollut.;
587 222: 356-366.
588

589 **Tables and Figures**

Table 1. Summary of method performance.

			Prochilodus lineatus			*Megaleporinus obtusidens*			*Salminus brasiliensis*		
	Precursor ion	Product ion	Recovery ± RSD %	MDL (µg/Kg)	MQL	Recovery ± RSD %	MDL (µg/Kg)	MQL	Recovery ± RSD %	MDL (µg/Kg)	MQL
β-blockers											
Atenolol	267 $[M+H]^+$	145	115% ± 10%	0.034	0.11	73% ± 10%	0.032	0.11	91% ± 12%	0.031	0.10
Carazolol	299 $[M+H]^+$	116	26% ± 10%	0.089	0.30	16% ± 4%	0.13	0.42	25% ± 3%	0.073	0.24
Metoprolol	268 $[M+H]^+$	133	104% ± 16%	0.043	0.14	49% ± 10%	0.18	0.60	46% ± 6%	0.080	0.27
Nadolol	310 $[M+H]^+$	254	97% ± 7%	0.048	0.16	50% ± 4%	0.038	0.13	55% ± 5%	0.026	0.087
Propranolol	260 $[M+H]^+$	116	26% ± 5%	0.047	0.16	16% ± 4%	0.097	0.32	34% ± 11%	0.057	0.19
Sotalol	273 $[M+H]^+$	255	119% ± 10%	0.017	0.056	63% ± 3%	0.030	0.10	78% ± 8%	0.024	0.08
Psychiatric drugs											
Carbamazepine	237 $[M+H]^+$	194	110% ± 12%	0.028	0.092	84% ± 8%	0.053	0.18	77% ± 14%	0.036	0.12
10,11-EpoxyCBZ	253 $[M+H]^+$	180	36% ± 7%	0.073	0.24	20% ± 4%	0.11	0.36	20% ± 6%	0.050	0.17
2-HydroxyCBZ	253 $[M+H]^+$	210	52% ± 12%	0.051	0.17	33% ± 4%	0.074	0.25	31% ± 6%	0.019	0.063
Diazepam	285 [M+H]+	193	75% ± 7%	0.024	0.08	61% ± 6%	0.085	0.29	63% ± 11%	0.035	0.12
Lorazepam	321 [M+H]+	275	49% ± 8%	0.10	0.35	43% ± 7%	0.58	1.9	41% ± 6%	0.33	1.1
Venlafaxine	278 [M+H]+	58	88% ± 10%	0.11	0.35	35% ± 2%	0.38	1.25	41% ± 11%	0.08	0.27
Antiplatelet agent											
Clopidogrel	322 $[M+H]^+$	212	27% ± 4%	0.098	0.33	26% ± 6%	0.11	0.37	41% ± 14%	0.049	0.16
To treat asthma											
Salbutamol	240 $[M+H]^+$	148	114% ± 6%	0.042	0.14	80% ± 7%	0.086	0.29	92% ± 12%	0.059	0.19
Analgesics/anti-inflammatories											
Codeine	300 $[M+H]^+$	152	40% ± 5%	0.093	0.31	21% ± 3%	0.13	0.43	36% ± 9%	0.17	0.57
Diclofenac	294 $[M-H]^-$	250	50% ± 17%	2.7	9.1	118% ± 15%	2.1	6.9	76% ± 10%	2.5	8.5
Diuretic											
Hydrochlorothiazide	296 $[M-H]^-$	269	72% ± 7%	0.063	0.21	64% ± 7%	0.062	0.21	56% ± 7%	0.072	0.239

Recoveries are expressed as the mean percentage and relative standard deviation (RSD) (n=3). MDL: Method Detection Limit, MQL: Method Quantification Limit.

Table 2. Overall concentrations and frequencies of the studied human pharmaceuticals in the muscle of the studied fish species from the Uruguay River.

	Mean	*SE*	*Max*	*Freq.*	*Class*
β-blockers					
Atenolol	0.088±	0.024	0.61	62%	II
Carazolol	0.054±	0.0062	0.21	4%	IV
Metoprolol	0.37±	0.13	2.4	38%	III
Nadolol	0.059±	0.020	0.49	27%	IV
Propranolol	0.19±	0.079	1.9	38%	III
Sotalol	0.020±	0.0055	0.15	12%	IV
Psychiatric drug					
Carbamazepine	0.19±	0.038	0.88	92%	II
Diazepam	0.048±	0.0068	0.19	8%	IV
10,11-EpoxyCBZ	0.053±	0.023	0.63	12%	IV
2-HydroxyCBZ	0.44±	0.39	10.2	23%	III
Lorazepam	0.21±	0.030	0.73	8%	IV
Venlafaxine	0.21±	0.061	1.6	23%	III
Antiplatelet agent					
Clopidogrel	0.066±	0.019	0.51	8%	IV
To treat asthma					
Salbutamol	0.082±	0.012	0.28	12%	IV
Analgesics/anti-inflammatories					
Codeine	0.13±	0.057	1.1	12%	III
Diclofenac			ND		
Diuretic					
Hydrochlorothiazide	0.31±	0.23	5.98	31%	III

Concentrations in µg/Kg (w/w). Mean: overall mean for all analyzed samples (values below detection or quantification limits were substituted by MDL/2 or (MQL-MDL)/2, respectively); SE: standard error; Number of samples = 26; Max: maximum concentration, Freq.: frequency of detection; ND: below MDL in all samples;
Class II: frequency above 50% and maximum concentration below 1 µg/Kg (w/w);
Class III: frequency below 50% and maximum concentration above 1 µg/Kg (w/w);
Class IV: frequency below 50% and maximum concentration below 1 µg/Kg (w/w).

591

592 **Figure 1. Map indicating the sampling sites along with the shared sector of the**

593 **Uruguay River between Argentina and Uruguay and showing the whole Uruguay River**

594 **(light gray) and Rio de la Plata (dark grey) basins.**

595

Figure 2. Relationships between the maximum concentrations (µg/Kg w/w) of the studied human pharmaceuticals measured in the muscle of fish from Uruguay River and the frequency of detection (A) and their physicochemical properties (B and C).

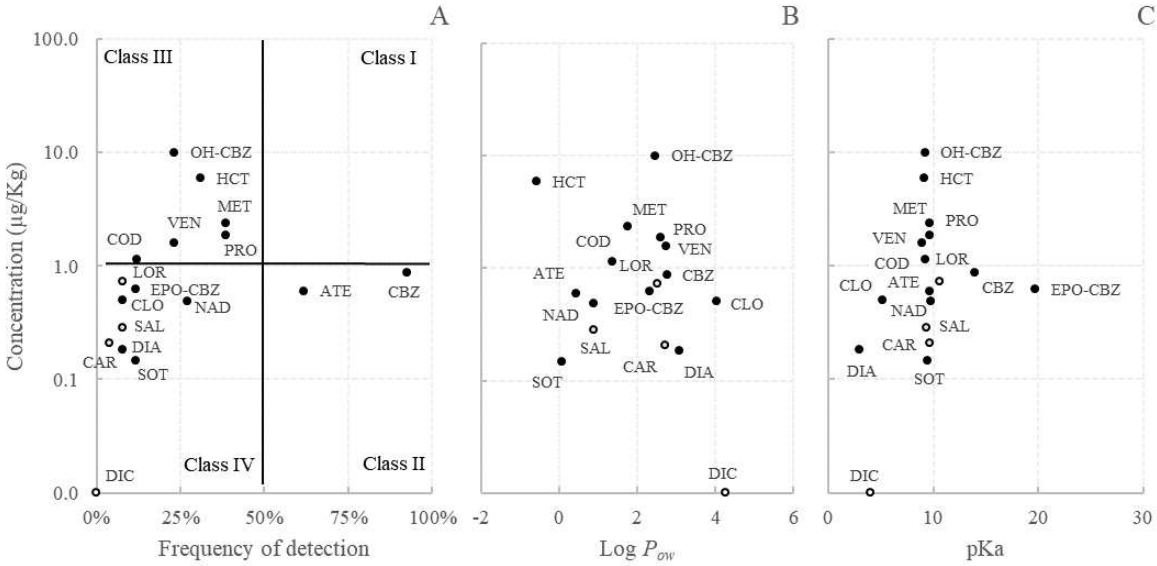
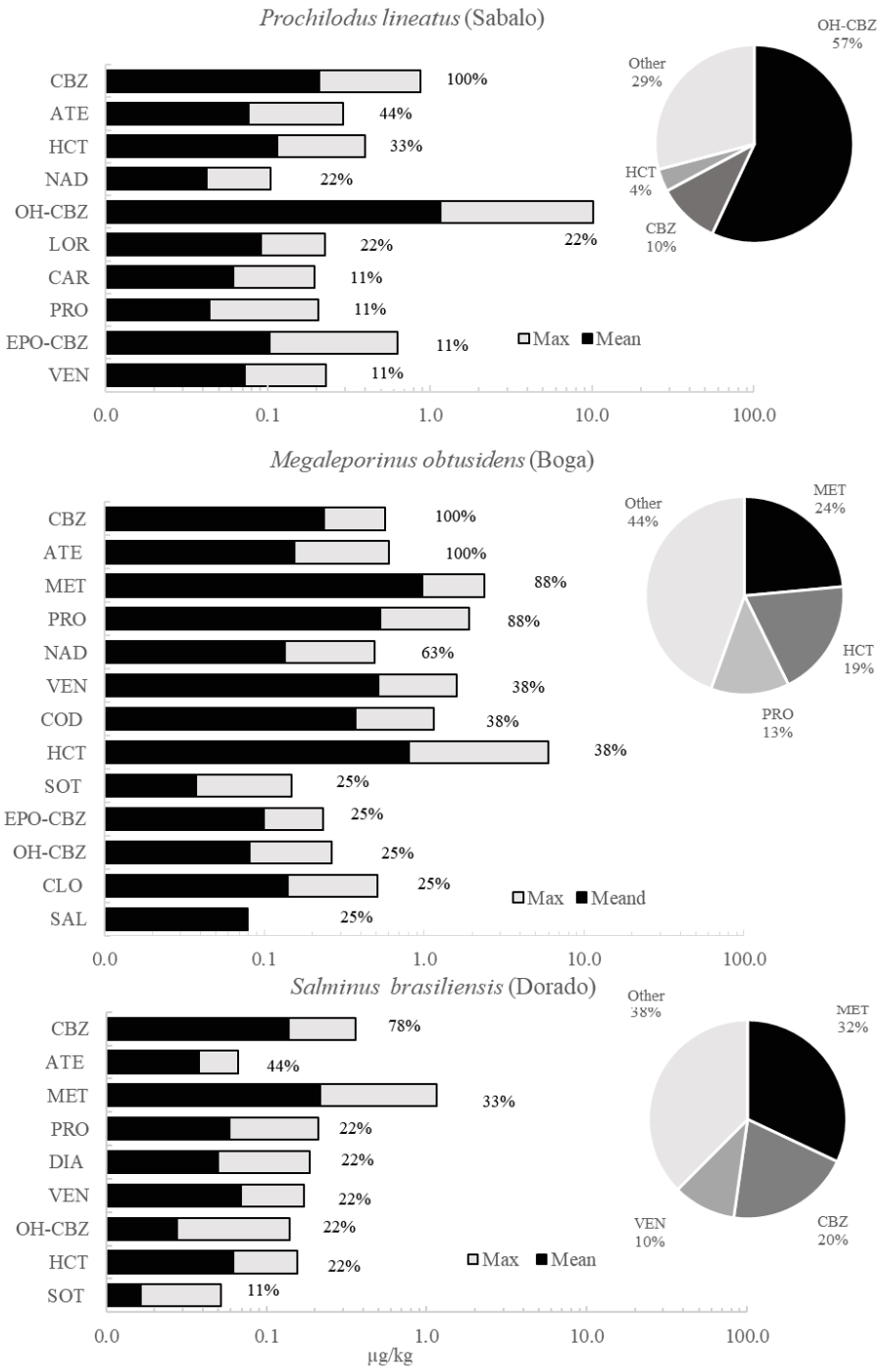
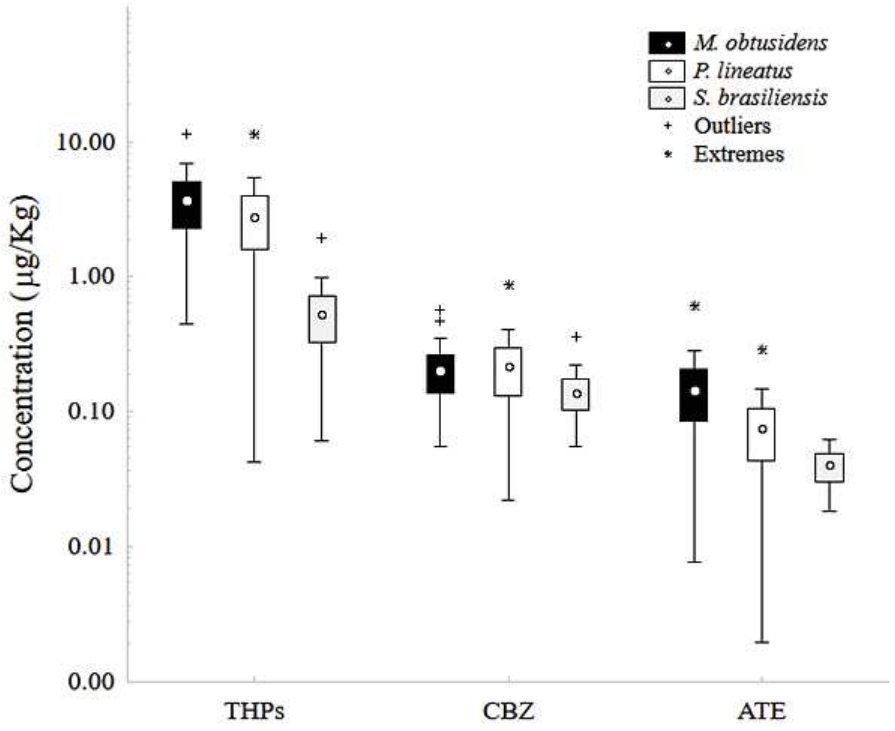


Figure 3. Characteristic accumulation patterns of studied human pharmaceuticals (HPs) in the muscle of studied fish species from the Uruguay River. Bar graph: maximum (grey) and mean (black) concentrations as µg/Kg w/w (samples below detection or quantification limits were substituted by <MDL/2 and (MQL-MDL)/2, respectively). N=9 for *P. lineatus* and *S. brasiliensis*, N=8 for *M. obtusidens*. Numbers beside each bar: frequency of detection (%); Pie graph: proportional contribution of each HP to the total HPs load (%).



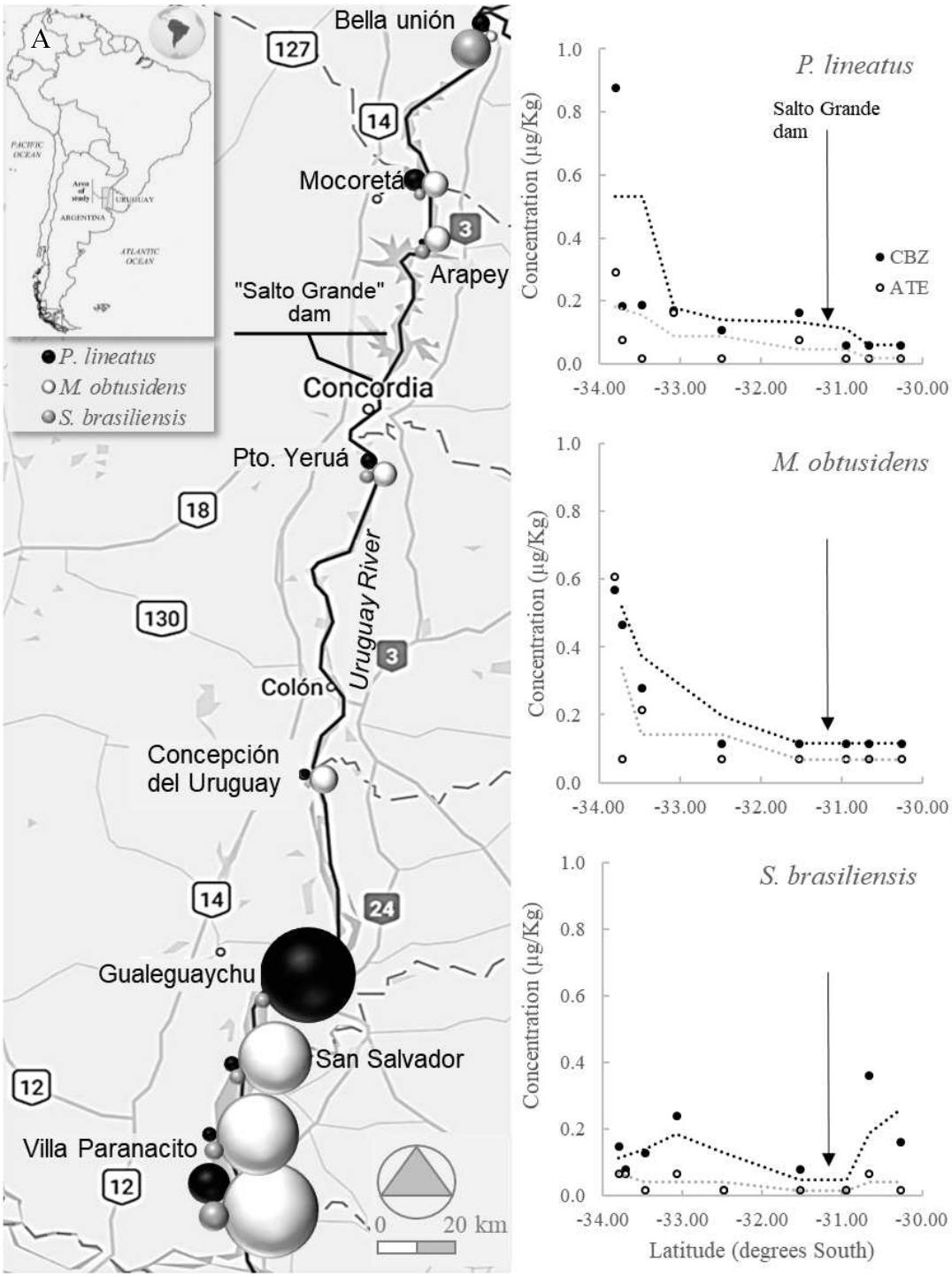
608 **Figure 4. Differences between the concentrations (µg/Kg w/w) of the most frequently**
609 **detected human pharmaceuticals and total pharmaceuticals load in the muscle of the**
610 **studied fish species from the Uruguay River.** THPs: total human pharmaceuticals load, CBZ:
611 carbamazepine, ATE: atenolol.



612

613

Figure 5. Geographical variations of the concentrations (µg/Kg w/w) of carbamazepine, atenolol and total pharmaceuticals load in the muscle of the three studied Uruguay River fish species. Panel A: total pharmaceuticals load (THPs) measured in each species at each sampling location along the studied sector of the Uruguay River, the bubble size is proportional to THPs concentration; Panel B: variation of carbamazepine (CBZ) and atenolol (ATE) concentrations with the latitude for the three studied species, dotted lines: moving average.



Supplemental Table 1. Data of fish collected during the fall and spring campaigns at each sampling locality and pooled for chemical analysis.

Season	Locality	Species	# Fish	SL (cm)	W (g)	# Pool
Fall	Villa Paranacito_1	*Prochilodus lineatus*	1	42	708	Pool_01
			2	33	773	
			3	32	825	
			4	31	842	
			5	37	844	
		Megaleporinus obtusidens	6	39	1,997	Pool_02
			7	40	1,936	
			8	37	1,611	
			9	36	1,223	
			10	41	1,997	
		Salminus brasiliensis	11	54	3,030	Pool_03
			12	37	1,844	
			13	45	1,905	
			14	39	1,205	
			15	48	2,078	
	San Salvador	*Prochilodus lineatus*	16	37	1,292	Pool_04
			17	33	986	
			18	36	1,406	
			19	38	1,498	
		Megaleporinus obtusidens	20	39	1,626	Pool_05
			21	41	2,028	
			22	37	1,048	
		Salminus brasiliensis	23	56	4,070	Pool_06
			24	54	3,314	
			25	53	3,312	
			26	37	950	
	Puerto Yeruá	*Prochilodus lineatus*	27	46	2,702	Pool_07
			28	34	1,216	
			29	34	1,352	
			30	32	1,084	
		Megaleporinus obtusidens	31	45	2,304	Pool_08
			32	38	1,324	
			33	41	1,454	
			34	49	2,638	
		Salminus brasiliensis	35	48	2,216	Pool_09
			36	47	1,706	
			37	42	1,492	
	Arapey	*Prochilodus lineatus*	38	38	1,498	Pool_10
			39	38	1,656	
			40	38	1,402	
		Megaleporinus obtusidens	41	27	1,426	Pool_11
			42	37	1,248	
			43	38	1,260	
			44	47	2,428	
		Salminus brasiliensis	45	49	2,242	Pool_12
			46	38	1,120	
			47	23	1,306	

Supplemental Table 1 (cont.). Data of fish collected during the fall and spring campaigns at each sampling locality and pooled for chemical analysis.

Season	Locality	Species	# Fish	SL (cm)	W (g)	# Pool
Fall	Bella Unión	*Prochilodus lineatus*	48	37	1,480	Pool_13
			49	30	1,668	
			50	43	1,806	
			51	34	1,246	
		Megaleporinus obtusidens	52	28	514	Pool_014
			53	31	714	
			54	33	983	
			55	35	1,104	
		Salminus brasiliensis	56	38	1,010	Pool_15
			57	55	2,766	
			58	30	1,506	
Spring	Villa Paranacito_2	*Prochilodus lineatus*	59	37	1,627	Pool_16
			60	36	1,045	
			61	34	1,078	
		Megaleporinus obtusidens	62	41	1,762	Pool_17
			63	32	1,088	
			64	37	1,484	
		Salminus brasiliensis	65	41	1,249	Pool_18
			66	40	1,160	
			67	35	922	
	Gualeguaychú	*Prochilodus lineatus*	68	30	764	Pool_19
			69	32	893	
			70	30	744	
		Megaleporinus obtusidens	71	36	1,152	Pool_20
			72	30	854	
			73	34	1,036	
		Salminus brasiliensis	74	47	2,194	Pool_21
			75	45	1,674	
			76	59	4,162	
	Concepción del Uruguay	*Prochilodus lineatus*	77	40	1,770	Pool_22
			78	34	1,068	
			79	36	1,148	
		Megaleporinus obtusidens	80	40	1,643	Pool_23
			81	41	1,494	
			82	36	1,005	
		Salminus brasiliensis	83	61	5,810	Pool_24
			84	41	1,409	
			85	41	1,382	
	Mocoretá	*Prochilodus lineatus*	86	29	690	Pool_25
			87	36	1,219	
			88	31	883	
		Megaleporinus obtusidens	89	30	648	Pool_26
			90	32	758	
			91	39	1,353	
		Salminus brasiliensis	92	48	2,166	Pool_27
			93	45	1,964	
			94	44	1,578	

Supplemental Table 2. Overall frequencies and concentrations of HPs measured in the muscle of the studied fish species from the Uruguay River (Figure 3).

Prochilodus lineatus

HP	Freq.	Mean	SE	Max	% THPs
Carbamazepine	100%	0.21	± 0.091	0.88	3%
Atenolol	44%	0.15	± 0.033	0.29	2%
Hydrochlorothiazide	33%	0.28	± 0.050	0.40	4%
Nadolol	22%	0.10	± 0.012	0.10	1%
2-HydroxyCBZ	22%	5.1	± 1.2	10.2	70%
Lorazepam	22%	0.23	± 0.027	0.23	3%
Carazolol	11%	0.19	± 0.018	0.19	3%
Propranolol	11%	0.21	± 0.021	0.20	3%
10,11-EpoxyCBZ	11%	0.63	± 0.070	0.63	9%
Venlafaxine	11%	0.23	± 0.021	0.23	3%

Megaleporinus obtusidens

HP	Freq.	Mean	SE	Max	% THPs
Carbamazepine	100%	0.24	± 0.065	0.57	3%
Atenolol	100%	0.15	± 0.067	0.61	2%
Metoprolol	88%	1.11	± 0.326	2.4	15%
Propranolol	88%	0.60	± 0.22	1.9	8%
Nadolol	63%	0.20	± 0.059	0.49	3%
Venlafaxine	38%	1.07	± 0.184	1.6	14%
Codeine	38%	0.89	± 0.16	1.1	12%
Hydrochlorothiazide	38%	2.08	± 0.74	6.0	28%
Sotalol	25%	0.11	± 0.017	0.15	1%
10,11-EpoxyCBZ	25%	0.23	± 0.030	0.23	3%
2-HydroxyCBZ	25%	0.21	± 0.030	0.26	3%
Clopidrogel	25%	0.37	± 0.057	0.51	5%
Salbutamol	25%	0.19	± 0.023	0.19	2%

Salminus brasiliensis

HP	Freq.	Mean	SE	Max	% THPs
Carbamazepine	78%	0.17	± 0.039	0.36	11%
Atenolol	44%	0.067	± 0.010	0.067	4%
Metoprolol	33%	0.57	± 0.13	1.2	35%
Propranolol	22%	0.17	± 0.023	0.21	10%
Diazepam	22%	0.16	± 0.023	0.19	10%
Venlafaxine	22%	0.17	± 0.021	0.17	11%
2-HydroxyCBZ	22%	0.091	± 0.015	0.14	6%
Hydrochlorothiazide	22%	0.16	± 0.019	0.16	10%
Sotalol	11%	0.052	± 0.0047	0.052	3%

HP: human pharmaceutical; Freq.: frequency; Mean: average concentration of each HP including all analyzed samples (concentration values of samples below detection or quantification limits were substituted by <MDL/2 or (values below detection or quantification limits were substituted by <MDL/2 or (MQL-MDL)/2, respectively); SE: standar error; Number of samples: *P. lineatus* and *S. brasiliensis* = 9 and *M. obtusidens = 8* ; Max: maximum concentration; %THP: percentage of the total HPs load.

Supplemental Table 3. Differences between the concentrations of THPs, CBZ and ATE (µg/Kg) in the muscle of the studied fish species from the Uruguay River (Figure 4).

THPs

Species	Mean ±	SE	N	Max	Median	Freq.	*p*-value
M. obtusidens	3.7 ±	1.3	8	11.5	1.00	100%	0.137
P. lineatus	1.7 ±	1.1	9	11.3	0.34	100%	
S. brasiliensis	0.52 ±	0.19	9	1.9	0.30	89%	

Carbamazepine

Species	Mean ±	SE	N	Max	Median	Freq.	*p*-value
M. obtusidens	0.20 ±	0.060	8	0.57	0.085	100%	0.667
P. lineatus	0.22 ±	0.079	9	0.88	0.16	100%	
S. brasiliensis	0.14 ±	0.034	9	0.36	0.13	78%	

Atenolol

Species	Mean ±	SE	N	Max	Median	Freq.	*p*-value
M. obtusidens	0.15 ±	0.056	8	0.61	0.07	100%	0.177
P. lineatus	0.075 ±	0.030	9	0.29	0.016	44%	
S. brasiliensis	0.040 ±	0.009	9	0.067	0.016	44%	

HP: human pharmaceutical; THPs: total HP load obtained as the summ of the conetrations of all detected HPs; Mean: average concentration ; SE: standar error; N: total number of analyzed samples; Median: median value considering all samples, Max: maximum concentration; Freq.: frequency of samples avobe the MDL (for THPs all samples with at least one HP above the MDL). *p*-value: One-way ANOVA analysis. The statistical parameters were obtained including all samples. Values for detectable but non-quantifiable or non-detectable samples were replaced by (MQL+MDL)/2 or MDL/2, respectively. MQL: method quantification limit; MDL: method detection limit.

Supplemental Table 4. Geographical variations of the CBZ, ATE and THPs concentrations (µg/Kg) in the muscle of the studied fish species from the Uruguay River (Figure 5).

P. lineatus

Locality	Latitud (degrees south)	Carbamazepine	Atenolol	THPs
Villa Paranacito_1	-33.715	0.185	0.074	0.259
Villa Paranacito_2	-33.802	0.877	0.292	2.103
San Salvador	-33.471	0.188	ND	0.299
Gualeguaychú	-33.078	0.171	0.162	11.332
Concepción del Uruguay	-32.484	0.106	ND	0.106
Puerto Yeruá	-31.527	0.162	0.074	0.340
Arapey	-30.949	DNQ	ND	DNQ
Mocoretá	-30.663	DNQ	ND	0.602
Bella Unión	-30.263	DNQ	ND	0.422

M. obtusidens

Locality	Latitud (degrees south)	Carbamazepine	Atenolol	THPs
Villa Paranacito_1	-33.715	0.569	0.607	8.462
Villa Paranacito_2	-33.802	0.467	DNQ	11.55
San Salvador	-33.471	0.278	0.214	6.815
Concepción del Uruguay	-32.484	DNQ	DNQ	0.997
Puerto Yeruá	-31.527	DNQ	DNQ	0.781
Arapey	-30.949	DNQ	DNQ	0.864
Mocoretá	-30.663	DNQ	DNQ	0.864
Bella Unión	-30.263	DNQ	DNQ	0.184

S. brasiliensis

Locality	Latitud (degrees south)	Carbamazepine	Atenolol	THPs
Villa Paranacito_1	-33.715	DNQ	DNQ	0.440
Villa Paranacito_2	-33.802	0.148	DNQ	1.073
San Salvador	-33.471	0.129	ND	0.302
Gualeguaychú	-33.078	0.239	DNQ	0.306
Concepción del Uruguay	-32.484	ND	ND	ND
Puerto Yeruá	-31.527	DNQ	ND	0.233
Arapey	-30.949	ND	ND	0.282
Mocoretá	-30.663	0.361	0.067	0.162
Bella Unión	-30.263	0.162	ND	1.924

Concentrations of the pooled sampled from each locality; THPs: total load of human pharmaceuticals calculated as the summ of all HPs above the MDL (detectable but non-quatifiable samples were replaced by (MQL+MDL)/2), DNQ: detectable not quantifiable (in the graph replaced by (MDL+MQL)/2), ND: not detectable (in the graph replaced by MDL/2). MQL: method quantification limit, MDL: Method detection limit.

Supplemental Table 5. Seasonal variations in the concentrations of THPs, CBZ and ATE (µg/Kg) in the muscle of studied fish species from the Uruguay River.

P. lineatus

HP	Season	Mean ± SE	N	Max	Median	*p* -value
THPs	Fall	0.23 ± 0.059	5	0.42	0.26	0.186
	Spring	3.6 ± 2.3	4	11.3	1.4	
Carbamazepine	Fall	0.13 ± 0.021	5	0.19	0.11	0.279
	Spring	0.32 ± 0.16	4	0.88	0.17	
Atenolol	Fall	0.027 ± 0.010	5	0.070	0.016	0.087
	Spring	0.14 ± 0.052	4	0.29	0.12	

M. obtusidens

HP	Season	Mean ± SE	N	Max	Median	*p* -value
THPs	Fall	3.4 ± 1.6	5	8.5	0.86	0.864
	Spring	4.0 ± 2.2	4	11.5	1.7	
Carbamazepine	Fall	0.22 ± 0.063	5	0.57	0.085	0.777
	Spring	0.18 ± 0.083	4	0.47	0.085	
Atenolol	Fall	0.21 ± 0.069	5	0.61	0.070	0.286
	Spring	0.070 ± 0.000	4	0.070	0.070	

S. brasiliensis

HP	Season	Mean ± SE	N	Max	Median	*p* -value
THPs	Fall	0.59 ± 0.31	5	1.9	0.30	0.743
	Spring	0.44 ± 0.18	4	1.1	0.27	
Carbamazepine	Fall	0.12 ± 0.056	5	0.36	0.085	0.657
	Spring	0.16 ± 0.027	4	0.24	0.16	
Atenolol	Fall	0.038 ± 0.012	5	0.070	0.016	0.798
	Spring	0.043 ± 0.013	4	0.070	0.043	

HP: human pharmaceutical; THPs: total HP load obtained as the summ of the concetration of all detected HPs; Mean: average concentration; SE: standar error; N: total number of analyzed samples; Median: median value, Max: maximum concentration; Freq.: frequency of samples avobe the MDL, for THPs all samples with at least one HP above the MDL. *p* -value: One-way ANOVA analysis. The statistical parameters and ANOVA were calculated including all samples (values below detection or quantification limits were substituted by <MDL/2 or (MQL-MDL)/2, respectively). MQL: method quantification limit; MDL: method detection limit.