

Negro River Environmental Assessment



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Abstract The Negro River comprises a multi compartmentalized environment which links Los Andes mountains with the Atlantic Ocean. Due to its relevance in terms of industries, intensive agriculture production and population is considered the second Argentinean river in importance; however, these economic activities have increased the environmental pressure, raising concerns. For instance, the intensive

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agriculture joint with urban settlements and industries have lead to the introduction of several Persistent Organic Pollutants, including legacy compounds (such as DDT and HCHs), new generation pesticides (endosulfan) and urban-industrial POPs (PCBs, PBDEs). In addition, several matrix have been shown to receive and accumulate heavy metal loads, including As, Cd, Cr, Co, Cu, Fe, Hg, Mn, Ni, Pb and Zn. Metal bioaccumulation and transference between environmental compartments (water, sediments, fish and mollusks) have been demonstrated to occur through the years. The anthropic activities have also induced changes in the aquatic macroinvertebrate assemblages of the Negro River, where both, water quality and invasive species, appear to be main drivers of the change. Added to this, from 17 fish species, only 9 are native, leading to a low zoogeographic integrity coefficient. Finally, the environmental quality threats, climate change and hydroelectric facilities pose synergistic new risks which are addressed in this chapter and further discussed.

Keywords Persistent organic pollutants · Heavy metals · Macroinvertebrates · Fish assemblages · Negro river · Patagonia

1 Introduction

The hydrographic system of the Negro River, along with the Limay and Neuquen rivers, is a set of water courses that form a natural environment, currently organized by different social, cultural and economic processes. Its headwaters are located at the west of Neuquén and Río Negro provinces. The area belongs to the Andes' lakes region and the main water source is precipitation occurring at the Andean zone, in the form of snow and rain. Water flows towards the main course, the Negro River, which outflows in the Atlantic coast. Once it leaves the **headwaters** area in the Andean region, there is a section that can be identified as the water catchment area outside the headwaters. This section is drained by two important water courses, one to the south, which is the Limay River and the other to the north, which is the Neuquén River. These rivers constitute the main tributaries of the Negro River (Fig. 1).

The Negro River is located in the third and last part of this hydrographic system, and it flows towards the Atlantic Ocean through the Upper, Middle and Low Valleys. It does not receive any tributary throughout its entire path to the Atlantic Ocean: it runs from the confluence point through the valley towards the east and crosses the entire Negro River province from west to east, including plateaus, fences, islands and plains throughout its extension. It is one of the main water courses in the country, and the most important in the province. Throughout its valleys, several economic activities are carried out, holding most of the population at its margins.

Three types of economic activities prevail throughout the Negro River catchment area. In the upper valley, intensive agricultural activities under irrigation are developed, such as fruits (especially apples and pears), grapes and other vegetables. The irrigated valley holds the largest concentration of population of the entire province along 100 km. The main cities include General Roca, Ingeniero Cipolletti, Villa

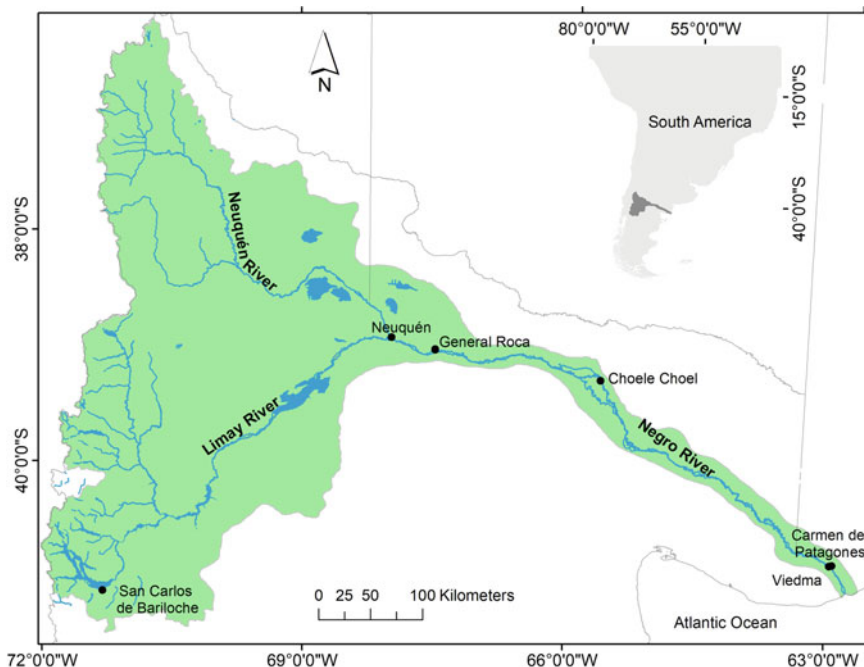


Fig. 1 The Negro River hydrographic scheme, including the Negro, Limay and Neuquén rivers, Patagonia, Argentina

Regina, Coronel Belisle, Darwin, Allen and Cinco Saltos. This area is followed by the middle valley, where the main activity is fruit and vegetables cultivation, interspersed with forages, vineyards and livestock breeding. Choele-Choele is one of the main cities in this area. Finally, the lower valley is emplaced the city of Viedma, which has a high public administration activity and several industries. Irrigated agriculture and livestock production are also developed in the area.

The growing population and the related economic activities have increased the environmental pressure over the freshwater ecosystems in the Argentinean Patagonia. While intensive agriculture is the second most important economic activity in region, the use of nonselective pesticides has significant implications to the environment quality (Loewy et al. 2011), particularly on aquatic macroinvertebrates assemblages (Macchi et al. 2014; Kohlmann et al. 2018). Extensive livestock farming, deforestation, wood and hydrocarbon extraction, flow regulation by dams, introduction of exotic species and the growth of unplanned urbanizations are also main threats currently impacting the area (Cazzaniga and Pérez 1999; Macchi 2008; Miserendino 2009; Miserendino and Brand 2009; Epele et al. 2018; Macchi et al. 2018).

Along the next sections a set of environmental impacts are revisited, aiming to review the Negro River environmental assessment from the past decades up to date. In this sense, along the first two sections both, persistent organic pollutants and heavy metal sources, occurrence, distribution and threats are explored. In the next section,

the macroinvertebrate assemblages of the Negro River are listed and analyzed as biological early warning sensors of the environmental status of the area. Finally, fish communities of the Negro River are revisited since they respond significantly and predictably to almost all kinds of anthropogenic disturbances. Their origin, current status and immediate and medium-term threats are addressed and discussed.

2 Persistent Organic Pollutants

Persistent Organic Pollutants (POPs) compounds belong to a group of substances of natural or anthropogenic origin, resistant to photolytic, chemical and biological degradation. They tend to bioaccumulate at different levels of the trophic web, with possible **upstream** biomagnification (Sangster 1989; Yalkosky 2010). The consequent toxicity includes various alterations in the reproduction, development, and some immune functions of animals and plants (Langston et al. 2010). Among the persistent compounds, the organochlorine compounds include the known pesticides: hexachlorocyclohexanes (HCHs), hexachlorobenzene (HCB), dichlorodiphenyl-trichloroethane (DDT), chlordanes and endosulphanes. Organochlorine pesticides have been banned or severely restricted by the sanitary or environmental Annexes in the III Rotterdam Convention (2004). Their use is also prohibited in Argentina, except for DDT which has a restricted use for certain applications (Table 1). For instance, HCHs are classified as carcinogenic to humans, DDT as probably carcinogenic to humans (2A) while HCB and chlordanes as possibly carcinogenic to humans (2B, IARC).

Table 1 Commonly used pesticides in the past at the Negro River catchment area

Compound	National Law	Resolution
Chlordane and Lindane	SAGPyA 513/98 Resolution	Prohibited for import, commercialization and use as phytosanitary products, as well as the products formulated based on these
DDT, Endrin, Aldrin	Law-ranking Decree No. 2121/90	Prohibited for import, manufacture, fractionation, commercialization and use of agricultural products formulated based on these active principles
Hexachlorobenzene (H.C.B)	SAGPyA No. 750/2000 Resolution	Total prohibition
Hexachlorociclohexane (H.C.H), Dieldrin	National Law 22.289	Total Prohibition
Endosulfan	ENASA 511/11 Resolution	Total Prohibition. Only re-export or destruction as of July 1, 2013

The Negro River basin is not excluded from the input of persistent organic pollutants. As mentioned above, the upper valley region of Negro River and Neuquen produces the 80 and 90% of the apples and pears of Argentina, respectively. Besides, the production of fine fruit, olive trees, nuts, and horticulture has been incorporated in recent years (LIBIQUIMA-CITAAC 2016). This level of production involves the application of multiple families of pesticides during a period that extends from September to February. Most used pesticides include organophosphates (OF), carbamates (CB), pyrethroids (PIR) and neonicotinoids (NN). Due to the fact that pesticides such as organochlorine pesticides (OC; e.g. DDT and endosulfan) have been extensively used in the last century and until the beginning of the twenty-first century, the area shows an extensive occurrence of OF and OC residues in soils and groundwaters in rural areas (Comahue 2016). Methylaziphos and chlorpyrifos have been frequently detected in water and the main compounds include methylaziphos and carbaryl, with levels up to 22.5 ppb and 45.7 ppb, respectively (Loewy et al. 2011). With methylaziphos banning in 2016 this trend has changed over time, decreasing the environmental levels in about 30%. It has been also shown that approximately 50% of the applied pesticides are lost in the environment without reaching the intended targets, moving in a very high proportion to canals, lakes and streams due to drifting, runoff, washing by rain and irrigation (LIBIQUIMA-CITAAC 2016). Along 2006–2007, Isla et al. (2010) showed a decreasing trend of organochlorine compounds (OCs) contents in river sediments from the upper valley to the inlet, with an increment at the beginning towards the lower valley. The higher concentrations were found in the Neuquen River and Paso Cordova location, reaching 18.1 and 7.5 ng/g dry weight, respectively (summatory of 10 compounds). While Neuquén River showed a prevalence of parental DDT, which was already illegal use during that period (Isla et al. 2010), DDE dominated along the Negro River pointing to a past use, with a higher diversity of compounds, including endosulfans and HCHs. Among endosulfans, the parental isomers dominated, indicating a widespread current use during that period. In the same period, Miglioranza et al. (2013) demonstrated the occurrence and distribution of OCPs, PCBs and PBDEs in several environmental matrices (soils, sediments, suspended particle matter and **macrophytes**) along the Río Negro. Similarly to Isla et al. (2010), authors concluded that the soil can be considered a hot spot of DDTs in the area while the *pp'*-DDE was dominant in all samples. While the occurrence of endosulfans with a relation α -/ β -isomers > 1 in all matrices denoted its current use in the region, their levels found in water are higher than the maximum values established for aquatic biota protection. During those years, DDTs, endosulfans, HCHs, chlordanes, PCBs and PBDEs were also detected at *Odontesthes hatchery* (patagonian silverside) from the Upper, Middle and Lower valleys of the Negro River (Ondarza et al. 2014). As a general outcome, all tissues showed decreasing levels from the upper to the lower regions. In agreement with another environmental matrix (Miglioranza et al. 2013), organochlorine pesticides were dominant (306–3449 ng g⁻¹ lipid) followed by Σ PCBs (65–3102 ng g⁻¹ lipid) and Σ PBDEs (22–870 ng g⁻¹ lipid), pointing to agriculture as the main source. Regarding organochlorine pesticides, DDT was dominant (90% *pp'*-DDE) followed by endosulfan (α - > β - > sulfate), γ -HCH and γ -chlordane. This pattern was still

confirmed in 2018, showing the prevalence of this legacy POPs in the river and the growing awareness for endosulfan, which in occasions exceeded the chronic exposure limit of the CCME (Arias et al. 2019).

Polychlorinatedbiphenyls (PCBs) are a set of compounds of environmental concern as they have a high half-life (from 60 days to 27 years in water, and 3–38 years in sediments; Sinkkonen and Paasivirta 2000), they are resistant to degradation by physical, chemical and biological processes and they bioaccumulate through the food web (Muir et al. 1988; Thomann 1989), generating adverse effects on both, the environment and human health (Jones and De Voogt 1999). Despite their prohibition and the consequent decrease in their global levels (USEPA 1999), PCBs are widely distributed and are among the most problematic and important pollutants in the world due to their strong presence in the environment. Urban areas at the Negro River highly impact the river by the introduction of several POPs, including PCBs > cyclodienes > DDTs (Miglioranza et al. 2013). Besides a retention effect of riparian vegetation which was demonstrated for pesticides and macrophytes, the proximity to dumping sites often correlates with an increase in PBDE levels; the mechanistic paths of inputs includes leaching from dumping sites and eventual river flooding and land wash-up (Miglioranza et al. 2013). Particularly, in terms of PCBs, Isla et al. (2010) showed a relatively constant concentration as a consequence of chronic pollution, with the Limay River contributing with a higher PCB load in comparison to Neuquen River.

Recent data have shown that a major anthropogenic pressure over the river is still caused by the use of DDT (Arias et al. 2019). In comparison to previous reports such as those of Miglioranza et al. (2013) and Isla et al. (2010), there is a decrease in the current values of HCHs and endosulfans transported onto the Suspended Particulate Matter (SPM) of the middle valley, while in the lower valley a slight increase in the contributions of HCHs and endosulfans were registered in comparison to 2006 (Fig. 2).

Regarding flame retardants (FRs), a set of compounds intended for thermal insulation, thermoplastics, textiles, plastic foams, etc., are based on chlorine and bromine. Bromine is currently used in a large number of products such as pesticides, gasoline additives, drilling fluids, and biocides, but currently the main application of bromine-based compounds is in the FRs production (Tombesi et al. 2017). In 2006, PBDE

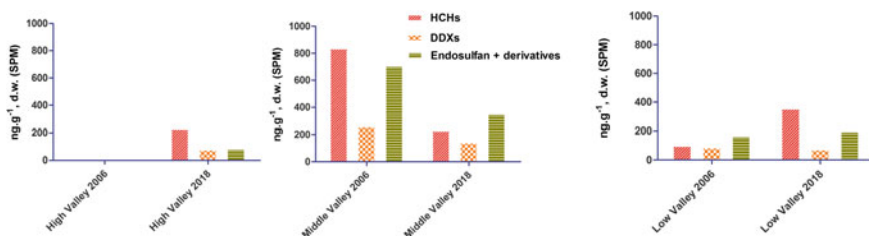


Fig. 2 HCHs, DDX and Endosulfans recorded at the Upper, Middle and Lower valleys of the Negro River in 2006 and 2018 (data from Miglioranza et al. 2013 and Arias et al. 2019)

could be associated to urban areas proximities and urban dumping sites (Miglioranza et al. 2013). Authors concluded that the proportion of PBDE congeners close to Viedma city would indicate a relatively recent use of penta-BDE mixtures due to the BDE-47/BDE-100 ratios. While BDE-100 is characterized by a high persistence, BDE-47 is one of the most ubiquitous BDE as a result of atmospheric transport. An air survey performed between 2010 and 2013 at the river basin can confirm the above mentioned patterns (Miglioranza et al. 2020). While endosulfan, trifluralin and DDT-related substances were the most prevalent pesticides in the Negro River **watershed**, low concentrations of industrial POPs were found (1.9 pg m^{-3} for $\Sigma 38$ PCBs, and $\Sigma 5$ PBDEs, respectively) and they were similar among sites.

3 Heavy Metal Pollution

Besides there are several essential metals which are commonly found in low concentrations and exhibit substantial implications to chemistry of natural ecosystems (e.g. Cu, Zn and Fe), some of them are toxic even in small concentrations (Cr, Pb, Cd, Hg, As) and pose a great concern due to their high acute and chronic effects and possible biomagnifications through the trophic web (Cai et al. 2011; Hu et al. 2013; Wang et al. 2015). This is the case for Negro River, where several research studies have been performed in this sense. In the next sections we describe the highlights of this research by matrix of study.

3.1 Water

Several challenges have been faced when comparing total or dissolved metals in the Negro River water (Phillips 1977); methods dispersion range from differences in sample pre concentration (filtration by membranes with several pore sizes) to the applied analytical methods (Inductively Coupled Plasma—optical spectrometry ICP-OES, ICP-Mass ICP-M, Graphite Furnace, etc.).

Regarding environmental levels, the most recent analysis based on the water soluble fraction showed low As, Cu and Zn levels, while traces of Cr, Ni, Cd, Pb were detected (Table 2; Abrameto 2019).

Historically, the Neuquén River has shown the maximum As levels ($5.6 \text{ } \mu\text{g L}^{-1}$), which tend to diminish through the Upper and Middle valley of Negro River to reach a media concentration of $3.0 \text{ } \mu\text{g L}^{-1}$ at the estuary zone. In many occasions, levels were shown to be above the water quality guidelines for aquatic life protection (Table 2) (CCME 2020). In general, the arsenic concentration is far from background levels and is remarkably close (or even higher) than the World Health Organization reference value for water sources destined to human consumption (WHO 2019; Table 2).

Table 2 Heavy metals and metalloids concentrations in freshwater of the Negro River

Authors	Location	Fraction	Concentration ($\mu\text{g L}^{-1}$)										
			Fe %	Hg	Mn	Co	Cr	Ni	Cd	Pb	Zn	As	Cu
Gaiero et al. (2002)	Conesa (MV)	Dissolved (0.22 μm)	11	-	1.6	0.08	0.9	1.7	-	03	1.6	-	1.4
		SPM (1996)	4.10	-	3.2	12	34	39	-	33	170	-	37
		SPM (1997-1998)	4.10	-	1.7	20	65	37	-	60	460	-	290
Abrameto et al. (2013)	GMitre (LV)	Total	-	-	-	-	-	-	-	-	n.d	2.42	2.4
	Drains (LV)		-	-	-	-	-	-	-	-	n.d	2.40	4.6
	Paloma Island-(LV)		-	-	-	-	-	-	-	-	n.d	1.16	4.9
	Maritime estuary		-	-	-	-	-	-	-	-	30	3.01	7.6
Abrameto et al. (2019)	Neuquén Vista Alegre	Dissolved (0.45 μm)	-	-	-	-	n.d	n.d	n.d	n.d	n.d	(3.16-4.32)	n.d
	Neuquén city		-	-	-	-	n.d	n.d	n.d	n.d	n.d	(6.63-8.36)	n.d
	Limay and Neuquén		-	-	-	-	n.d	n.d	n.d	n.d	n.d	n.d	n.d
	Cipoletti (UV)		-	-	-	-	n.d	n.d	n.d	n.d	n.d	(5.0-7.9)	n.d
	Fernandez Oro (UV)		-	-	-	-	n.d	n.d	n.d	n.d	n.d	(3.5-3.8)	n.d
	Allen Sewer (UV)		-	-	-	-	n.d	n.d	n.d	n.d	n.d	1.95	n.d
	Allen pipeline (UV)		-	-	-	-	n.d	n.d	n.d	n.d	n.d	3.5	n.d
	Allen Water catchment (UV)		-	-	-	-	n.d	n.d	n.d	n.d	n.d	4.5	n.d
	Roca (UV)		-	-	-	-	n.d	n.d	n.d	n.d	n.d	n.d	n.d
	Villa Regina (UV)		-	-	-	-	n.d	n.d	n.d	n.d	4.0	(3.2-14.7)	n.d

(continued)

Table 2 (continued)

Authors	Location	Fraction	Concentration ($\mu\text{g L}^{-1}$)													
			Fe %	Hg	Mn	Co	Cr	Ni	Cd	Pb	Zn	As	Cu			
	Chichinales (UV)		-	-	-	-	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
	Chelforo (UV)		-	-	-	-	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
	Choele Choel (MV)		-	-	-	-	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	(4.4-8.6)	1.7
	Pomona (MV)		-	-	-	-	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	3.6	n.d
	Conesa (MV)		-	-	-	-	n.d	n.d	n.d	n.d	n.d	n.d	n.d	69.5	(3.7-5.3)	n.d
	Guardia Mitre (LV)		-	-	-	-	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	(3.4-8.9)	n.d
	Viedma (LV)		-	-	-	-	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	(3.8-7.5)	1.3
	Paloma Island—(LV)		-	-	-	-	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	(7.0-15.7)	8.5
	Patagones (LV)		-	-	-	-	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	11.4	n.d

Metal values are separated by different sectors and by sampling year, where (-) is Not analyzed
n.d not detected, *UV* upper valley, *MV* middle valley, *LV* lower valley

Regarding heavy metals transport mechanisms through the Negro River, Gaiero et al. (2002) showed that Cu would be mainly bonded to the particulate fraction and tends to increase its total concentration (dissolved + particulate) from the inland to the maritime estuary (Abrameto et al. 2013). In general, the partition coefficient (calculated among dissolved and suspended loads, $K_d = \text{HM}_{\text{dis}}/\text{HM}_{\text{spm}}$) shows a general decreasing tendency to the ocean for Zn, Cu and Pb, confirming the gradual adsorption increment on suspended particulate matter (Gaiero et al. 2002). Finally, in terms of Cr, the General Conesa City area showed a dissolved mean concentration of $0.9 \mu\text{g L}^{-1}$; wide above the international aquatic life protection limits (CCME 2020).

3.2 Sediments

Sediments act as an end reservoir of metals that came into the aquatic system from several ways: through creeks, drainage systems, atmospheric deposition and leaching (Botté et al. 2010, 2013). While recording the past and present biogeochemical variability, they have an important role in the transport and storage of potentially dangerous metals (Zhang et al. 2014). The Negro River sediments have shown a set of heavy metals, including As, Cd, Cr, Co, Cu, Fe, Hg, Mn, Ni, Pb and Zn.

Firstly, Arribére et al. (2003) performed a study on heavy metals contents in the $< 63 \mu\text{m}$ fraction of sediments and biota of the Upper Negro River. Recorded levels were higher at the coast of Neuquén city and the Limay confluence area, decreasing towards Allen and Regina City. Mercury maximum levels showed $0.28 \mu\text{g g}^{-1}$ (d.w.) with a mean of $0.22 \mu\text{g g}^{-1}$ (d.w.) in the river upper basin. Arsenic levels ranged from 12 to $5.2 \mu\text{g g}^{-1}$ (d.w.) and Ni and Zn exhibited a media concentration of 29.2 and $106.3 \mu\text{g g}^{-1}$ (d.w.) respectively, along the basin.

For the middle valley, Gaiero et al. (2002), showed that Ni and Zn values in a similar range to those (21 and $101 \mu\text{g g}^{-1}$) reported by Arribére et al. (2003), while the mean concentrations of Co, Cr, Cu, Pb, Mn and Fe were the following: 20, 39, 36, 21, $1049 \mu\text{g g}^{-1}$, 5.2% respectively. Considering the upper valley, Abrameto (2004) reported Hg, Ni and Zn levels which were preferably associated to the $< 63 \mu\text{m}$ fraction, with remarkable Zn levels at Fernández Oro, which presented a maximum of $163.9 \mu\text{g g}^{-1}$. Simultaneously, Cd, Mn and Ni showed constant mean concentrations throughout the area ($0.2\text{--}0.97 \mu\text{g g}^{-1}$; $0.14\text{--}0.19\%$ and $5.6\text{--}9.43 \mu\text{g g}^{-1}$, respectively) while Cu and Pb peaked at Fernández Oro site, with an average of $39.1 \mu\text{g g}^{-1}$ and $29.9 \mu\text{g g}^{-1}$, confirming a potential heavy metal hotspot in the area. Finally, Fe levels showed a mean value of 9.6% along the upper valley sediments.

Ward (2007) evaluated the occurrence of heavy metals in sediments of the upper Negro River valley ($<63 \mu\text{m}$). The mean Hg concentration was $0.06 \mu\text{g g}^{-1}$ with peak levels ranging from 0.18 to $0.28 \mu\text{g g}^{-1}$, exceeding the ISQG of $0.17 \mu\text{g g}^{-1}$ (CCME 2020). Heavy metal levels were even higher at the confluence area, showing 6.3, 2.6, 35.9, 100.1, 79.9 and $46.8 \mu\text{g g}^{-1}$ for As, Cd, Co, Cr, Pb and Zn, respectively. Abrameto et al. (2013) investigated the “total” content of heavy metals in bed

sediments from the Low Negro River Valley. In that occasion, hot spots were detected at “El Molino drainage”, “Inlet maritime” and Patagones city coast (for As and Cu) and La Paloma island (Zn). The environmentally high levels for these metals raised a concern: 3–28.7 $\mu\text{g g}^{-1}$ for As, 6.4–37.5 $\mu\text{g g}^{-1}$ for Cu, and 17.1– 50.1 $\mu\text{g g}^{-1}$ for Zn. According to Ward (2007), mercury concentrations decrease towards the end of the upper valley, while higher Cd and As levels are detected at the lower valley.

Table 3 revises the heavy metals levels in Negro River bed sediments and other locations around the world. As shown, while Cr levels (Neuquén) were lower than those reported for other places, these levels were higher than those reported for the Bahía Blanca Estuary, Samborombón Bay, the Yangtze basin (China), and Río de La Plata sediments. A similar situation was observed for As: its levels at the upper basin and the coast of Neuquén were lower than those reported by Yi et al. (2008) for the Yangtze River. While Ni levels in bottom sediments of the Negro River were higher than those indicated for the Bahía Blanca Estuary, they were lower than those reported for the Samborombon Bay. Pb levels were comparable to the data recorded at Río de La Plata and the Bahía Blanca Estuary, but lower than those obtained in the Yangtze (China) and Dipsiz (Turkey) rivers. Regarding Hg levels, they were in the range of those registered within the Bahia Blanca Estuary (Argentina) and the middle basin of the Yangtze River, but higher than those indicated for the lower basin of the Yangtze River. Cu, Fe and Mn levels were at the same order of magnitude than those reported by other authors in rivers and estuaries worldwide.

3.3 Fish

There are three pathways by which fish can intake metals from the environment: by the tegument, gills or through diet. While the highest intake of metals occurs through the gills (Alam et al. 2002), they can also accumulate suspended particles from water by food ingestion. Abrameto (2004) investigated the levels of Ni, Cd, Hg, Pb, Zn, Fe and Mn in *O. hatcheri* and *P. colhuapiensis* collected in the upper and middle Negro River basin. Only Hg, Mn, Pb and Zn have been reported as bioaccumulated heavy metals (Table 4). The Zn range for muscle tissue in the upper basin was from 5.49 to 7.08 $\mu\text{g g}^{-1}$ (w.w.) while fish samples from Beltran city averaged 10 $\mu\text{g g}^{-1}$ (w.w.). These values were lower than those reported by Arribere et al. (2003). Hg in the muscle of *O. hatcheri* showed similar levels throughout the upper and middle basin of the Negro River, with comparable levels to those reported by Arribere et al. (2003) and by Marcovecchio and Moreno (1993) on fish from the Río de la Plata estuary. Similarly to Zn patterns, Pb concentration in muscle tissues was higher in individuals collected from the Beltran city area (0.14 $\mu\text{g g}^{-1}$ w.w.) than those collected from Allen city area (0.05 $\mu\text{g g}^{-1}$ w.w., middle valley). Finally, Hg and Pb reported concentrations were lower than those contained in *O. bonariensis* in the Río de la Plata (Avigliano et al. 2015). Finally, Pb and Hg levels in Negro River fish could exceed the current daily recommended intake by the US Environmental Protection Agency (EPA 2020).

Table 3 Mean concentrations of heavy metals in sediments from Negro River basin compared to rivers and estuaries in Argentina and the world

Authors	Location	Concentration $\mu\text{g g}^{-1}$ d.w.										
		Fe %	Hg	Mn %	Co	Cr	Ni	Cd	Pb	Zn	As	Cu
* Arribére et al. (2003)/Abrameto (2004)/ *Ward (2007)	Neuquén Upper Valley	13.63	0.07	23.10	–	17.93	13.56	0.34	7.53	53.88	6.56*	7.60
*Gaiero et al. (2002)/Abrameto et al. (2012)	Middle Valley	5.20	–	0.10	20.00	39.00	21.00	8.30	21.00	74.25	15.97	22.63
Abrameto et al. (2012)	Lower Valley	–	–	–	–	–	–	3.46	13.46	36.00	–	21.34
Janiot et al. (2001)	Río de la Plata	–	–	–	–	17.4/22.7	–	0.05/ 0.13	10.9/20.0	–	–	12.65/ 37.7
Demirak et al. (2006)	Dipsiz stream Yatagan basin	–	–	–	–	19.70	–	0.80	83.60	37.00	–	13.00
Yi et al. (2008)	Middle Yangtze River	–	0.16	–	–	61.38	–	0.11	37.38	109.1	14.38	38.25
Yi et al. (2008)	Lower reach of the Yangtze River	–	0.02	–	–	66.40	–	0.19	34.2	75.8	66.8	37.00
Tatone et al.(2015)	Río de la Plata	3.35	–	0.06	–	21.20	15.1	–	15.4	80.8	–	23.3
PMCA-EBB (2016)	Bahia Blanca Estuary	–	0.09	–	–	10.70	7.7	0.04	6.13	32.75	–	12.3

*Fractions < 63 μm

PMCA-EBB: Environmental Quality Monitoring Program of the Bahía Blanca Estuary

Table 4 List of studies in Negro River and other rivers reporting bioaccumulation of heavy metals and metalloids in fishes

Authors	Location	Species	Organ	Concentration $\mu\text{g g}^{-1}$ w.w.													
				Fe	Hg	Mn	Co	Cr	Ni	Cd	Pb	Zn	As	Cu			
Abrameto (2004)	Neuquén	<i>Odontesthes hatcheri</i>	Liver	14.2	-	4.38	n.d	n.d	n.d	n.d	n.d	n.d	n.d	27.9	-	n.d	
			Muscle	2.44	-	3.42	-	-	-	-	-	-	-	n.d	7.08	-	-
		<i>Percichthys colhuapiensis</i>	Liver	60.99	-	3.79	-	-	-	-	-	-	-	n.d	26.60	-	-
			Muscle	3.25	-	3.12	-	-	-	-	-	-	-	-	n.d	5.49	-
	Allen (UV)	<i>Odontesthes hatcheri</i>	Liver	-	0.18	-	-	-	-	n.d	n.d	0.13	130	-	-	-	-
			Muscle	-	n.d	-	-	-	-	-	n.d	n.d	0.05	6.10	-	-	-
	Fernández Oro (UV)	<i>Odontesthes hatcheri</i>	Liver	-	0.36	-	-	-	-	-	-	-	-	1.43	91.2	-	-
			Muscle	-	0.13	-	-	-	-	-	n.d	n.d	-	-	-	-	-
	Beltrán (MV)	<i>Odontesthes hatcheri</i>	Liver	-	0.20	-	-	-	-	n.d	n.d	1.59	113	-	-	-	-
			Muscle	-	0.12	-	-	-	-	-	n.d	n.d	0.14	10	-	-	-
Arribéret al. (2003)	Río Negro (UV)	<i>Odontesthes hatcheri</i>	Muscle	-	0.077-0.384	-	0.016-0.025	-	<2	<0.3	-	-	-	12-39	0.056-0.118	-	-
Marcovecchio and Moreno (1993)	Río De La Plata	<i>Rhamdia</i>	Muscle	-	0.13	-	-	-	-	n.d	-	-	-	21.15	-	-	-
Avigliano et al. (2015)	Río De La Plata	<i>Odontesthes bonariensis</i>	Muscle	0.30	-	-	-	-	-	-	-	-	0.19	-	0.03	-	-

(-) Not analyzed; n.d. Not detected; UV upper valley; MV middle valley; LV lower valley

3.4 Mollusks

Abrameto et al. (2012) investigated the presence of Cd, Zn, As, Cu in *C. fluminea* collected from the middle and lower basins of the Negro River, and reported Cd concentrations from 0.7 to 0.13 $\mu\text{g g}^{-1}$, Zn levels between 10.5 and 29.2 $\mu\text{g g}^{-1}$, As concentrations from 0.16 to 0.87 $\mu\text{g g}^{-1}$, and Cu levels between 2.8 and 7.2 $\mu\text{g g}^{-1}$. Hünicken et al. (2019) reported lower concentrations of heavy metals, ranging from 3 to 3.68 $\mu\text{g g}^{-1}$ for Cu and from 10.5 to 11.9 $\mu\text{g g}^{-1}$ for Zn. Although spaced in time, there is solid evidence of the heavy metal impact in water, sediments, SPM and transference to the aquatic biota at the Negro River basin, including bioaccumulation and biomagnification processes (Table 5). This supports the urgent need for serial and tiered monitoring programs over the area.

4 Freshwater Macroinvertebrates Assemblages

Several studies have addressed the effects of anthropic activities (involving changes in the land use/cover) on the aquatic macroinvertebrate assemblages of Patagonian streams and rivers (Miserendino 2001; Macchi and Dufilho 2008; Miserendino and Masi 2010; Miserendino et al. 2011; Horak et al. 2020). As a result, biological criteria based on the analyses of aquatic macroinvertebrates have been added to the traditional physical–chemical monitoring methods to assess the quality of regional aquatic ecosystems (Miserendino and Pizzolón 1999; Brand and Miserendino 2015; Mauad et al. 2015; Kohlmann et al. 2018; Miserendino et al. 2020).

Despite an increase in the knowledge about the composition and structure of macroinvertebrate assemblages, the Negro River basin remains poorly studied in this way. The first documented survey was carried out by Wais (1990), who made a description of the macroinvertebrate assemblages at the Negro River, from the headwaters to the mouth, dividing it into the upper, middle and lower basin. A total of 63 families and 129 macroinvertebrate taxa were described, of which only 29 were recognized in the Negro River basin (lower valley). The richest taxa in the assessment were Mollusca and Diptera (Chironomidae family) and the analysis of functional groups showed a greater abundance of collector-gatherers throughout the basin, which were dominant along the entire basin (Wais 1990). Unfortunately, in that occasion, species–environment relationships were not analyzed.

In the following years, macroinvertebrate taxa received special attention: this was the case of the widespread Chironomidae (Paggi and Capítulo 2002; Paggi 2003) and the invasive mollusk species *Corbicula fluminea* (Archuby et al. 2015; Cazzaniga and Perez 1999; Martín and Estebenet 2002; Molina et al. 2015; Hünicken et al. 2019), particularly at the Limay and Negro rivers. Changes in the composition and structure of Chironomidae were related to water flow fluctuations due to the dams located upstream, in the Limay River. Main results showed the overlapping and return from

Table 5 List of some research in Negro River basin reporting bioaccumulation of heavy metals and metalloids in *Corbicula fluminea*

Authors	Location	Concentration $\mu\text{g g}^{-1}$ w.w.										
		Fe	Hg	Mn	Co	Cr	Ni	Cd	Pb	Zn	As	Cu
Abrameto et al. (2012)	Conesa (MV)	-	-	-	-	-	-	0.11	-	19.10	0.16	4.30
	Zanjon de Oyuela-San Javier (LV)	-	-	-	-	-	-	0.13	-	29.20	0.24	5.80
	Isla La Paloma-Viedma (LV)	-	-	-	-	-	-	0.08	-	18.50	0.87	2.50
	Balneario-Viedma (LV)	-	-	-	-	-	-	0.07	-	23.90	0.69	7.20
Hünicken et al. (2019)	Dren El Molino-Viedma (LV)	-	-	-	-	-	-	-	-	11.68	-	3.68
	Puente Nuevo-Viedma (LV)	-	-	-	-	-	-	-	-	10.50	-	3.60
	Balneario-Viedma (LV)	-	-	-	-	-	-	-	-	11.40	-	3.00

(-) Not analyzed; UV upper valley; MV middle valley; LV lower valley

lotic environments with typical species (*Cricotopus*, *Thienemanniella*, *Limaya*) to more lentic species (*Ablabesmia*, *Dicrotendipes*) (Paggi and Capítulo 2002).

Invasive species are one of the main threats to freshwater biodiversity (Sala et al. 2000). For instance, *C. fluminea* population inhabiting the Negro River propagate in upstream direction to the Limay River at a rate of aprox. 8.8 km year⁻¹ (Labaut 2021). This invasive bivalve causes biotic and structural changes influencing macroinvertebrates assemblages (Vaughn and Hakenkamp 2001; Gutiérrez et al. 2003; Werner 2008). Additionally, fishing with live incubators clams as bait could be an important potential vector for potential invasive species throughout Patagonian freshwater ecosystems (Belz et al. 2012).

There are several studies related to macroinvertebrates in upstream localities, including the Limay and Neuquén rivers, main tributaries of the Negro River. For instance, Luchini (1981) studied the assemblages and their relationships with temperature and water level fluctuations at the upper Limay River. Added to this, significant changes in the composition and decreased taxa richness of macroinvertebrate assemblages have been associated to poor water quality from the Duran stream, a tributary of the Limay River which flows through the city of Neuquén (Province of Neuquén) near to the confluence of Negro River. While in the upstream direction a high taxa richness (38 taxa) was correlated with high dissolved oxygen levels and the occurrence of particularly sensitive species to organic pollution (e.g. Leptoceridae -Trichoptera- and Leptophlebiidae -Ephemeroptera-), in the **downstream** direction, poor oxygenated waters-with values close to anoxia- were associated to low biodiversity (5 taxa). These last taxa belong to families tolerant to organic enrichment, such as Tubificidae, Glossiphoniidae and Chironomidae (Macchi 2008).

Macchi et al. (2018) recorded 43 taxa in the macroinvertebrate assemblages of irrigation and drainage system of Neuquén River basin, including Diptera (mainly Chironomidae), Ephemeroptera, Trichoptera and Gastropoda as the richest taxa. In this study, a decrease in biodiversity and abundance of macroinvertebrates (mainly the sensitive Baetidae -Ephemeroptera-) were associated with higher levels of chlorpyrifos and azinphosmethyl in surface waters. This coincided with an increase of tolerant species/groups such as *Hyaella curvispina* (Amphipoda), subfamily Chironominae and Gastropoda (Macchi et al. 2018). When comparing exposed sites to the insecticide with pristine ones (Anguiano et al. 2008) in Limay and Neuquén rivers, these species showed different levels of resistance to azinphosmethyl in non-target populations of *H. curvispina* and *Simulium* spp. (Diptera). Added to this, Lares et al. (2016) also found populations of *H. curvispina*, *Heleobia* sp. (Gastropoda) and *Girardia tigrina* (Tricladida) in sites with higher chlorpyrifos LC₅₀ values than those previously reported.

River macroinvertebrates are influenced by both, habitat complexity and heterogeneity (Vinson and Hawkins 1998). In the Negro River the types of habitats varied according to the size of the substrate, with a predominance of boulders, cobble and pebbles upstream and higher content of gravel and sand downstream. Also, is common the presence of dense submerged macrophyte patches, generally monospecific, with a coverage close to 10% (Macchi et al. 2019). The most frequent species were *Myriophyllum aquaticum*, *Stuckenia pectinata*, *Stuckenia striata* and *Elodea*

callitrichoides. The substrate also showed litter and woody materials, which comes from riparian forest. The riparian forest patches in the floodplain of the regional rivers are dominated by exotics *Salix alba* and *Populus nigra*, next to *S. humboldtiana* (only native species), *S. rubens* and *P. deltoids*, as companion species (Datri et al. 2016).

Macchi et al. (2019) reported the first approach to establish the entire macroinvertebrate assemblages of Negro River basin, analyzing the relationships with other environmental variables. For the first time, the authors registered 72 macroinvertebrate taxa of 30 families along this river, with Diptera (30), Ephemeroptera (8) and Mollusca (8) as the richest taxa. The most abundant taxa were Chironomidae (mainly *Eukiefferiella*, *Pseudochironomus*, *Tanytarsus*, *Limaya longitarsis* and *Cricotopus*), Ephemeroptera (mainly *Americabaetis alphus* and *Meridialaris* spp.) and *H. curvispina* (Fig. 3). Results indicated that the size of the substrate, conductivity, nutrients and turbidity were the most important environmental variables driving the macroinvertebrate assemblages in the Negro River basin (Macchi et al. 2019).

Macroinvertebrates have been used for environmental monitoring in rivers and streams worldwide. The application of the **biotic index BMPS-RN** (Biological Monitoring Patagonian Streams Negro River; Miserendino and Pizzolón 1999 adapted by Macchi et al. 2019) has allowed to identify the hot spots in the Negro River (Fig. 4, sites in red and orange colors). These were often closely related to drains that collect excess irrigation from agriculture, containing pesticide and fertilizer residues. In some cases, these drains are also used by urban waste treatment plants as an effluent

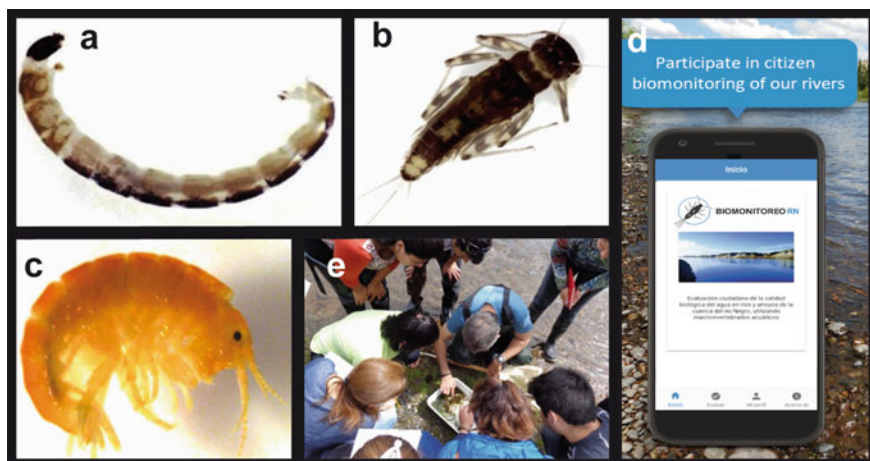


Fig. 3 a–c. Some of most abundant taxa in the Negro River. **a** *Cricotopus* (Chironomidae); **b** *Meridialaris chiloeensis* (Ephemeroptera); **c** *Hyalella curvispina* (Amphipoda). **d** Mobile application developed to enable involvement of the local population in the protection of the Negro River environment. **e** Using the app to provide data on biological water quality in participatory biomonitoring projects in the Negro River

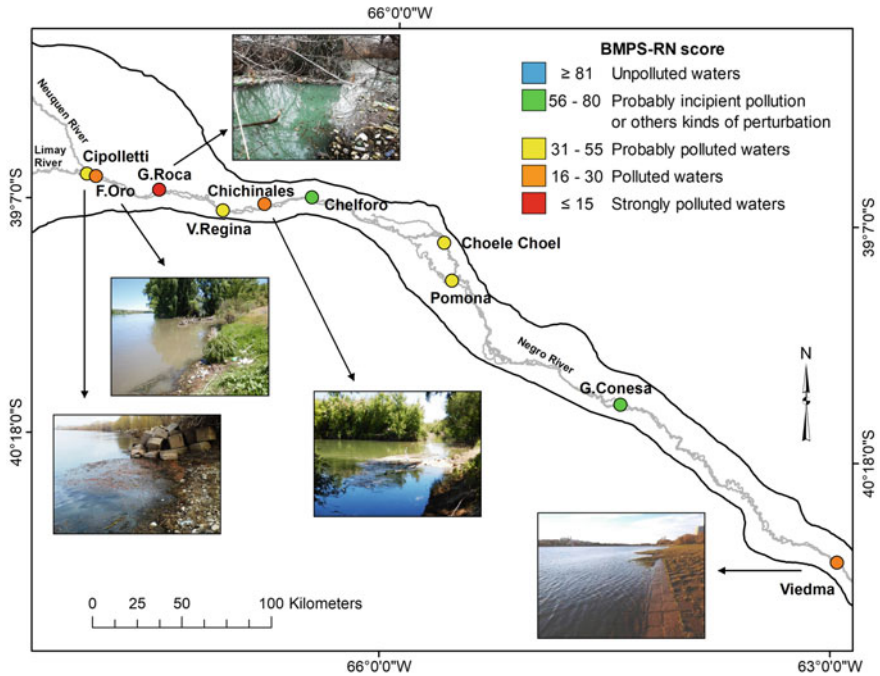


Fig. 4 Biological water quality monitoring based on BMPS-RN index at the Negro River

reception system, with little or no-treatment joint to industrial effluents and clandestine domestic sewage inputs. These contribute to a diffuse source of organic matter, nutrients, heavy metals and POPs into the Negro River. At impacted sites/locations, results showed a reduction in the taxonomic richness due to the elimination of sensitive species and an increase in the abundance of tolerant taxa such as Oligochaeta and Chironominae. The increased numbers of these Rasmussen taxa at most of the degraded sites were consistent with those reported in worldwide moderately polluted rivers (Rasmussen et al. 2013; Horak et al. 2020).

The growing concern about the deterioration of water quality of the Negro River has led to a requirement for routine monitoring and the development of rapid testing that can be used by local governmental water management agencies. It is also important that citizens could get empowered towards sharing a sustainable environment and that all the community stakeholders take up the challenge of conserving and managing river water quality (Macchi and Maestroni 2020). Considering this, a mobile phone application (www.biomonitoreo.com.ar) has been developed by Macchi et al. (2019). This app, called RN Biomonitoring, is an initiative to allow the collective citizen participation in the monitoring of the biological quality of the water of the Negro River, oriented to enhance ownership and emphasize citizen responsibilities in the sustainable management of the national freshwaters (Fig. 3). These actions encourage the construction of a more active role for citizens in environmental monitoring of

natural resources, with greater commitments and reflective approaches to the problem of water.

5 Freshwater fish communities

5.1 *Introduced Species: Status and Potential Problems*

A total of 17 species have been identified in the Negro River; while 9 of these are native, 8 have been introduced from other Argentinean courses (*Corydoras paleatus*, *Cnesterodon decemmaculatus*, *Cheirodon interruptus*, *Psalidodon pampa*, and *Odontesthes bonariensis*) and other world regions (*Oncorhynchus mykiss*, *Salmo trutta*, and *Cyprinus carpio*) (Baigún et al. 2002, Casciotta et al. 2005, Alvear et al. 2007, Aigo et al. 2008, Solimano et al. 2019, Soricetti et al. 2020) (Table 6). This leads to a zoogeographic integrity coefficient of 0.52 (Elvira 1995). In the upper valley of the Negro River, the species assemblage is similar to those of the Andean zone of the Patagonia, including the Percichthyidae, Galaxiidae, Diplomystidae, and Salmonidae families. In particular, *S. trutta* is only found in the upper valley of the river. On the one hand, the abundance of *O. mykiss*, *Galaxias maculatus*, *Diplomystes viedmensis* and *Percichthys trucha* shows a decrease from west to east, with scarce catches in the lower valley (Soricetti et al. 2020). Conversely, *Jenynsia lineata*, *Odontesthes hatcheri*, and *C. carpio* are abundant throughout the entire river. The first report of *C. carpio* was documented at the lower valley by Alvear et al. (2007); after that, this species advanced to the upper valley in just a few years (Alvear et al. 2007, Solimano et al. 2019, Soricetti et al. 2020). Marine species can be found at the lower valley, such as *Paralichthys orbignyanus*, *Mugil liza* and *Genidens barbuis*: while their abundance decrease from east to west, they are not present in the middle and upper valleys. On the other hand, *Odontesthes bonariensis*, freshwater specie, was probably introduced in the lower valley, but it presents a similar pattern of distribution of the marine species.

The two species of the Characidae family, *Psalidodon pampa* and *Cheirodon interruptus*, are rare in the upper valley of the Negro River, although they have been captured at irrigation channels at Allen city area (Solimano et al. 2019). These species are easily found in irrigation channels both in the middle and lower valleys, where they form shoals together with *Corydoras paleatus* (Baigún et al. 2002, Soricetti et al. 2020). It is believed that *C. interruptus* has invaded the Negro River due to illegal releases by recreational fishers, since it is used as live bait for silverside fishing (Maiztegui et al. 2009). Shoals of *C. paleatus* are also very common and are distributed throughout the entire Negro River (Alvear et al. 2007, Solimano et al. 2019, Soricetti et al. 2020). Finally, the species *Cnesterodon decemmaculatus* is rare and can be caught in flooded areas or low lagoons adjacent to the river (Soricetti et al. 2020). Table 6 shows the order, family, species, ordinary name, origin, and relative abundance of fish species at the different valleys of the Negro River.

5.1.1 Salmonids

Salmonids were stocked at the Negro River since 1904, for fishing purposes (Marini 1936). The first stocked species were *Coregonus clupeaformis*, *Salvelinus fontinalis*, *Salvelinus namaychus*, *Salmo salar*, and *Onchorinchus mykiss*, while *Salmo trutta*, *C. clupeaformis*, and *S. namaycush* were not able to establish self-sustainable populations (Macchi et al. 2008; Macchi and Vigliano 2014). Up to date, only *S. trutta* and *O. mykiss* can be found at the river. It is worth to mention that *O. mykiss* is the most abundant salmonid at the Negro River, mainly due to competition and the increase numbers of stocked individuals during the 40 s and 50 s (Macchi et al. 2008; Macchi and Vigliano 2014).

Salmonids are found in all freshwater environments of the Patagonia (Otturi et al. 2020) and their effect on native fauna has been difficult to assess due to the lack of data prior to their introduction (Casalino et al. 2017). Besides, *O. mykiss* and *S. trutta* are on the list of the 100 most harmful invasive alien species in the world (Lowe et al. 2004). Several authors have stated that the introduction of salmonids produces large impacts on native communities (Aigo et al. 2008; Arismendi et al. 2009, 2014; Vigliano et al. 2009; Habit et al. 2010, 2012; Correa et al. 2012). At the Negro River, for instance, *O. mykiss* has been shown to compete for food with the native *P. trucha*, producing a decrease in the growth rate and abundance of the native species (Otturi et al. 2020). The effect of *O. mykiss* on *Galaxias* sp. occurs through two pathways, the first is by trophic competition between *Galaxias* ssp with *O. mykiss* juveniles (Tagliaferro et al. 2015), while the second is related to the predation by *O. mykiss* and *S. trutta* (Macchi et al. 2007; McDowall 2006). In addition, salmonids may be prone to feed on lamprey eggs or larvae (Arakawa and Lampman 2020).

5.1.2 Cyprinus Carpio

C. carpio is found almost everywhere in Argentina (Maiztegui 2016). It was introduced in the early twentieth century, for ornamental and aquaculture purposes (Mac Donagh 1948). Its introduction in the Negro River dates back to 2002 (Alvear et al. 2007) with not known rationale; however, there are some hypotheses by which they could have been used as an ecological answer to control the biomass of macrophytes in the irrigation channel systems. Table 6 shows the order, family, species, ordinary name, origin, and relative abundance of fish species at the different valleys of the Negro River.

The common carp is widely considered an ecosystem engineer (Crooks 2002) since it influences the availability of resources for other organisms (Jones et al. 1994), modifying the structure and functioning of the communities (McCollum et al. 1998; Usio and Townsend 2004). It represents one of the most damaging species for the aquatic systems and is listed between the eight worst invasive fish species in the world according to the IUCN (Lowe et al. 2004). It has a benthic feeding behaviour that produces alterations on the bottom (Tatrai et al. 1994); this sediment removal normally produces a suspension of solids and nutrients into the

Table 6 Order, Family, Species, Ordinary name, Origin, and relative abundance of fish species at the different valleys of the river

Order	Family	Species	Ordinary name	Origin	Relative Abundance		
					Upper valley	Middle valley	Lower valley
Petromyzontiformes	Geotriidae	<i>Geotria macrostoma</i>	Patagonian lamprey	Native	*	*	*
Characiformes	Characidae	<i>Psalidodon pampa</i>	Tetra	Exotic B	*	**	***
		<i>Cheirodon interruptus</i>	Tetra	Exotic B	*	**	***
Siluriformes	Diplomystidae	<i>Diplomystes vielmensis</i>	Velvet catfish	Native	*	*	*
		<i>Corydoras paleatus</i>	Peppered corydoras	Exotic B	**	**	***
		<i>Genidens barbatus</i>	White sea catfish	Native			*
		<i>Cyprinus carpio</i>	Common carp	Exotic C	***	***	***
Cypriniformes	Galaxiidae	<i>Galaxias maculatus</i>	Small puyen	Native	***	*	*
		<i>Salmo trutta</i>	Brown trout	Exotic C	*		
Salmoniformes	Salmonidae	<i>Oncorhynchus mykiss</i>	Rainbow trout	Exotic C	***	*	*
		<i>Odontesthes hatcheri</i>	Patagonic silverside	Native	***	***	***
Atheriniformes	Atherinopsidae	<i>Odontesthes bonariensis</i>	Silverside	Exotic B			***
		<i>Cnesterodon decemmaculatus</i>	Ten spotted live-bearer	Exotic B	*	*	*
		<i>Jenynsia lineata</i>	Onesided livebearer	Native	***	***	***

(continued)

Table 6 (continued)

Order	Family	Species	Ordinary name	Origin	Relative Abundance		
					Upper valley	Middle valley	Lower valley
Perciformes	Percichthyidae	<i>Percichthys trucha</i>	Creole perch	Native	***	***	**
Mugiliformes	Mugilidae	<i>Mugil liza</i>	Mullet	Native			**
Pleuronectiformes	Paralichthyidae	<i>Paralichthys orbignianus</i>	Flounder	Native			**

Built from Alvear et al. (2007), Aigo et al. (2008), Solimano et al. (2019), Soricetti et al. (2020)

* = Rare; ** = Common; *** = Abundant. B = Exotic of Brasilic origin C = Exotic from another continent

water column, thus generating a decrease in transparency and an increase in the algae population through “bottom-up” effects (Tatrai et al. 1990, 1996), modifying chlorophyll-a levels (Matsuzaki et al. 2007). It should be noted that the increase in the number of carp is directly proportional to the degree of river regulation (Gehrke 1997). Currently, *C. carpio* has showed a significant trophic overlap with *P. trucha* in summer and with *O. hatcheri* in spring (Alvear et al. 2007; Crichigno et al. 2013; Conte-Grand et al. 2015). Further impacts on endemic species will depend mostly on the carp biomass (Weber et al. 2010) and will range from decline to local extinction of native species (Crichigno et al. 2016; Koehn et al. 2000).

5.2 Native Species

5.2.1 Geotria Macrostoma

In recent years, its abundance has declined, a phenomenon which has been shown for several lamprey species worldwide (Maitland et al. 2015; Boulêtreau et al. 2020). Despite the fact that two decades ago it was common to observe large shoals of lampreys at the Negro River, recent studies have shown a scarcity or even absence of lamprey larvae. Alvear et al. (2007), Aigo et al. (2008), Solimano et al. (2019) and Soricetti et al. (2020) reported no captures, while Riva-Rossi et al. (2020) mentioned the capture of 3 juveniles of *G. macrostoma* in the lower valley. Hypotheses on the diminished abundance of this native species range from habitat modification by dams, climate change and the introduction of predators into the river (Mateus et al. 2012; Maitland et al. 2015; Hansen et al. 2016; Boulêtreau et al. 2020) such as the introduction of *C. carpio*. For instance, Arakawa and Lampman (2020) found that there is a high rate of consumption of ammocetes larvae by this species. Finally, the native species *G. macrostoma* has disappeared from the upper and middle reaches of the Limay River, upstream the dams (Pascual et al. 2007; Cussac et al. 2016).

5.2.2 Aplochiton Spp

On the one hand, the understanding of what has happened to the species of the genus *Aplochiton* in several Patagonian rivers, particularly in the Negro River, is extremely complex since there is no baseline knowledge or recorded population status prior to the entry of the salmonids (Casalinuovo et al. 2017). On the other hand, no individuals of this genus have been obtained in Patagonian rivers since 1945. *Aplochiton zebra* was reported for the Negro River by Pozzi (1945) and by Marini (1936) and Sorçaburu at Puerto Blest (captures performed in 1930 and 1933 respectively; Piacentino 1999; Cussac et al. 2020). In recent studies, no specimens of this species were caught (Alvear et al. 2007; Solimano et al. 2019; Soricetti et al. 2020) for both, the Negro and Limay rivers. In areas with still known occurrence, Patagonian rivers with Pacific catchments, it is commonly observed that they are

highly affected by salmonids (Lattuca et al. 2008; Arismendi et al. 2009). They are also abundant species at Chilean lakes where there is no presence of salmonids (Soto et al. 2006; Arismendi et al. 2009).

5.2.3 *Diplomistes Viedmensis*

The family Diplomystidae is considered the first lineages to diverge from the ancestor of all living Siluriformes (Arratia 1987; Sullivan et al. 2006; Muñoz-Ramírez et al. 2014). Endemic from southern South America is distributed at both sides of the Andes (Muñoz-Ramírez et al. 2014) and consist of seven species, most of them facing conservation issues or extinct (Muñoz-Ramírez et al. 2010, 2014; Arratia and Quezada-Romegialli 2017). It is poorly known in Argentina (Bello and Ubeda 1998) where has been suggested the need of maximum conservation priority (Bello and Ubeda 1998; López et al. 2002). In the Negro River, *D. viedmensis* is still present but faces the anthropic use of the habitat and introduced species as relevant threats (Arratia 1987; López et al. 2002).

5.3 *Environmental Threats for Fish Assemblages*

Considering the current climate change predictions, two main processes can be expected to occur: the southward expansion of the Brazilian fauna and other introduced species (*P. pampa*, *Ch. interruptus*, *C. carpio*) and the extinction of southern fauna such as galaxids and salmonids (Cussac et al. 2020). An example of this type of displacement is the decrement of *G. barbuis* communities in southeastern Brazil (Araújo et al. 2018) and its increment of the abundance of this species in the Negro River area, the southern part of its distribution (Solimano et al. 2019, Soricetti et al. 2020) and this may pose risks for the native *G. macrostoma*, a fact demonstrated to occur, with the interaction of predators siluriforms and lamprey, in southern France (Cucherousset et al. 2018) and Columbia River (Close et al. 1995).

Regarding future dams, while it is well known that flow fluctuations can generate biological effects on taxa that depend on flood or riparian habitats (Pringle et al. 2000), no reliable consequences on communities are still known (Macchi et al. 1999; Temporetti et al. 2001; Cussac et al. 2016).

Typically, the biodiversity crisis addresses species loss through extinction, although on a sub-global scale, the loss of populations through local extirpation and invasion by exotic species may be of most concern (Olden et al. 2011). Climate change is one of the main drivers of biotic homogenization, causing a change in species composition, but not in species richness (Magurran et al. 2015). If we take into account the studies conducted at the Negro River, the results show that the diversity is reduced and most of the inhabiting species are not native (Alvear et al. 2007; Solimano et al. 2019; Soricetti et al. 2020). Climate change and direct anthropic interventions such as live bait, recreational fishing, aquarium escapes or other uses

have led to a shift of the species transition zone to the south (Pérez and Cazorla 2008; Soricetti et al. 2020).

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