

Longitudinal patterns in water quality and fish assemblage structure in a low-gradient, temperate Neotropical stream disrupted by a point-source urban effluent

Ignacio Masson^{1,2,3,*} , Andrea Bertora^{3,4,5} , Juan José Rosso^{4,6} , José González Castellain^{1,3} , Sabrina Dubny^{1,4} , Natalia Othax^{1,3,4}  and Fabio Peluso^{1,2,3} 

¹ Instituto de Hidrología de Llanuras “Dr. Eduardo Usunoff”. Azul, Buenos Aires, Argentina.

² Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CIC), La Plata, Argentina.

³ Universidad Nacional del Centro de la Provincia de Buenos Aires (UNCPBA), Tandil, Argentina.

⁴ Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Ciudad Autónoma de Buenos Aires, Argentina.

⁵ Instituto Multidisciplinario sobre Ecosistemas y Desarrollo Sustentable (UNCPBA-CIC), Universidad Nacional del Centro de la Provincia de Buenos Aires, Pje. Arroyo Seco, s/n, Campus Universitario, Tandil, B7000, Buenos Aires, Argentina.

⁶ Grupo de Biotaxonomía Morfológica y Molecular de Peces (BIMOPE), Instituto de Investigaciones Marinas y Costeras, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, CONICET, Mar del Plata, Argentina.

* Corresponding author: imasson.ihlla@gmail.com

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ABSTRACT

Longitudinal patterns in water quality and fish assemblage structure in a low-gradient, temperate Neotropical stream disrupted by a point-source urban effluent

Low-gradient, prairie streams from the Pampa Plain, Argentina, constitute the southernmost geographic occurrence for many Neotropical fish species. Fish assemblages in Neotropical streams have shown drastic responses to the modification of habitat and water quality. Under this scenario it could be hypothesized that the water quality will be influenced by the anthropic activities and the natural conditions and that the ichthyofauna will be responsive. Therefore, understanding the functioning of these ecosystems is crucial for their conservation in a world with ever-growing anthropic pressures. In order to explore patterns in fish assemblage and water quality under urbanization pressure, the Del Azul stream (Buenos Aires, Argentina) was sampled. The objectives of the study were: (1) to explore trends in water quality and fish assemblage structure along the stream, (2) to study the relationship between fish assemblage structure and water quality, and (3) to pinpoint individual species with potential for biomonitoring. Seven sites were selected along the stream, and they were sampled twice during each of five consecutive summer periods. Through the use of multivariate analysis, we revealed trends in water quality and fish assemblage structure where water quality characteristics were more distinctive of a basin sector than the fish species abundances. Irrespective of that, the Indicator Species Analysis evidenced some key species indicative of given groups of sites sharing similar water quality characteristics. Namely, *Cnesterodon decemmaculatus* was associated to polluted reaches downstream from the point-source urban effluent, and *Cheirodon interruptus*, *Pimelodella laticeps* and *Oligosarcus jenynsii* to lower salinities and less polluted reaches upstream from the point-source urban effluent. All these species could be useful for biomonitoring temperate Neotropical streams.

Key words: Pampa Plain, Neotropical fish, temperate streams, water quality, biomonitoring, point source pollution

RESUMEN***Patrones longitudinales en la calidad del agua y la estructura del ensamblaje de peces en un arroyo Neotropical de bajo gradiente y clima templado afectado por un efluente urbano puntual***

*Los arroyos de bajo gradiente de la llanura pampeana, Argentina, constituyen la ocurrencia geográfica más austral para muchas especies de peces Neotropicales. Aquí, las poblaciones de peces han mostrado respuestas drásticas a la modificación del hábitat y de la calidad del agua. En este escenario se podría hipotetizar que la calidad del agua estará influenciada por actividades antrópicas y condiciones naturales y que la ictiofauna será sensible. Por lo tanto, comprender el funcionamiento de estos ecosistemas es crucial para su conservación. Con el fin de explorar patrones en el ensamble de peces y la calidad del agua bajo la presión de urbanización, se muestreó el Arroyo del Azul (Buenos Aires, Argentina). Los objetivos del estudio fueron: (1) explorar tendencias en calidad del agua y estructura de la población de peces a lo largo del arroyo, (2) estudiar la relación entre la estructura de la población de peces y la calidad del agua, y (3) identificar especies con potencial para biomonitorio. Se muestrearon siete sitios en la época estival, dos veces durante cinco años. Mediante el uso de análisis multivariado, hallamos tendencias en la calidad del agua y la estructura de ensamblajes de peces, donde la calidad del agua fue más distintiva de sectores de cuenca que la abundancia de especies. Por otro lado, el análisis de especies indicadoras reveló especies clave. En concreto, *Cnesterodon decemmaculatus* asociada a tramos contaminados aguas abajo del efluente urbano puntual, y *Cheirodon interruptus*, *Pimelodella laticeps* y *Oligosarcus jenynsii* a tramos de menor salinidad y menos contaminados aguas arriba del efluente urbano puntual. Estas especies podrían ser útiles para el biomonitorio de arroyos Neotropicales templados.*

Palabras clave: *llanura pampeana, peces Neotropicales, arroyos templados, calidad de agua, biomonitorio, efluentes*

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INTRODUCTION

Anthropic pressures on the environment have globally intensified since the advent of the industrial revolution (Jackson, 2017). Freshwater ecosystems are particularly susceptible to pollution due to large amounts of the waste and contaminants produced on land end up in water bodies. Water pollution, habitat alteration, overfishing, exotic species introduction, agricultural and urban expansion are some of the ever-growing threats faced by freshwater ecosystems (Albert *et al.*, 2021). Despite warnings based on scientific evidence (Winemiller *et al.*, 2016), these threats persist. Certainly, these ever-growing anthropic pressures interact with local aspects of ecosystems to regulate the biotic structure (Zalewski, 2015; Collier *et al.*, 2019).

The fact that environmental factors influence the biotic composition of aquatic ecosystems (Marshall & Elliott, 1998; Selleslagh & Amara, 2008; Göthe *et al.*, 2013) constitutes the basis of biomonitoring, which implies the use of biota to gauge and track changes in the environment (Frigberg *et al.*, 2011). Common organisms used for biomonitoring include bacteria, protozoans, diatoms, algae, macrophytes, benthic macroinverte-

brates and fish (Abbasi & Abbasi, 2012). Despite the use of macroinvertebrates for biomonitoring freshwater ecosystems has been the most globally widespread method (Rosenberg & Resh, 1993; Bae *et al.*, 2005; Buss *et al.*, 2015), the use of fish has some advantages. Unlike macroinvertebrates, fish species identification usually does not require so much expertise and often can be done on site during sampling (Karr, 1981). Fish also have longer lifespans than macroinvertebrates, they live in the water all their life, and therefore, they continually inhabit the water body and integrate the chemical, physical and biological histories of the aquatic ecosystems (Karr, 1981; López & Sedeño, 2015). Besides, due to their position at the top of the aquatic food web in relation to the macroinvertebrates, they provide an integrative view of the watershed environmental quality (Karr, 1981). For these reasons, analyzing the fish assemblage structure was postulated as being effective for inferring the ecological condition of streams (Karr, 1981; Fausch *et al.*, 1990; Scardi *et al.*, 2006).

Fish assemblages in Neotropical streams have shown drastic responses to the modification of habitat and water quality triggered by human activities. This has been more exhaustively studied in tropical ecosystems at lower latitudes (Cunico

et al., 2006; Araujo et al., 2009; Alexandre et al., 2010; Teresa et al., 2015). Conversely, temperate streams have been less explored (Granitto et al., 2016; Crettaz-Minaglia & Juarez, 2020; Paracampo et al., 2020; Paredes del Puerto et al. 2021; Bertora et al., 2021a, 2022a). Unlike Neotropical streams at lower latitudes which harbor a highly diversified ichthyofauna, temperate Neotropical streams have fish assemblages represented by a very impoverished subset of species (Ringuelet, 1975; Menni, 2004). The reduction in species numbers is further strengthened beyond the La Plata River, where many fish families have their southernmost distribution (López et al., 2002). In this scenario of pauperized fish assemblages (Menni et al., 1996; Paracampo et al., 2015, 2020), the magnitude and direction of the response of fish species to environmental conditions, if any, could not be easily anticipated. Just recently, the response of fish fauna at these latitudes to the combined effects of exotic species, river regulation and water quality was assessed (Bertora et al., 2021a). Similarly, major patterns in fish assemblages' structure in relation to urbanization and agricultural activities were also surveyed (Paredes del Puerto et al., 2021; Bertora et al., 2022a). In spite of the relevance of these recent findings mostly derived from case studies in single streams, more evidence is needed to accurately understand fish assemblage patterns at a regional scale.

In this paper, we studied major physicochemical water quality variables and fish community composition along a low-gradient temperate Neotropical prairie stream running through an urbanized area. As water quality varies along a stream course conditioned by anthropic activities and intrinsic characteristics of the basin (Amuchástegui et al., 2016; da Rocha et al., 2018; Bertora et al., 2022b) and fish assemblages respond to those water quality changes (Karr, 1981; Teresa & Casatti, 2012; Bertora et al., 2022a) we expected that stream reaches belonging to the same sectors of the basin would share similar water quality characteristics and fish assemblage structure. In addition, pollution would increase as water flows downstream, with stream reaches immediately downstream from the urban zone being more polluted whereas those further downstream from it (i.e., lower basin reaches) showing some water

quality recovery. We also expected some species would be more represented at certain water quality conditions (e.g., more saline, less polluted waters) than others. Accordingly, we foresee linking individual species to certain water quality conditions to potentially use them as bioindicators.

Based on our expectations, the objectives of the study were: (1) to explore trends in water quality and fish assemblage structure along the stream course, (2) to study the relationship between fish assemblage structure and water quality, and (3) to pinpoint individual species with potential for biomonitoring.

METHODS

Study area

The Salado del Sur River Basin (34° 7' S to 35° 59' S) represents the southernmost geographic occurrence for some Neotropical fish species (Menni, 2004; Gómez, 2015). This basin with a surface area of 170 000 km² comprises most of the freshwater ecoregion known as Bonaerensean Drainages (Abell et al., 2008), and it is included within the Pampean ecoregion of Argentina (Matteucci, 2012). The streams of the Pampa Plain are characterized by low current velocities, lack of riparian forests, high nutrient levels, no dry periods or exposure to extreme temperatures, fine substrates and dense and rich macrophyte communities (Giorgi et al., 2005). A list of fish species inhabiting the basin was published by Gómez (2015) which accounts for a total of 46 species.

The Del Azul stream (Azul, Buenos Aires province, Argentina) is 160-km long and runs north-east (Fig. 1). Its flow rate under stationary conditions is 2-3 m³/s. The mean annual precipitation in the basin is 908 mm and the mean annual temperature is 14.5 °C (Varni et al., 2019). Azul city with approximately 70 000 inhabitants is developed on both margins of the stream, 60 km downstream from the headwaters. The Del Azul basin has a surface area of 6000 km² (Entraigas & Vercelli, 2013). The main productive activities in the basin are agriculture and cattle farming. Agriculture predominates in the upper and middle basin; the main crops are soybean, wheat and corn. The cattle farming sector is more developed in the

lower basin where soil conditions limit agriculture (Ares *et al.*, 2007). Upper basin land slopes are 0.5-2 % whereas lower basin land slopes decrease to 0.2-0.1 % (Sala *et al.*, 1987). In the urban area, the stream receives many pluvial network discharges, the effluent of the sewage treatment plant (STE) and non-point source pollutants common to urban areas. The natural landscape in the urban area has been modified, with roads close to the stream, bridges, rest and workout stations, parks and a beach (Grosman & Merlos, 2011). In addition, some urban and post-urban segments have been widened and dredged to prevent city flooding in cases of extreme precipitation events. At the sampling sites, stream widths ranged from 8 to 10 m. Artificially-reshaped segments were excluded from the study.

Twenty-two fish species are known for this ecosystem (Grosman & Merlos, 2011; Bertora *et al.*, 2018a) including some occasional visitors from the paranoplatense fish fauna. The occur-

ence of these uncommon fish species in temperate Neotropical streams has been linked to man-made channels that allow the connection of these environments with the Samborombón Bay (Bertora *et al.*, 2018b) during environmental and climatological conditions that produce a freshwater dominance in the Bay (Jaureguizar *et al.*, 2016).

Sampling protocol

The study was conducted at seven reaches along the Del Azul stream, covering its upper, middle and lower basin (Fig. 1). Urban, pre-urban and post-urban areas of the basin were considered into the analysis as well (Table 1). Sampling sites sharing similar aquatic vegetation, water depth, width, substrate type and lack of canopy cover were selected for the study. Macrophytes such as *Ludwigia* sp., *Hydrocotyle* sp. and *Typha* sp., were characteristically present throughout the sampled reaches. Maximum depths at the sampling loca-

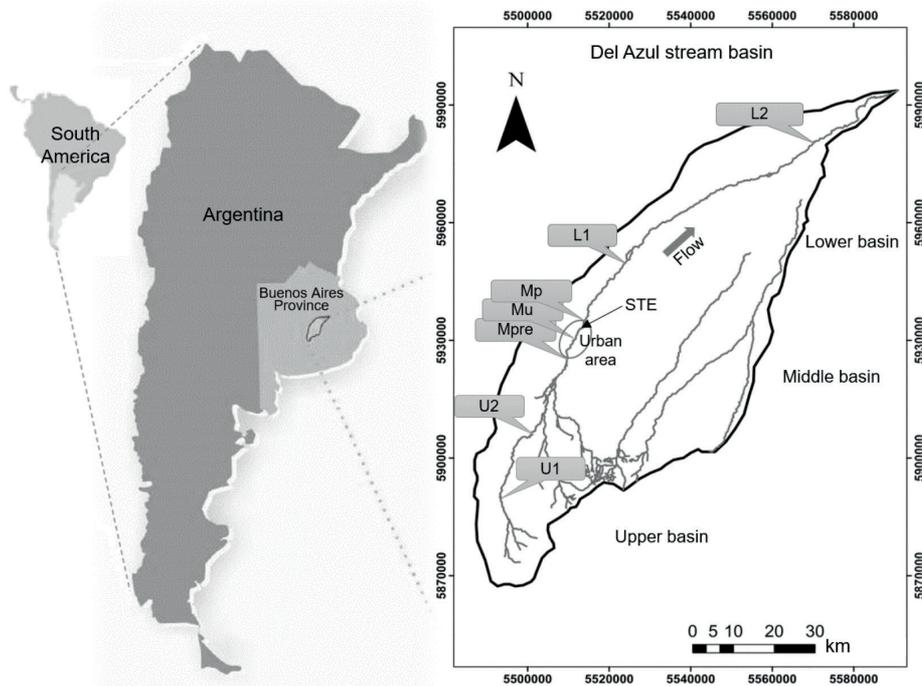


Figure 1. Location of the sampled reaches. U1: Upper basin 1; U2: Upper basin 2; Mpre: Middle basin pre-urban; Mu: Middle basin urban; Mp: Middle basin post-urban; L1: Lower basin 1; L2: Lower basin 2; STE: sewage treatment plant effluent. *Localización de los sitios muestreados.* U1: cuenca alta 1; U2: cuenca alta 2; Mpre: cuenca media pre-urbano; Mu: cuenca media urbano; Mp: cuenca media post-urbano; L1: cuenca baja 1; L2: cuenca baja 2; STE: efluente de la planta de tratamiento cloacal.

tions ranged from 0.75 to 1 m. Stream substrate is characterized by the coexistence of patches of fine sediments (sand, silt and clay) and limestone. According to the Strahler method (Strahler, 1954), the two upper basin sampling sites are on third order segments whereas the rest of the sampling stations are on fourth order segments (Table 1). Sampling campaigns were conducted twice during each of five consecutive summer periods: December 2015, February 2016, December 2016, February 2017, December 2017, February 2018, December 2018, February 2019, December 2019 and February 2020.

Fish were sampled with a seine net (5 x 1 m², length x width) covering a 50-m² surface area per reach in each of the sampling campaigns. Captured fish were kept alive, identified to species, counted and released on site. Fish species abundance was expressed as the total number of individuals of each species captured at each reach per sampling campaign.

The water quality variables evaluated in this study were: pH (pH), dissolved oxygen (DO), electric conductivity (EC), Na⁺, K⁺ (K), SO₄²⁻ (SO₄), NO₂⁻ (NO₂), NO₃⁻ (NO₃), NH₄⁺ (NH₄), total phosphorous (TP), total solids (TS), suspended solids (SS), turbidity, 5-d biochemical oxygen demand (BOD) and total coliforms (TC). DO, EC and pH were measured on site using portable equipment: YSI model 58 oximeter, Altronix CT2 conductivity meter and Altronix TPA IV pH meter, respectively. For the rest of the variables, subsurface, midstream water samples were taken, stored at 4 °C and transported to the laboratory for analysis. Analytical protocols from APHA (2017) were followed for the determinations.

Data Analyses

Differences in mean values of the water quality variables among reaches considering their position along the stream were evaluated using Kruskal-Wallis tests before determining that the Normal distribution criteria was not met by any of the variables. Only those that showed significant differences ($p < 0.05$) in mean values among reaches were used in the multivariate analyses. Further, multivariate analyses were conducted with non-redundant water quality variables (i.e.,

Table 1. List of the studied reaches, their distances from the stream origin and sectors of the basin where they are located. The stream orders were assigned according to the Strahler method (Strahler, 1954). *Lista de tramos estudiados, distancias a la naciente y sectores de la cuenca en los que se ubican. Los órdenes de los tramos de arroyo se asignaron según el método de Strahler (Strahler, 1954).*

Reach name	Code	Distance from stream origin (km)	Sector of the basin	Stream order
Upper 1	U1	16	Upper	3 rd
Upper 2	U2	41	Upper	3 rd
Middle pre-urban	Mpre	59	Middle	4 th
Middle urban	Mu	65	Middle	4 th
Middle post-urban	Mp	69	Middle	4 th
Lower 1	L1	88	Lower	4 th
Lower 2	L2	148	Lower	4 th

those not showing multicollinearity). Redundant variables were detected through correlation by setting R and p threshold levels at 0.75 and 0.05, respectively. Only one variable of a correlated group was used in the multivariate analyses. Prior to applying multivariate techniques, the values of the water quality variables were standardized to zero mean and unit variance and fish species relative abundances were arcsin-square root transformed (Zar, 1999).

In order to explore water quality resemblance patterns among sampled reaches along the stream course (i.e., objective 1), a cluster analysis (CA) with Ward's method for clustering was conducted. This method uses an analysis of variance approach to evaluate the distances between clusters, attempting to minimize the sum of squares of any two hypothetical clusters that can be formed at each step. It is considered efficient for the evaluation of spatial and temporal patterns in water quality (Wunderlin et al., 2001). Differences among samples were measured using city-block distances which according to McCune & Grace (2002) is less sensitive to outliers than Euclidean distances. Following the same approach, CA was also performed to study differences in fish species relative abundances among reaches. Statistica v.8.0 software (StatSoft Inc., 2007) was used

for the CA analyses. Further, the RELATE routine (Clarke & Gorley, 2015) was used to assess whether the observed changes in water quality and fish assemblages respond to a longitudinal continuum. This analysis evaluates the level of association (R = Spearman's correlation coefficient) between the Euclidean distance matrix of the water quality variables or the specific abundances of

fish and the serial model matrix of the distances between reaches. A total of 9999 random permutations were used to evaluate the significance of Spearman's correlation between both models. The distances between sites were calculated from Table 1. This analysis was performed with the software PRIMER.5 (Plymouth Routines In Multivariate Ecological Research).

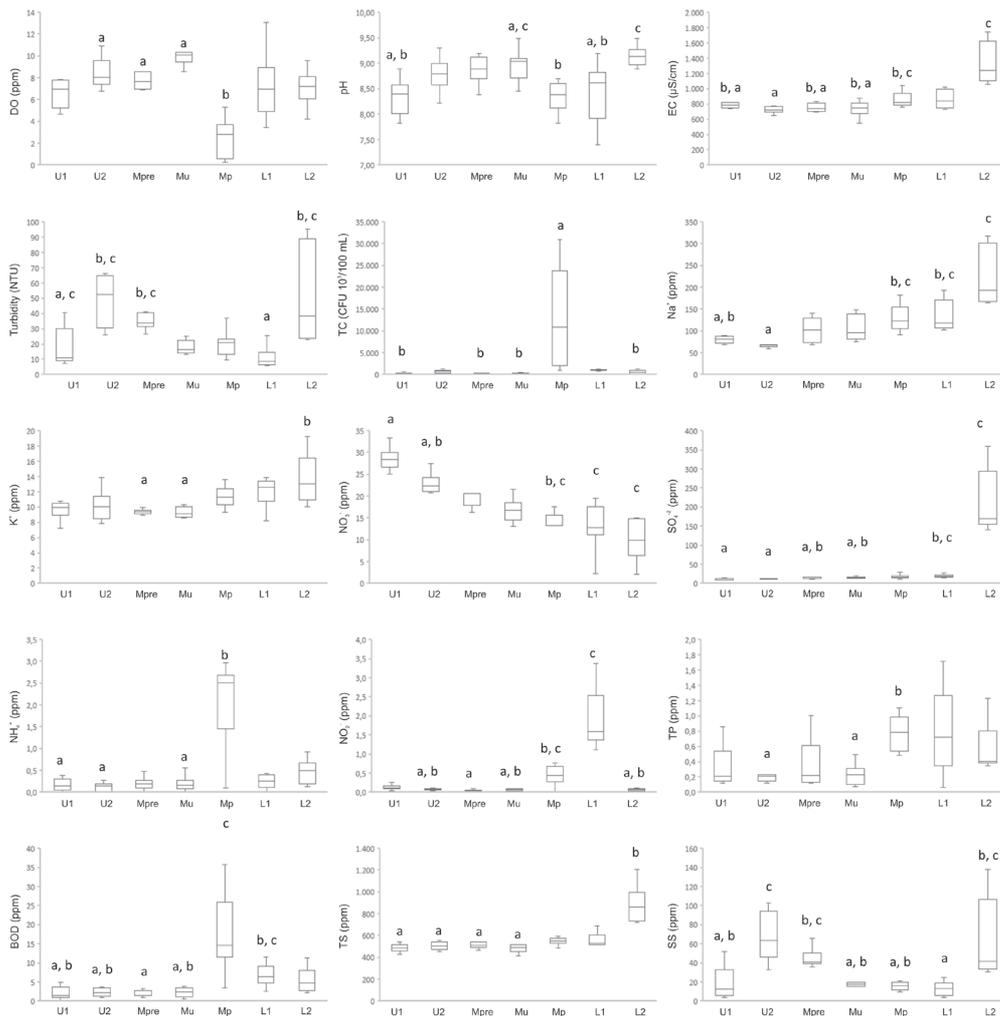


Figure 2. Box-whisker plots for water quality variables along the Del Azul stream. Different letters above box plots denote significant differences in mean values ($p < 0.05$). Reaches codes: U1, upper 1; U2, upper 2; Mpre, middle pre-urban; Mu, middle urban; Mp, middle post-urban; L1, lower 1; L2, lower 2. DO: dissolved oxygen. EC: electric conductivity. TC: total coliforms. TP: total phosphorous. BOD: 5-d biochemical oxygen demand. TS: total solids. SS: suspended solids. *Diagramas de caja y bigotes para para las variables de calidad de agua del arroyo del Azul. Letras distintas indican diferencias significativas en los valores medios ($p < 0.05$). Códigos utilizados para los tramos de arroyo: U1, cuenca alta 1; U2, cuenca alta 2; Mpre, cuenca media pre-urbano; Mu, cuenca media urbano; Mp, cuenca media post-urbano; L1, cuenca baja 1; L2, cuenca baja 2. DO: oxígeno disuelto. EC: conductividad eléctrica. TC: coliformes totales. TP: fósforo total. BOD: demanda bioquímica de oxígeno 5-d. TS: sólidos totales. SS: sólidos suspendidos.*

To study the relationships between fish assemblage structure and water quality (i.e., objective 2), a detrended correspondence analysis (DCA) was performed and, as data showed a linear response, a Canonical Correspondence Analysis (CCA) was applied (Legendre & Legendre, 1998).

Finally, to pinpoint individual species with potential for biomonitoring (i.e., objective 3) we performed Indicator Species Analysis (IndVal) for identifying species indicative of given groups of sites (Dufrêne & Legendre, 1997). Sites were grouped according to their water quality similarities based on the results of the CA. Paleontological Statistics (PAST) version 4.12 software was used for the analysis (Hammer & Harper, 2006).

RESULTS

The water quality variables that showed significant differences ($p < 0.05$) in mean values among reaches were: pH, DO, EC, Na^+ , K^+ , SO_4^{2-} , NO_2^- , NO_3^- , NH_4^+ , TP, ST, SS, turbidity, BOD and TC (Fig. 2). From these, two pairs of variables showed multicollinearity: the pair EC and Na^+ ($R = 0.80$, $p < 0.05$) and the pair SS and turbidity ($R = 0.78$, $p < 0.05$). To avoid redundancy, the former variable of each redundant pair was retained for use in the multivariate analyses (CA and CCA).

The CA dendrogram for the water quality variables evidenced two discrete main clusters (Fig. 3). Cluster 1 grouped all reaches upstream from

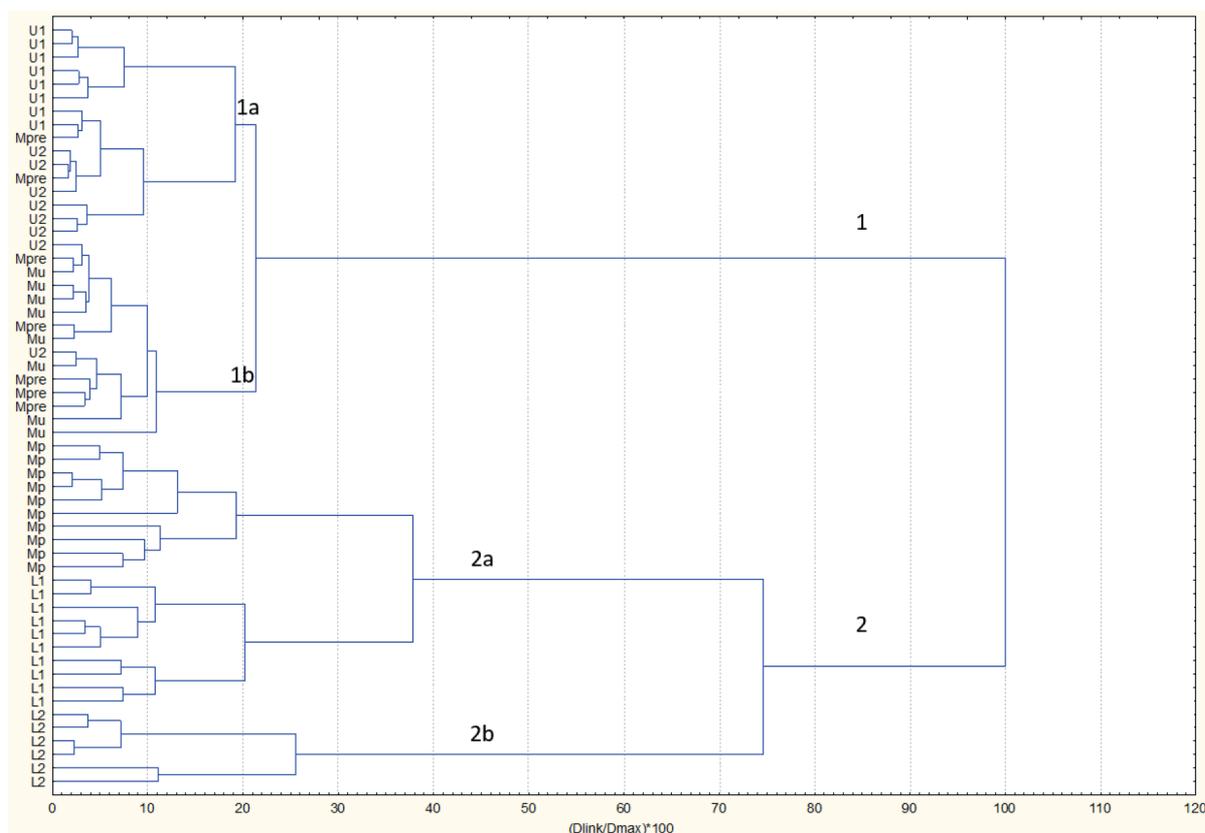


Figure 3. Dendrogram showing clusters of samples from different reaches based on the similarity for the water quality variables. Numbers and letters above branches are used in the text for reference. Reaches codes: U1, upper 1; U2, upper 2; Mpre, middle pre-urban; Mu, middle urban; Mp, middle post-urban; L1, lower 1; L2, lower. *Dendrograma de agrupamiento de muestras de diferentes tramos basados en la similitud en sus variables de calidad de agua. Los números y letras sobre las ramificaciones son utilizados en el texto como referencia. Códigos utilizados para los tramos de arroyo: U1, cuenca alta 1; U2, cuenca alta 2; Mpre, cuenca media pre-urbano; Mu, cuenca media urbano; Mp, cuenca media post-urbano; L1, cuenca baja 1; L2, cuenca baja 2.*

Table 2. Species composition and abundances of fish at each sampling reach. Number of sampling events shown between parentheses next to the reach code. The abundance represents the number of fish captured at a certain reach for the entire sampling period. In-between parenthesis next to the abundance value is the mean abundance and the standard error per sampling event. U1: Upper basin 1; U2: Upper basin 2; Mpre: Middle basin pre-urban; Mu: Middle basin urban; Mp: Middle basin post-urban; L1: Lower basin 1; L2: Lower basin 2. *Composición de especies y abundancia de peces en cada tramo muestreado. El número de eventos muestrales se presenta entre paréntesis al lado del código de tramo. La abundancia representa el número de peces capturados en un tramo para la totalidad del periodo estudiado. Los valores medios de abundancia y el error estándar por evento muestral se presentan entre paréntesis a un lado del valor de abundancia. U1: cuenca alta 1; U2: cuenca alta 2; Mpre: cuenca media pre-urbano; Mu: cuenca media urbano; Mp: cuenca media post-urbano; L1: cuenca baja 1; L2: cuenca baja 2.*

Order	Species (code)	U1 (8)	U2 (8)	Mpre (7)	Mu (8)	Mp (10)	L1 (10)	L2 (6)	Total
		Abundance (mean±SE)							
	<i>Pinelodella laticeps</i> (Pl)	32 (4.00±1.69)	32 (4.00±2.47)	0	95 (11.88±4.07)	1 (0.10±0.10)	34 (3.40±1.42)	6 (1.00±0.52)	200
	<i>Rhamdia quelen</i> (Rq)	3 (0.38±0.18)	7 (0.88±0.44)	0	0	4 (0.40±0.22)	23 (2.30±1.27)	0	37
SILURIFORMES	<i>Corydoras paleatus</i> (Cp)	27 (3.38±0.86)	31 (3.88±0.90)	19 (2.71±0.92)	30 (3.75±1.24)	16 (1.60±0.62)	2 (0.20±0.20)	1 (0.17±0.17)	126
	<i>Loricariichthys anus</i> (La)	0	0	2 (0.29±0.29)	22 (2.75±2.47)	3 (0.30±0.30)	2 (0.20±0.13)	0	29
	<i>Hypostomus commersoni</i> (Hc)	0	7 (0.88±0.48)	3 (0.43±0.43)	21 (2.63±1.24)	11 (1.10±0.80)	17 (1.70±0.91)	3 (0.50±0.34)	62
	<i>Psalidodon pampa</i> (Pp)	38 (4.75±1.53)	33 (4.13±1.62)	79 (11.29±5.78)	121 (15.13±6.07)	24 (2.40±1.50)	531 (53.10±41.22)	262 (47.60±14.28)	1,088
	<i>Bryconamericus theingi</i> (Bi)	80 (10.00±3.09)	75 (9.39±3.95)	95 (13.57±7.36)	25 (3.13±1.54)	0	237 (23.70±17.18)	55 (9.17±7.82)	567
	<i>Cheirodon interruptus</i> (Ci)	105 (13.13±4.80)	144 (18.00±6.07)	603 (86.14±38.88)	109 (13.63±3.70)	31 (3.10±1.93)	330 (33.00±19.47)	3 (0.50±0.22)	1,325
CHARACIFORMES	<i>Oligosarcus jenynsii</i> (Oj)	43 (5.38±1.91)	5 (0.63±0.32)	44 (6.29±3.25)	5 (0.63±0.32)	6 (0.60±0.43)	9 (0.90±0.43)	14 (2.33±0.76)	126
	<i>Cyphocharax voga</i> (Cv)	0	1 (0.13±0.13)	0	0	1 (0.10±0.10)	0	9 (1.50±0.96)	11
	<i>Hoplias argentinensis</i> (Ha)	0	0	0	1 (0.13±0.13)	5 (0.50±0.40)	5 (0.50±0.17)	0	11
CYPRINIFORMES	<i>Cyprinus capio</i> (Cc)	0	0	14 (2.00±1.84)	0	0	1 (0.10±0.10)	0	15
CYPRINODONTIFORMES	<i>Jenynsia lineata</i> (Jl)	160 (20.00±11.70)	105 (13.13±4.04)	207 (29.57±8.68)	80 (10.00±3.60)	321 (32.10±11.97)	537 (53.70±29.62)	0	1,410
	<i>Chesterodon decemmaculatus</i> (Cd)	6 (0.75±0.62)	33 (4.13±0.97)	54 (7.71±4.50)	3 (0.38±0.26)	454 (45.40±23.18)	138 (13.80±8.68)	1 (0.17±0.17)	689
SYBRANCHIFORMES	<i>Sybranchus marmoratus</i> (Sm)	0	0	0	0	3 (0.30±0.15)	4 (0.40±0.22)	0	7
CICHLIFORMES	<i>Australoheros facetus</i> (Af)	4 (0.50±0.19)	4 (0.50±0.27)	9 (1.29±0.47)	238 (29.75±11.03)	40 (4.00±1.44)	29 (2.90±1.03)	1 (0.17±0.17)	325
Total	16 species	498	477	1,129	750	920	1,899	355	6,028

STE and cluster 2 grouped all reaches downstream from STE. Further, cluster 2 split into two sub clusters differing 75 % from each other: one identified as subcluster 2b was formed exclusively by all L2 samples and the other identified as subcluster 2a was formed by all L1 and all Mp samples. Further, within 2a, samples from Mp and L1 formed separate subclusters as well. Cluster 1 also split into two subclusters. Subcluster 1a grouped uppermost samples, containing all U1 samples, 75 % of U2 samples and two Mpre samples, the latter belonging to the middle reaches. Subcluster 1b grouped most middle basin samples upstream from STE, containing all Mu samples, 71 % of Mpre samples and two U2 sam-

ples, the latter belonging to the upper basin sector.

Overall, 21 % of the total variation in water quality was significantly explained by the longitudinal position of reaches in the stream network ($R = 0.206$, $p = 0.007$).

A total of 6028 individuals were captured throughout the study (Table 2). The only exotic species documented was *Cyprinus carpio*.

The CA dendrogram for the fish assemblage revealed two separate main clusters (Fig. 4). Roughly, cluster 1 accounted for fish assemblages of upper and middle reaches upstream from STE but also all samples for the lowermost reach at L2. Conversely, cluster 2 mostly grouped samples from middle basin reaches with very few samples

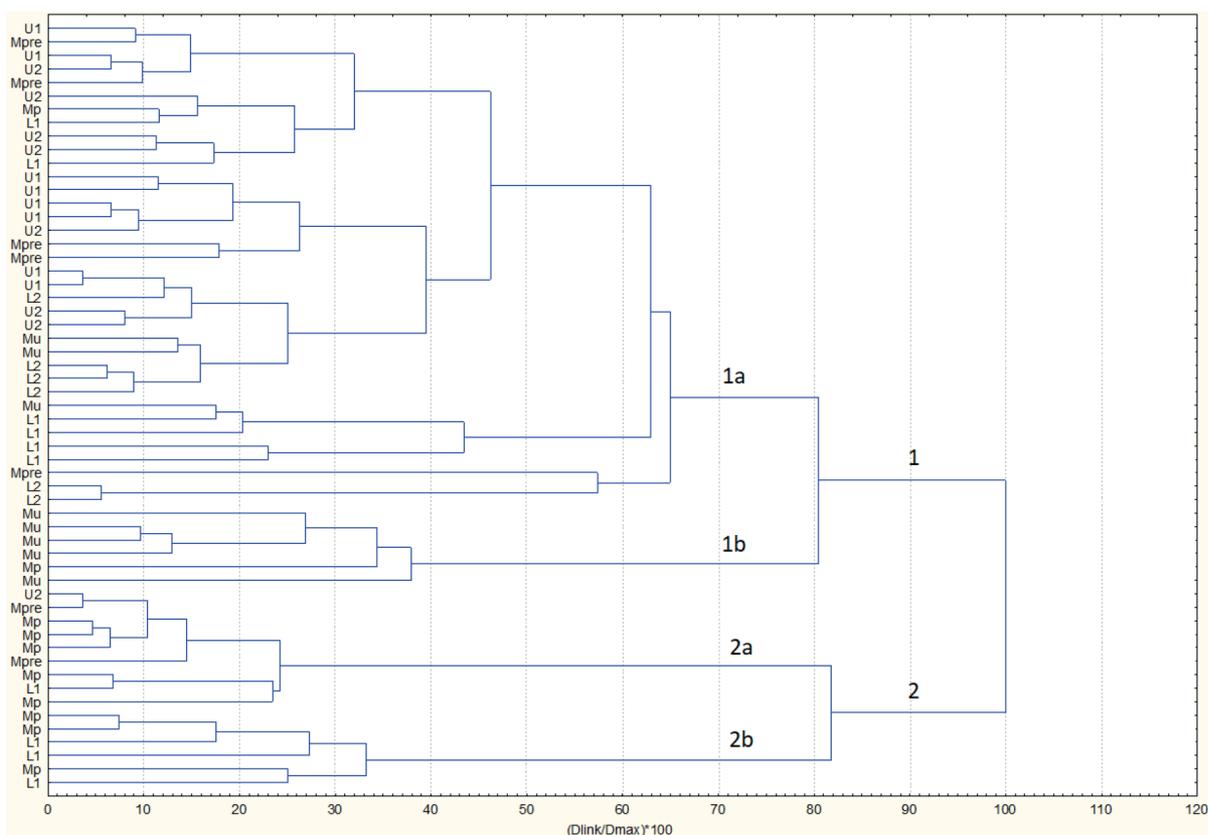


Figure 4. Dendrogram showing clusters of samples from different reaches based on the similarity for the fish species relative abundances. Numbers and letters above branches are used in the text for reference. Reaches codes: U1, upper 1; U2, upper 2; Mpre, middle pre-urban; Mu, middle urban; Mp, middle post-urban; L1, lower 1; L2, lower. *Dendrograma de agrupamiento de muestras de diferentes tramos basados en la similitud de las abundancias relativas de especies de peces. Los números y letras sobre las ramificaciones son utilizados en el texto como referencia. Códigos utilizados para los tramos de arroyo: U1, cuenca alta 1; U2, cuenca alta 2; Mpre, cuenca media pre-urbano; Mu, cuenca media urbano; Mp, cuenca media post-urbano; L1, cuenca baja 1; L2, cuenca baja 2.*

lained 31.06 and 23.35 % of the variance of the data, respectively (Fig. 5). The relation between environmental variables and fish species was significantly high ($R = 0.87, p = 0.002$).

Analyzing the CCA triplot and focusing on the water quality vectors, a general trend could be considered: organic pollution increases from left to right and salinity increases from bottom to top of the coordinate system. Following this ordination, the right half of the coordinate system (positive values on axis 1) could be thought of as the organic pollution side of the system due to BOD, TC, TP and NH₄ vectors mainly point to the right (higher scores on axis 1 than on axis 2). In contrast, the left half of the coordinate system (negative values of axis 1) could be thought of as the least organically polluted side of the system for being diametrically opposed to the organically polluted side and for having the DO vector pointing mainly to the left (higher absolute scores on axis 1 than on axis 2). The pH vector follows a similar trend to that of the DO which could be associated to least organically polluted and more oxygenated waters. This agrees with a drop in pH at Mp (always above 7) which could be attributed to organic pollution (Fig. 2). Similarly, the upper half of the coordinate system (positive values of axis 2) could be interpreted as higher salinity waters due to SO₄, EC, K and TS vectors point this way. Analogously, the lower half of the coordinate system could be thought of as the least saline waters side of the system for being diametrically opposed to the above mentioned side. Combining both the salinity trend with the organic pollution trend we can characterize each quadrant of the triplot as follows: upper right quadrant associated with higher salinity and higher organic load, upper left quadrant associated with higher salinity and lower organic load, lower right quadrant associated with lower salinity and higher organic load and lower left quadrant associated with lower salinity and lower organic load.

Following multivariate ordination of fish species and sites along the environmental scenario depicted in the CCA triplot, Mp could be seen as a moderate salinity and highly organically polluted reach due to a great number of samples from this reach showed high scores on axis 1 and low to moderate scores on axis 2, falling within the upper

right quadrant of figure 5. Species correlated with these conditions were *C. decemmaculatus* and, to a lesser extent, *J. lineata* and *H. argentinensis*, all of which had greater scores on axis 1 than on axis 2. Samples from L2 and, to a lesser extent, from L1 could be associated with higher salinity and lower organic load waters as some of those samples positioned in the upper left quadrant. Eighty-three percent of L2 samples and 20 % of L1 samples fell within this quadrant. Regarding L1 samples, some of these also positioned in other quadrants of the coordinate system but mostly in the lower left quadrant, suggesting lower salinities and less organically polluted waters. These differences in how L1 samples scattered across quadrants could be attributed to fluctuations in the discharges from STE. Sixty-six percent of L2 samples had the highest absolute scores on both axis 1 and 2. This agrees with the highest mean values for SO₄²⁻ and EC found at L2 along with high DO and pH mean levels (Fig. 2). The species correlated with these water quality variables were *C. voga*, *P. pampa* and, to a lesser extent, *A. faceutus*, *L. anus* and *H. commersoni*.

Samples from the uppermost section of the basin (U1, U2 and Mpre samples), were mostly distributed between the lower right and lower left quadrants. This suggests these reaches have moderate to low salinity for being positioned in the lower half of the coordinate system. However, it is hard to categorize them in terms of their organic load because some were on the right side and others on the left side of the coordinate system suggesting fluctuating conditions. Fifty percent of U1, U2 and 43 % of Mpre samples positioned within the lower left quadrant with high loads of SS and NO₃ vectors. The species correlated with these water quality variables were *C. interruptus*, *C. carpio*, *O. jenynsii*, *P. laticeps* and *C. paleatus*.

Nitrites (NO₂) was the only water quality variable positioned in the lower right quadrant, having low scores on both axes but an almost null score on axis 2. By opposition, the lower right quadrant could be defined by lower salinity waters with increasing levels of organic pollution. Forty-three percent of Mpre samples, 37.5 % of U2 samples and 25 % of U1 samples fell within the quadrant. *S. marmoratus*, *R. quelen* and *B. iheringii* positioned within the quadrant as well,

with *S. marmoratus* having higher scores on both axes, followed by *R. quelen* which had a higher score on axis 2 than on axis 1 whereas *B. iheringii* had a low score on axis 1 and an almost null score on axis 2.

Regarding Mu samples, they were scattered among the four quadrants with relatively low absolute scores on both axes suggesting fluctuating moderate conditions in terms of salinity and organic loads.

For the indicator species analysis, we used the results of the CA for the water quality variables to group sites according to their similarities. Due to CA showed two distinctive main clusters based on the water quality variables (i.e., cluster 1 formed by all sites upstream from STE and cluster 2 formed by all sites downstream from STE, Fig. 3), we grouped sites according to their relative position to STE. Following this criterion, we ended up with group 1 (all sites upstream from STE) and group 2 (all sites downstream from STE). The species that showed significant indicator values (IndVal) were Cp (66.55%, $p=0.0001$), Ci (64.44%, $p=0.0192$), Pl (49.34%, $p=0.007$) and Oj (42.81%, $p=0.036$) for the site group upstream from STE and, Cd (60.95%, $p=0.0034$), Ha (24.84%, $p=0.0122$) and Sm (23.08%, $p=0.0061$) for the site group downstream from STE.

DISCUSSION

Overall, our study supported the well-known ecological pattern that water quality in streams is drastically affected downstream from urban zones as the water course receives its effluents. Accordingly, the fish assemblage structure reflected that change in water quality. In addition, we were able to identify some key species distinctive of certain water quality conditions which could be useful for biomonitoring purposes.

Water quality

Our study showed water quality differences among the sampled reaches, with a general trend given by reaches upstream from STE being similar among them and differing from reaches downstream from STE. This was mainly reflected through the CA where reaches from both sec-

tors (i.e., upstream and downstream from STE) formed separate main clusters. Furthermore, the three reaches downstream from STE (Mp, L1 and L2) seemed to be more unique and distinguishable from each other regarding their water quality characteristics since they formed more homogeneous subclusters. Moreover, Mp samples were more similar to L1 than to L2 samples since they fell within the same subcluster (i.e., subcluster 2a). This observation agrees with their relative locations along the stream course and their distance to STE. Mp receives the STE water almost directly since it is located 1 km downstream from the STE discharge. L1 is 20 km downstream from Mp whereas L2 is 60 km downstream from L1. As the water travels downstream from STE towards the lower basin, water quality tends to recover, likely due to natural auto-depuration mechanisms of lotic ecosystems. Auto-depuration or self-purification mechanisms are natural recovery processes of stream water quality where biodegradable organic matter loads are reduced through physical (dilution and sedimentation), chemical (oxidation) and biological (decomposition of organic matter) processes (Antunes *et al.*, 2018). Irrespective of that, some variables such as NO_3^- , NO_2^- and TP showed to be less sensitive to the auto-depuration processes in L1 they did not recover values comparable to those upstream from STE, as it was observed for DO, NH_4^+ , BOD. Overall, the proximity between Mp and L1 in comparison to the larger distance separating L1 from L2 helps explain why water quality from L1 is more similar to that from Mp than to that from L2. In fact, 20 % of the variability in water quality was explained by the arrangement of the sections along the stream. On the other hand, there was also a trend of increasing salinity downstream from Mp evidenced by EC, SO_4^{2-} and Na^+ levels. Even when it could be expected an important effect of STE on the concentration of salts, the aquifer in the lower sector of the basin is saline and shallow, exerting influence on the surface water characteristics due to it discharges into the stream (Zabala *et al.*, 2015; Varni *et al.*, 2019). In relation to this, the increase in salinity-related variables towards the lower basin sector could be partially explained by both the STE and the nature of the groundwater. The highest nitrate levels

were found in the upper basin, probably attributed to the intense agricultural production in this area (Ares et al., 2007). For this reason, most samples from U1 and U2 both belonging to a region where agricultural activities prevail, fell within the lower half of the coordinate system.

Fish assemblages

About 30 % of the total variation in fish assemblages was explained by the longitudinal spatial arrangement of sampled reaches. Longitudinal variation in fish communities is a common phenomenon in streams (Matthews, 1998) as response to gradual increase in habitat complexity and the physical and chemical changes of water (Gorman & Karr, 1978; Vannote et al., 1980). However, the natural functioning expected for these systems is disrupted by the effects from different surrounding land uses on their ecological integrity (Bertora et al., 2022b). Our results show that the presence of the city in the middle section of the basin promotes a strong effect on water quality and also in some key attributes of the fish assemblages. Indeed, although the grouping of sampled reaches regarding fish assemblages was not as robust as the trend for water quality variables in the CA, 80 % of the Mp samples fell within the same cluster (main cluster 2) further suggesting an important effect of the discharge from the STE in the fish assemblage organization.

Relationship between fish assemblage structure and water quality

Some species correlated with major water quality scenarios in terms of salinity and organic loads. Spatial ordination at the upper right quadrant of the CCA plot shows that *C. decemmaculatus* and to a lesser extent *J. lineata* and *H. argentinensis*, are correlated with organic pollution (high scores on axis 1) and moderately saline waters (low scores on axis 2). *C. decemmaculatus* and *J. lineata* are two Cyprinodontiformes collectively known as livebearers which besides being viviparous are prolific, have an omnivorous diet and are tolerant to low DO levels (Rosso, 2006). The ability to cope with low levels of dissolved oxygen is related to their mouth pointing upwards allowing

them to breathe from the uppermost water layer richer in oxygen (Lewis, 1970). The dominance of these two species in impaired environments has also been reported in lake Rodó (Montevideo, Uruguay), a eutrophic lake in an urbanized area (Quintans et al., 2009). Likewise, high proportions of these two species were reported from the most impaired urban reaches of the Suquia River (Córdoba, Argentina) (Hued & Bistoni, 2005). Moreover, Cyprinodontiformes are widely distributed in the Neotropical region and are notorious for inhabiting deteriorated habitats where few species can thrive (Araujo et al., 2009; Dias et al., 2020). Regarding *H. argentinensis*, it is also known to withstand low DO levels and to occur in poorly oxygenated waters (Rantin et al., 1992).

Although not positioned in the same quadrant of the plot, the swamp eel (*S. marmoratus*) is on the right side of the coordinate system which is associated with higher organic loads and lower oxygen levels as already pointed out. This agrees with studies indicating that these eels present intestinal and buccopharyngeal adaptations which favor the uptake of atmospheric oxygen. Further, they are considered tolerant (Bozzetti & Schulz, 2004; Casatti et al., 2009; Costa & Schulz, 2010) or moderately tolerant (Hued & Bistoni, 2005) to water pollution.

The upper left quadrant of the plot includes species correlated with higher salinity and lower organic loads. Among these species, *C. voga* had the greatest score on axis 2 suggesting it was more abundant in ionic-rich waters. Menni et al. (1996) reported an EC maximum tolerance for *C. voga* of 284 $\mu\text{S}/\text{cm}$. In our survey, we captured this species at ECs between 700 and 1600 $\mu\text{S}/\text{cm}$. These values of water conductivity are still low for this species. It has been already shown that *C. voga* is able to thrive at more than 4000 $\mu\text{S}/\text{cm}$ (Rosso & Quirós, 2009). Nevertheless, at these conditions, the species was precluded to colonize harsh abiotic conditions of high conductivity (over 5000 $\mu\text{S}/\text{cm}$) during dry summers and was predominantly collected at lower salinities. Although not as marked as for *C. voga*, *P. pampa* followed a similar pattern showing it was also predominantly collected at these abiotic conditions associated with the lower basin. Both of these species also showed high absolute scores on

axis 1 (negative values), demonstrating empirical correlations with DO and pH.

Other species such as *A. facetus*, *H. commersoni* and *L. anus* showed moderate scores in both axes compared to *C. voga* and *P. pampa*. *A. facetus* is known to tolerate saline waters up to 25 000 $\mu\text{S}/\text{cm}$ and it is considered an invasive species in European streams (Baduy *et al.*, 2019). Despite *H. commersoni* and *L. anus* are both Siluriformes of the Family Loricariidae, only the former is known to be tolerant to low DO levels (Franco, 1994) which agrees with a lower absolute score on axis 1 than for *L. anus*, which is less tolerant of water quality impairment (Bertora *et al.*, 2018a).

As previously mentioned, the lower left quadrant of the CCA plot represents a scenario of lower salinities and better water quality, including higher DO levels. The species that loaded high at this quadrant were *C. interruptus*, *C. carpio*, *P. laticeps*, *O. jenynsii* and *C. paleatus*, with the latter having almost a null score on axis 1. Except for *C. carpio* and *C. paleatus* which are considered species that can tolerate water quality impairment to some extent (Laird & Page, 1997; Bertora *et al.*, 2021b, respectively), the other species could not be considered tolerant. The Characiformes *C. interruptus*, *P. pampa* and *O. jenynsii* are considered intolerant to poor water quality conditions (Bozzetti & Schulz, 2004; Costa & Schulz, 2010; Bertora *et al.* 2018a). Likewise, the small silurid *P. laticeps* is also considered an intolerant species inhabiting reaches with good water quality conditions (Hued & Bistoni, 2005 and 2007). Moreover, *P. laticeps* is one of the most vulnerable species when exposed to arsenic (Rosso *et al.*, 2013) and toxic trace metals (Rosso *et al.*, 2022) in abiotic matrices, accumulating the highest concentrations of these elements from the environment.

The lower right quadrant associated with a lower salinity and a higher organic load contained *S. marmoratus*, *R. quelen* and *B. iheringii*. Of the three species, *S. marmoratus* showed the highest absolute scores on both axes, *R. quelen* scored moderately on axis 2 (i.e., the axis related to salinity) and low on axis 1 (i.e., the axis related to organic load) whereas *B. iheringii* scored low on axis 1 and it got an almost null score on axis 2. The latter species is considered intolerant to ad-

verse water quality conditions whereas the other two are considered tolerant (Bozzetti & Schulz, 2004; Costa & Schulz, 2010).

Other relevant variables for fish such as water depth and substrate composition that could also affect species distribution (Mueller & Pyron, 2010; Teresa & Casatti, 2012) were not addressed in this study. However, we ensured sampling sites were alike in terms of these characteristics, although minor variations are possible.

Indicator species

Despite the water quality variables being more distinctive of a basin sector than the fish assemblage structure, there were some key species that could be indicative of water quality as suggested by the IndVal analysis. For instance, *C. paleatus*, *C. interruptus*, *P. laticeps* and *O. jenynsii* were associated with less polluted reaches upstream from STE whereas *C. decemmaculatus*, *H. argentinensis* and *S. marmoratus* were associated with more polluted reaches downstream from STE. From the first group, it is arguable to find *C. paleatus* here since, as it was already stated, this species is considered tolerant to water quality impairment. However, the other three species in the group agree with the idea of them being intolerant species as supported by literature (see previous section) and thus their potential use as bioindicators of environmental health of aquatic ecosystems. On the other hand, the other group of species associated with poor water quality was formed by three species already known to be tolerant to pollution (see previous section) however *H. argentinensis* and *S. marmoratus* had IndVal scores below 25 % in contrast to *C. decemmaculatus* which had an IndVal score of 60.95 %. *C. decemmaculatus* can withstand pollution and is reported as the only species found in the most polluted reaches of the Matanza-Riachuelo basin, the most contaminated basin in the pampean region of Argentina (Paredes del Puerto *et al.*, 2021)

In conclusion, our study revealed trends in water quality and fish assemblage structure along a prairie Neotropical stream where reaches located upstream from an urban point-source effluent were more similar, less polluted and less saline than downstream reaches. Some key aspects of

fish assemblage structure accompanied main patterns of changes in water quality. Likewise, species *C. decemmaculatus*, *C. interruptus*, *P. laticeps* and *O. jenynsii* showed great potential as bioindicators of temperate Neotropical streams and should be taken into account for future bio-monitoring programs.

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