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Implications of wastewater discharges on environmental features and fish communities in an urban river

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Abstract

Wastewater effluent discharges from treatment plants (WTP) are among the most pervasive stressors in urban rivers, however, their impact on the different levels of biological organization is poorly understood. This study examined the effects of wastewater effluent outflow on different trophic levels in an urban river and measured physicochemical features; bacterial, periphyton, and riparian forest composition; as well as the abundance, density, and biomass of freshwater fish at both pre- and post-WTP effluent discharge sites. Strong modification of the riparian vegetation was seen downstream from the WTP with a dominance in the richness of exotic plants. The highest values of conductivity, total bacterial composition, periphyton, and nutrients were detected in post-WTP sites. The freshwater fish recorded in the pre-WTP reaches had the highest abundance values for all species recorded. However, the exotic Brown Trout appeared to be indirectly favoured by the nutrient subsidy from the WTP discharges since the biggest individuals were recorded at post-WTP river reaches. Although the river assessed runs through an underdeveloped region in the country; our findings indicated a strong deterioration in environmental conditions and a complex relationship between the biotic responses to effluent pollution. Adequate treatment of effluent discharges is a crucial issue for urban rivers. Management actions are required because the consequences of chronic exposure on aquatic wildlife are unknown. Mostly, Patagonian aquatic systems have not yet reached an ecological tipping point, so it is recommended that restoration plans incorporate improvements in sewage treatment to avoid further degradation of ecosystem services.

Keywords Wastewater effluent · Nutrient increase · Bacteria contamination · Freshwater fish abundance · Urban pollution gradient

Introduction

Urbanization is considered one of the main environmental stressors in producing habitat degradation associated with channel straightening and realigning, the increase of rate erosion, sedimentation, water temperature, and runoff, as well as the nutrient enrichment and the alteration of the flow regimes (Boët et al. 1999; Paul and Mayer 2001; Miserendino et al. 2011). Another consequence of urbanization is the removal of riparian vegetation, which is crucial to maintaining the in-stream temperature, buffering nutrients and sediments, and linking processes between aquatic and terrestrial ecosystems (Munné et al. 1998). Several studies conducted in different regions around the world have reported drastic impacts on the populations of microbes (Flies et al. 2020), plants (Aronson et al. 2014), and fish (Paul and Meyer 2001).

As urbanization grows, the reliance on wastewater treatment plants (WTPs) does it and will continue rising. The consequences that effluent discharge will have on the wildlife that resides in these habitats is a real concern (Grimm et al. 2008). The WTPs are the major source of aquatic pollution and affect downstream water quality (Medhi et al. 2018). Effluent discharges result in several environmental stressors

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on aquatic ecosystems, affecting multiple levels of biological organization (Nikel et al. 2021). Their continuous release into watercourses can substantially impair ecosystems via the chronic exposure of biota to excess nutrients, dissolved oxygen reduction, eutrophication, and the physical changes to the habitats, whether due to flow alteration, turbidity increases, or changes in the thermal regimes (Hamdhani et al. 2020).

Regarding fish, several studies examining the effects of urbanization on fish communities revealed that species responses might differ according to their origin (native or non-native), region, or population status (Wang et al. 2001; Dyer et al. 2003; McEwa et al. 2009; among others). In south-eastern North America, a marked decline in endemic fish species as a result of exurban development was reported by Scott (2006). A detailed study of the River Seine over 30 years revealed that water quality degradation causes noticeable decreases in fish species richness downstream from Paris. Authors observed that low flow conditions and storm events resulted in decreased dissolved oxygen levels, causing massive fish mortality (Boët et al. 1999). Furthermore, it had documented that downstream of the nutrient input point source, Brown Trout (*Salmo trutta*) and Rainbow Trout (*Oncorhynchus mykiss*) biomass might increase in wastewater-enriched rivers, not so the total sportfish (Askey et al. 2007). Fish abundance and richness can also result negatively influenced by degraded water quality associated with WTP effluent through increased levels of ammonia, higher temperatures, increased periphyton, and changes in pH (Northington and Hershey 2006).

In South America, Fierro et al. (2019) found that environmental changes downstream outflow of domestic wastewater were major stressors on the composition of fish assemblages, with an increase seen in more tolerant species. A study comparing fish populations at 15 streams subjected to different land uses revealed that urban streams showed only exotic fish species without native specimens (Di Prinzio et al. 2009). Similarly, Miserendino et al. (2008) documented that urbanization increased the biomass of exotic fish caused essentially for nutrient enrichment. In addition, the highest values of fish abundance were observed at disturbed sites, which might have been explained by the opportunistic behaviour displayed by these communities, which allows them to take advantage of the increased trophic resources in these environments (Miserendino et al. 2011).

The Patagonian region is characterized by a low population density, being the metropolises and mega-factories scarce. Development linked with urban activities is relatively contemporary and dates back to around 1800; however, nowadays, the strong concern regarding accelerated urban expansion in certain areas is evident (INDEC 2010). In a study of 103 sampling sites conducted at streams from the most relevant basins of North West Chubut province, Williams-Subiza et al. (2021) found that degradation in water quality was strongly related to the point source of pollution, probably associated with WTPs failures. In this sense, recent studies have warned about significant inefficiencies in WTPs functioning (Manzo et al. 2020, 2022). The main objective of the present study was to assess the urbanization effects on habitat conditions and fish assemblages in a Patagonian urban basin. With this in mind, we assessed nutrient enrichment, changes in riparian and aquatic vegetation. Also, the abundance, density, biomass, and condition of fish were compared at sites upstream and downstream from the sewage discharge point. Urban impact on aquatic systems is significant, and it is necessary to address this problem jointly among scientists, resource managers, political authorities, and the society that benefits from this resource. It will allow obtaining pathways toward enhancing the future integrity of the region´s water resources.

Materials and methods

Study area

Quemquemtreu is a sixth-order river whose catchment (273 km^2) is located between 41º57´S-71º32´W and 42º0´S-71º35´W, on the eastern side of the Andes Mountains in Argentina (Fig. 1). It is a sub-basin of the binational Puelo-Manso drainage system that discharges into the Pacific Ocean (Chile) through the Puelo River (Coronato and Del Valle 1988). The Quemquemtreu is dominated by glacio-fluvial deposits from the Holocene. Precambrian crystalline bedrock and granitoids are also well represented. Quaternary volcanic ashes are widespread. The surrounding landscape is characterized by the Subantarctic Forest, which is mostly composed of native species: *Nothofagus pumilio*, *N. dombeyi*, *N. antarctica*, *Austrocedrus chilensis*, *Chusquea culeou*, *Lomatia hirsuta*, *Maytenus boaria*, *Myrceugenia apiculata* and *Aristotelia maqui*. Exotic vegetation includes *Salix* sp., *Rosa eglanteria* and *Retama sphaerocarpa* and these tend to invade areas of forests burned and affected by human activities (Pizzolón 2013). The area's mean annual rainfall is < 1000 mm year^{-1}. Quemquemtreu River has a pluvio-nival regime with two seasonal peaks in flow related to winter rainfalls and spring snowmelt. The mean annual discharge is approximately 10.3 m^3 s^{-1} and the substrate is composed mainly of boulder and cobble beds. Quemquemtreu River runs from north to south, crossing the town of El Bolsón (20,000 inhabitants INDEC, 2010). Tourism, forestry, cattle rearing, organic cultivation, and

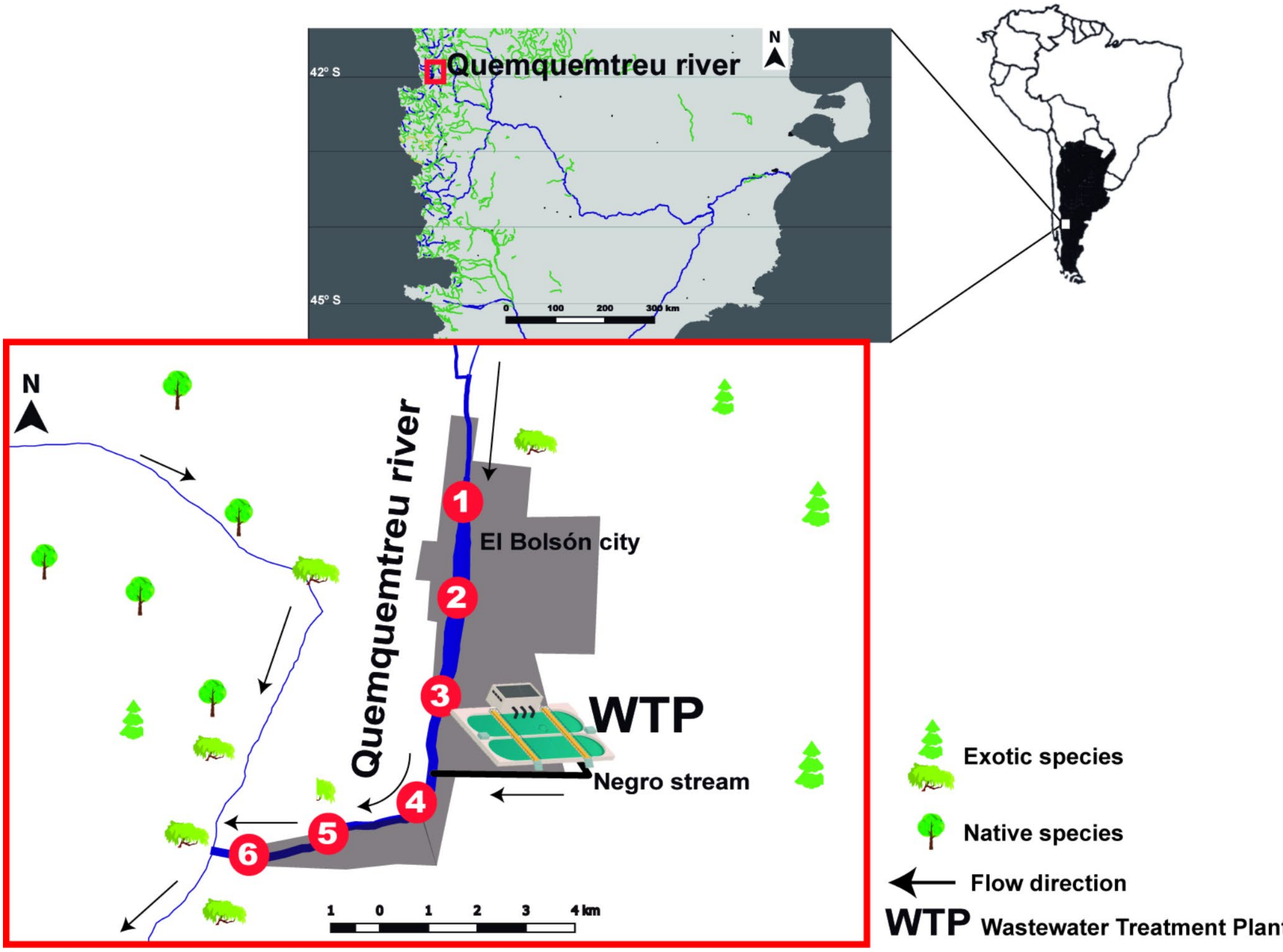

Fig. 1 Location of the Quemquemtreu River in El Bolsón city (Río Negro, Argentina). Pre- and post-WTP (Wastewater Treatment Plant) studied sites are consigned

family farming are the dominant economic activities in the catchment.

Environmental data

The wastewater treatment plant (WTP) was constructed based on oxidation ditch technology and was planned in the year 1980, finally reaching completion in 2010. However, by 2010 only 59% of the town's population was served by the wastewater collection system (Martin 2012) and the town has still not achieved 100% coverage. As a result, the excess of the plant´s effluent (crude) is discharged into the Negro stream that drains into the Quemquemtreu River (Fig. 1). To evaluate the effects of the WTP, six sites were established. Site selection was based on accessibility and convenience under stable environmental conditions; samples were not taken after rainstorms or extremely high discharge events. Three sites were located upstream (pre-WTP), and three sites were located downstream (post-WTP) of the WTP's effluent discharge point: Q3 and Q4 were located 1 km from the outflow; Q2 and Q5 were 2 km from the outflow and Q1 and Q6 were 3 km from the outflow point.

All sites were sampled seasonally ($n = 4$) during 2018. On each sampling date, current velocity (m s^{-1}) was measured in mid-channel on three occasions by timing a float (average of three trials) as it moved over a known distance, and the water temperature was recorded (Gordon et al. 2004). The average depth was estimated from five measurements on a transect across the channel with a calibrated stick. Discharge was obtained by combining depth, wet width, and current velocity as per Gordon et al. (2004). Specific conductance (µ $S.cm^{-1}$), pH, salinity (‰), total dissolved solids (TDS) and dissolved oxygen (mg $O_2.l^{-1}$) were measured with a multi-parameter probe (Hach HQ40d). For nutrient analysis, water samples were collected below the water surface and kept at 4 °C before analysis. Nutrients analysed included the ionic form of nitrogen, ammonium (NH_4^+) and nitrate plus nitrite nitrogen (NO_3^--NO_2^-), as well as dissolved inorganic phosphorus, determined as soluble reactive phosphorus (SRP), measured by spectrophotometric standard methods for the examination of water and wastewater (APHA 1998). Total suspended solids (TSS) (mg.l^{-1}) were estimated gravimetrically by filtering a known volume of water with pre-weighted fibreglass filters, drying (110 °C, 4 h), and re-weighing (APHA 1998). Pigment analysis was performed to estimate algal biomass. Samples of periphyton were taken at each site in duplicate. Each sample consisted of scraping a known area (20 cm^2) on the exposed surface of three randomly selected rocks. The collected material was preserved in water from the site and cooled while transported in dark containers to the laboratory, where they were filtered on GF/FF filters. All samples were stored at -20 ºC until analysis. Chlorophyll (Chl *a*) was extracted from filters in 90% acetone, and measured spectrophotometrically with correction by phaeopigments, according to standard methods (APHA 1999; Wetzel and Likens 1991).

Biological sampling

Bacteriological characterization

For microbiological studies, water samples were collected seasonally under sterile conditions and placed in sterile, screw-capped glass bottles, following the standard methods of handling water samples. Both biological oxygen demand (BOD) and bacteriological analysis were assessed. Bacteriological analysis of water samples was done within 24 h of collection, including for colony forming units (CFU/100 ml water) of presumptive total coliforms and *Escherichia coli* (CFU/100 ml water). Medium and simple concentrations of Lauryl Triptose were used as a culture medium in the presumptive stage and Brilliant Green Bile Lactose broth (BGBL) in the confirmatory stage following American Water Works Association methods (AWWA 1998).

Characterization of riparian and aquatic plant vegetation

At each study site, a riparian plant species inventory (herbs, shrubs, and trees) was conducted. In addition, aquatic plants were also sampled. This included aquatic macrophytes, taking all functional groups present in each site into consideration: emergent (plants rooted, morphologically adapted to growing in a waterlogged or submersed substrate), floating-leaves (plants rooted in the substrate with floating leaves), submersed (plants with photosynthetic tissue entirely submersed) and free-floating (an unattached plant which is entirely suspended on the water), (Correa 1999; Cronk and Fennessy 2001). Voucher specimens for all species were gathered and kept in collection bags and then pressed for later laboratory identification. The species were observed under a LEICA MZ6 stereomicroscope and identified using regional bibliography (Correa 1978–1999). Species were also classified as native, endemic or exotic, according to the Catalogue of the Vascular Plants from the Southern Cone (Zuloaga et al. 2008).

Fish assemblage

To assess fish abundance, density (ind m^{-2}), and biomass (g m^{-2}) a 100 m reach was sampled at each selected site, using an electro-fishing machine fitted with a battery-powered backpack, and a hand net operated by the same handler. All fish caught were identified to species level following Ringuelet (1975), measured for total length, and weighed. In the laboratory, the condition factor of each individual was calculated using length-weight Fulton type relationships (10^5 x W L^{-3}; W = weight in g, L = total length in cm) (Anderson and Gutreuter 1992). To speed up processing and reduce handling stress, the first 15 individuals of a given species recorded at each sampling site were individually measured and weighted, with the remaining individuals of that species being identified to species level, counted, and then batch-weighed. All fish were immediately released back to their site of collection.

Statistical approach

The specific composition of both macrophyte and riparian plant species was determined according to presence/absence cluster analysis using the average linkage algorithm and Jaccard distance (Kreb 1989). Contingency Tables were performed to categorize the values of *Escherichia coli* (< 30–91,> 91–360,> 360–7300), total Coliform bacteria (< 30–230,> 230–1400,> 1300–14,000) (Feachem et al. 1983, DPN 1540/16 Pcia Chubut) and BOD (1–3 low, 4–6 medium, 9–14 high). A Principal Component Analysis (PCA) was performed to identify which physicochemical parameters explain the patterns observed in fish assemblages according to their scores on the PCA axes (Ludwing and Reynolds 1988). The environmental data were log (x + 1) transformed and standardized. Generalized linear mixed models (GLMs) were employed to evaluate disparities in response variables (chemical variables and fish abundance, biomass, and density) as a function of the distance from the WTP effluent discharge point, with ‘time of year’ (seasons) nested as a random effect variable. Akaike’s criterion (AIC) was used to select the model with the best fit, given the data. To compare means, a Di Rienzo, Guzman and Casanoves test (DGC) was run, which uses the multivariate technique of cluster analysis (significance level = 0.05). The data were analysed using the R-package interfaced with InfoStat statistical software version 2019 (Di Rienzo et al. 2019).

Table 1 Mean values of the physicochemical variables seasonally measured (n = 24) at pre- and post-WTP (wastewater treatment plant) sites in the Quemquemtreu River during 2018. The standard deviation values are shown in parentheses

	Summer		Autumn		Winter		Spring	
	pre-WTP	post-WTP	pre-WTP	post-WTP	pre-WTP	post-WTP	pre-WTP	post-WTP
Water Temperature (ºC)	20.2 (± 1.3)	14.3 (± 0.8)	7.8 (± 0.3)	8.2 (± 0.5)	8.4 (± 0.4)	9.0 (± 0.4)	12.1 (± 0.1)	12.1 (± 0.0)
Conductivity (µS.cm^{-1})	72.8 (± 3.2)	50.5 (± 39.5)	46.5 (± 0.5)	53.0 (± 1.0)	80.0 (± 0.6)	94.2 (± 1.7)	65.5 (± 0.7)	77.2 (± 1.3)
Salinity (‰)	0.0 (± 0.0)	0.1 (± 0.1)	0.1 (± 0.0)	0.1 (± 0.0)	0.0 (± 0.0)	0.0 (± 0.0)	0.0 (± 0.0)	0.0 (± 0.0)
Total Dissolved Solids (mg.l^{-1})	38.2 (± 0.6)	30.7 (± 24.2)	32.8 (± 0.3)	37.2 (± 1.3)	37.7 (± 0.3)	44.5 (± 0.9)	30.7 (± 0.3)	36.4 (± 0.6)
PO_4^- (µg.l^{-1})	2.0 (± 1.0)	20.2 (± 2.7)	3.9 (± 1.5)	13.3 (± 1.0)	0.8 (± 0.4)	4.6 (± 0.7)	3.4 (± 0.4)	13.8 (± 2.5)
NH_4^+ (µg.l^{-1})	12.0 (± 2.1)	176.1 (± 105)	5.2 (± 0.3)	79.3 (± 37.3)	0.2 (± 0.4)	39.4 (± 13.5)	3.4 (± 0.6)	83.2 (± 11.2)
NO_2^--NO_3^- (µg.l^{-1})	24.5 (± 18.8)	1529.7 (± 461)	12.9 (± 7.3)	68.6 (± 8.4)	19.4 (± 1.9)	352.3 (± 36.0)	25.6 (± 12.6)	530.0 (± 257.8)
Total Suspended Solids (mg.l^{-1})	1.7 (± 1.3)	1.0 (± 0.5)	2.5 (± 0.6)	4.2 (± 0.4)	1.2 (± 0.2)	3.3 (± 0.9)	2.1 (± 0.2)	3.9 (± 1.3)

Results

Environmental characterization: pre- vs. post-WTP sites

Several water quality parameters showed differences between pre- and post-WTP sites (Table 1). The highest values of conductivity, ammonium, phosphorus, nitrate plus nitrite nitrogen and total suspended solids were observed in post-WTP sites. However, the highest values of water temperature were recorded in pre-WTP sites during the summer season. Significant differences ($p < 0.05$) between the sites were seen for SRP, NH_4^+, NO_3^--NO_2^- and Chl *a* (Fig. 2). The highest values for SRP and NO_3^--NO_2^- were recorded at sites Q4 and Q5 (located at 2 and 3 km downstream from the WTP), while the peak value for NH_4^+ was detected at site Q4, immediately downstream from the WTP (1 km) (Fig. 2). The highest values of Chl *a* were observed at Q4 and Q5, located 2 and 3 km downstream from the WTP, respectively (Fig. 2). Overall, the majority of the parameters recorded appear to be lower at the pre-WTP sites.

Bacterial analysis

The mean concentration of total coliforms and *E. coli* differs significantly between pre- and post-WTP reaches. The highest values of both total coliforms (14,000 CFU) and *E. coli* (7300 CFU) were observed downstream from the WTP at Q4 during the summer season; bacteria values were significantly lower upstream (Chi^2, $p < 0.05$ Table 2). Biological oxygen demand (BOD_5) values were low ($\bar{x}$ = 1.5) at pre-WTP sites, whereas the values recorded at post-WTP sites were moderate ($\bar{x}$ =7.2) (Table 2).

Plant assemblage comparison at pre- and post-WTP sites

A total of 50 plant species were recorded, of which 83% were exotic and 17% were native species. Most native species were observed at pre-WTP sites whereas exotic ones dominated at the post-WTP section (Table 3). The plant richness native/exotic ratio was 3 (21/7) at the pre-WTP sites and 7 (35/5) at the post-WTP sites. Exotic species comprised 52.5% of the total inventory at the pre-WTP sites whereas at the post-WTP sites was 87.5%. Arboreal and shrub species such as *Salix* spp., *Rubus ulmifolius* and *Cytisus scoparia* occurred at all sampled sites. The herbaceous stratum included the exotics *Brassica nigra*, *Lupinus polyphyllus*, *Saponaria officinalis* and *Plantago lanceolata*, all of which were among the most frequent species recorded. A total of 4 species of macrophytes were recorded, three were emergent and one submersed (*Myriophyllum quitense*). Assemblage composition analysis identified 2 plant groups that characterized pre- and post-WTP sectors ($R^2 = 0.75$, LSD $_{\text{Fisher Test}} < 0.05$) (Fig. 3). Species richness was higher at post-WTP sites, probably due to the greater presence of herbaceous species, as well as the variety of plant life-forms, which included many exotic annual, biannual, and perennial species (DGC test < 0.05). Significant differences ($\chi 2 = 6$, $p < 0.05$) were observed between the pre- and post-WTP sites with regard to the presence or absence of exotic species (mostly relating to *Conium maculatum*, *Hypericum perforatum*, *Medicago polymorpha* and *Tripleurospermum inodorum*). Interestingly, the endemic shrub *Baccharis linearis* was only recorded in the pre-WTP stretch.

Fish in pre- and post-WTP reaches

Fish were present in all samples (24) taken in the Quemquemtreu River during 2018. A total of 548 fish were

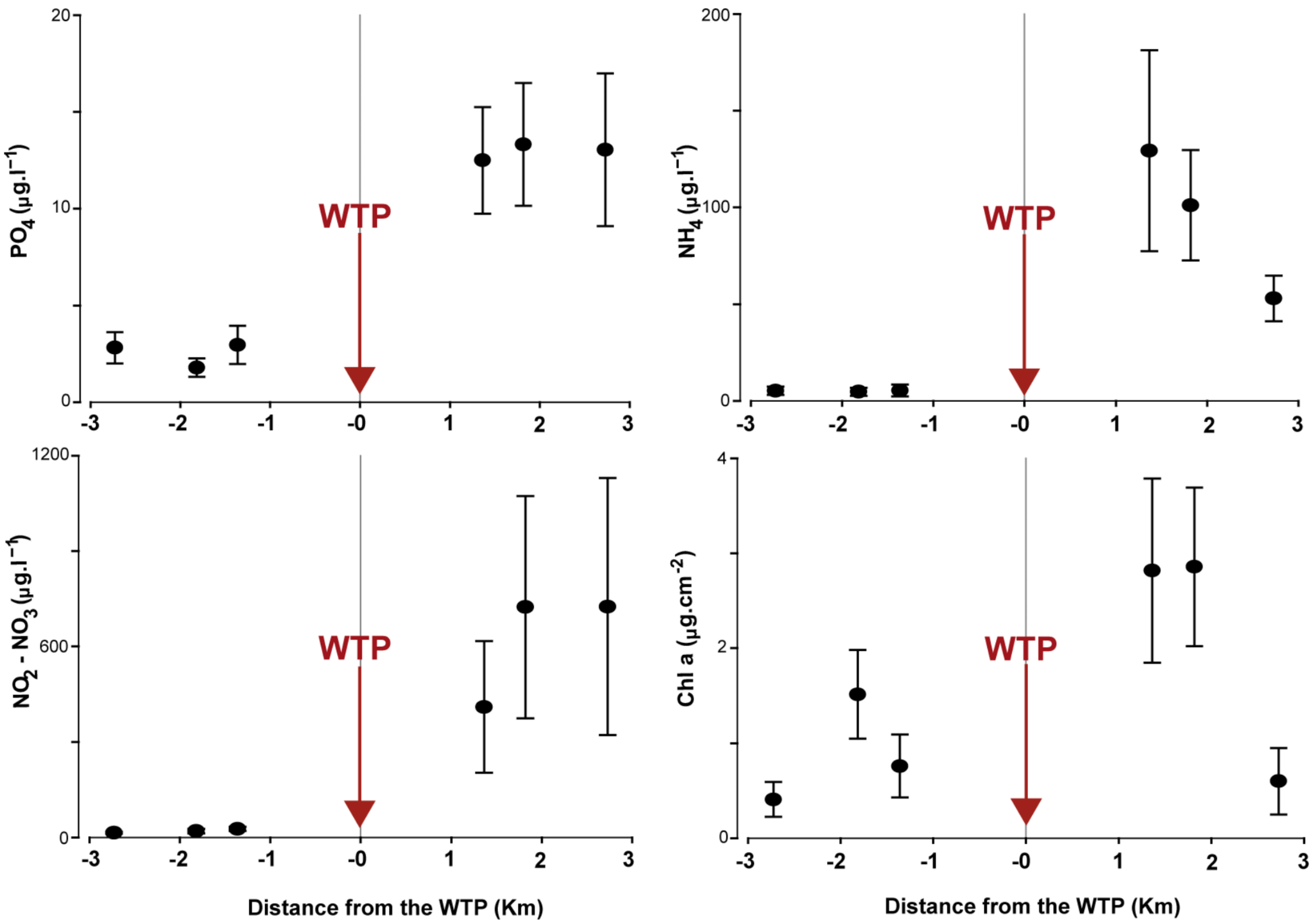

Fig. 2 Flux patterns of the main nutrients and chlorophyll *a* at pre- and post-WTP sites at different distances from the wastewater effluent discharge (WTP) on the Quemquemtreu River. Data are mean values (n = 4, ±Standard deviation) during the study period 2018

Table 2 Resume of the mean values, ranges (minimum and maximum) and the Biological oxygen demand (BOD), bacteria *Echerichia coli* (EC) and Total coliforms bacteria (BCT) categorized as low (1–3), medium (4–6) and high (9–14) at pre- and post-WTP sites in the Quemquemtreu River (2018)

Variable	Sector	Mean values	Min. – Max. values	Category			Chi^2 (*p*)
				low	medium	high	
BOD	pre-WTP	1.5	1–3	4	0	0	12
	post-WTP	7.2	4–14	0	5	3	(0.002)
EC	pre-WTP	46.7	< 30–91	4	0	0	8.4
	post-WTP	1478.9	91–7300	1	3	4	(0.015)
BCT	pre-WTP	57.5	< 30–91	4	0	0	12
	post-WTP	5332.5	230–14,000	0	3	5	(0.0025)

obtained and included exotics - Rainbow Trout (*O. mykiss, n* = 234) and Brown Trout (*S. trutta, n* = 306) - and natives, of which only the Catfish (*Hatcheria macraei, n* = 8) was collected. The smallest individuals recorded were Rainbow Trout (2.7 cm-0.13 g) and the largest individuals were also Rainbow Trout (37.4 cm-568.1 g). The total length of Brown Trout individuals ranged between 5.5 and 27.7 cm, and they had a weight range of between 2.0 and 170.5 g. On the other hand, the total length of the native Catfish individuals caught ranged between 3.4c and 8.5 cm, and they weighed between 0.3 and 3.3 g. Taking the entire sample into consideration, significant differences were observed in terms of total length (KW $p < 0.05$) and weight (KW $p < 0.05$) as a function of the distance from the WTP. The condition factor of the exotic fish did not vary in a consistent manner ($p = 0.33$). Rainbow Trout did not present differences in weight and length in relation to the distance from the WTP; however, a significant difference was detected among Brown Trout individuals (KW $p < 0.05$). Due to the low Catfish specimens captured, this comparison was not considered. The longest (Fig. 4A)

Table 3 Plant inventory was recorded in pre- and post-WTP sites considering the habitat: subshrub (SA), grass (H), shrub (AR), tree (A); life cycle: Perennial (P), annual (A), annual/biannual (AB), and origin: exotic (E), native (N). Species of macrophytes were identify with *

			pre-WTP			post-WTP		
Species		Habit	Q1	Q2	Q3	Q4	Q5	Q6
Artemisia absinthium	*Exotic*	SAPE				x		
Artemisia verlotorum	*Exotic*	HPN	x		x	x	x	
Baccharis linearis	*Native*	ARPN	x		x			
Brassica nigra	*Exotic*	HAE	x	x	x		x	x
Brassica rapa	*Exotic*	HAE					x	
Carduus thoermeri	*Exotic*	HBAE				x	x	
Centaurea cyanus	*Exotic*	HAE						x
Chenopodium album	*Exotic*	HAE	x					
Cirsium vulgare	*Exotic*	HBAE						x
Conium maculatum	*Exotic*	HBAE				x	x	x
Convolvulus arvensis	*Exotic*	HPE	x					x
Crepis capillaris	*Exotic*	HAE	x			x		x
Cytisus scoparius	*Exotic*	ARPE	x	x	x	x	x	x
Dactylis glomerata	*Exotic*	HPE						x
Diostea juncea	*Native*	ARPN			x			
Epilobium brachycarpum	*Exotic*	HAE						x
Eschscholzia californica	*Exotic*	HPE				x		x
Gunnera tinctorea	*Native*	HPN	x					
Hypericum perforatum	*Exotic*	HPE				x	x	x
Hypochaeris radicata	*Exotic*	HPE	x			x	x	x
Impatiens glandulifera	*Exotic*	HAE					x	x
*Juncus balticus**	*Native*	HPE			x			
*Juncus involucratus**	*Native*	HPN	x					
Lactuca serriola	*Exotic*	HBAE	x		x		x	x
Lupinus polyphyllus	*Exotic*	HPE		x	x	x	x	x
Malva sylvestris	*Exotic*	HAE	x	x				
Medicago polymorpha	*Exotic*	HAE				x	x	x
Medicago sativa	*Exotic*	HPE					x	
Melilotus albus	*Exotic*	SABAE		x		x		x
*Myriophyllum quítense**	*Native*	HPN	x			x		
*Nasturtium officinale**	*Exotic*	HPE				x	x	
Nothofagus dombeyi	*Native*	APN					x	
Oenothera odorata	*Native*	HAN		x	x	x		
Pinus ponderosa	*Exotic*	APE		x				
Pinus radiata	*Exotic*	APE		x				
Plantago lanceolata	*Exotic*	HPE	x	x	x	x		x
Polygonum lapathifolium	*Exotic*	HPE					x	
Populus sp.	*Exotic*	APE		x			x	
Rosa rubiginosa	*Exotic*	ARPE	x	x		x	x	
Rubus ulmifolius	*Exotic*	ARPE	x	x	x	x	x	x
Rumex acetosella	*Exotic*	HPE				x		x
Rumex crispus	*Exotic*	HPE	x			x		x
Salix spp.	*Exotic*	APE	x	x	x	x	x	x
Saponaria officinale	*Exotic*	HPE	x	x		x	x	x
Schinus patagonicus	*Native*	ARPN				x		
Solidago chilensis	*Native*	HPN				x		
Trifolium pratense	*Exotic*	HPE	x					
Tripleurospermum inodorum	*Exotic*	HAE				x	x	x
Verbascum thapsus	*Exotic*	HBAE				x	x	
*Veronica anagallis-aquatica**	*Exotic*	HAE				x		
Total Richness			**20**	**14**	**12**	**27**	**23**	**24**

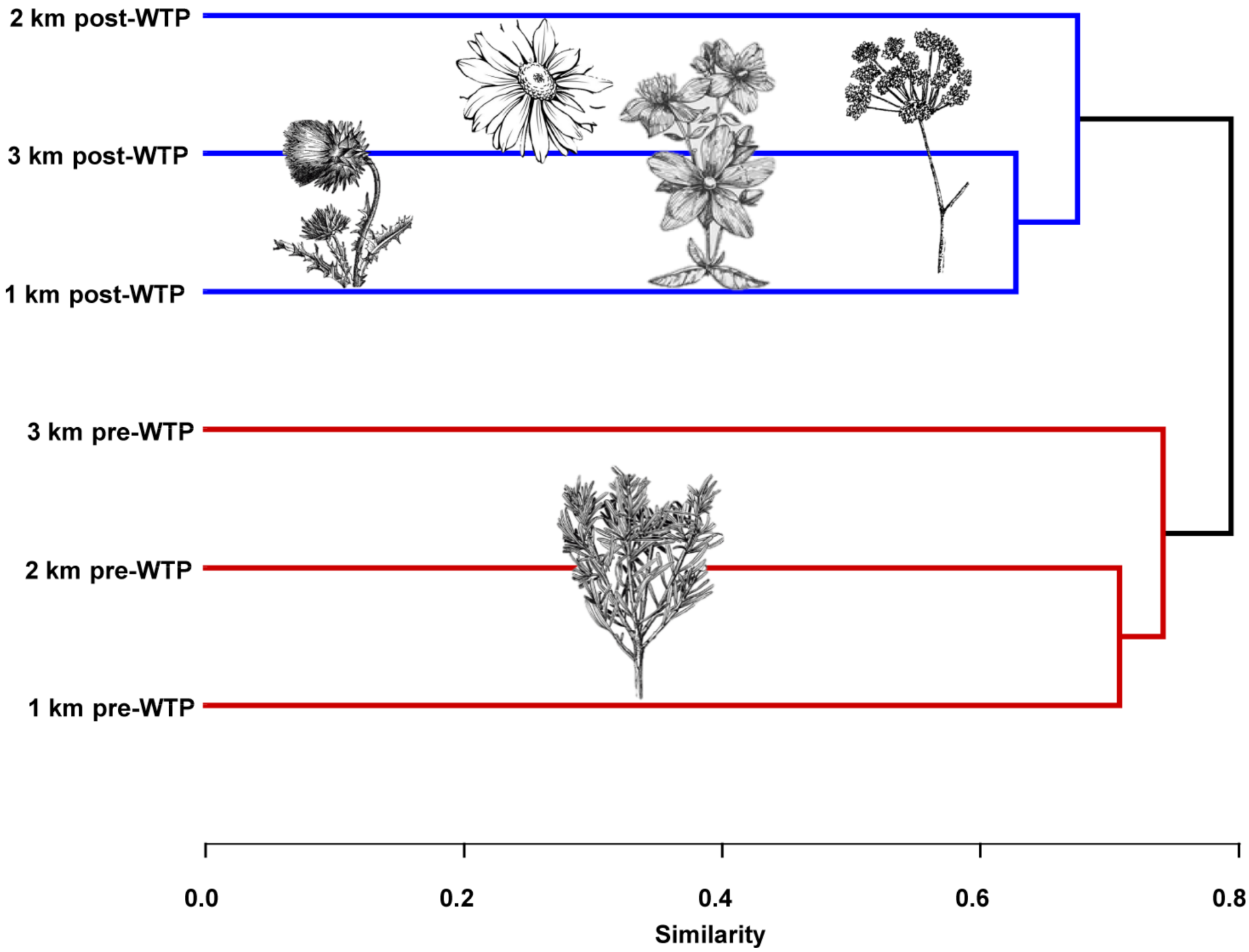

Fig. 3 Specific composition of aquatic, riparian and terrestrial plants inventoried along the Quemquemtreu River showing significant differences between pre- and post-WTP zone

and heaviest (Fig. 4B) individuals of this species were recorded at site Q5, whereas the individuals with the best condition score were recorded at sites Q3 and Q6 (Fig. 4C).

A spatial variation in total fish abundance was observed, with a decrease seen in the post-WTP sector: a marked reduction in fish abundance occurred at Q5 (Fig. 4D). Even though an increase in total fish abundance was observed at the farthest site from the WTP discharge point, Q6, it was lower than the level of abundance recorded in the pre-WTP stretch. Despite no significant correlation being detected between WTP distance and total fish biomass ($p = 0.08$) and density ($p = 0.5$), our PCA analysis (with eigenvalues larger than 1, explaining about 76.6% of the total variance) indicated the existence of two environmental gradients. The first component, which explains 50.9% of the total variance within the dataset, was characterized by high positive loadings for ammonium and phosphates and a negative correlation with total fish abundance, total fish biomass and total fish density. The second component, which explains 25.8% of the variance, was characterized mainly by a high positive loading for the fish parameters (Fig. 5 Table 4). The biological variables considered in this study appear to be positively affected by distance from the WTP, particularly at 3 km and 2 km upstream from the WTP: those sites showed the lowest values of ammonium, phosphates, nitrate plus nitrite nitrogen, and chlorophyll.

Discussion

Our study demonstrates that urbanization and WTP effluent discharges significantly altered several environmental conditions, biota, and ecosystem features in the Quemquemtreu River. The impacts detected were; nutrient enrichment, increased of bacteria counts, alteration of riparian vegetation, and changes in fish assemblages. Specifically, among main environmental changes, this research shows that in the farthest sampling site from the

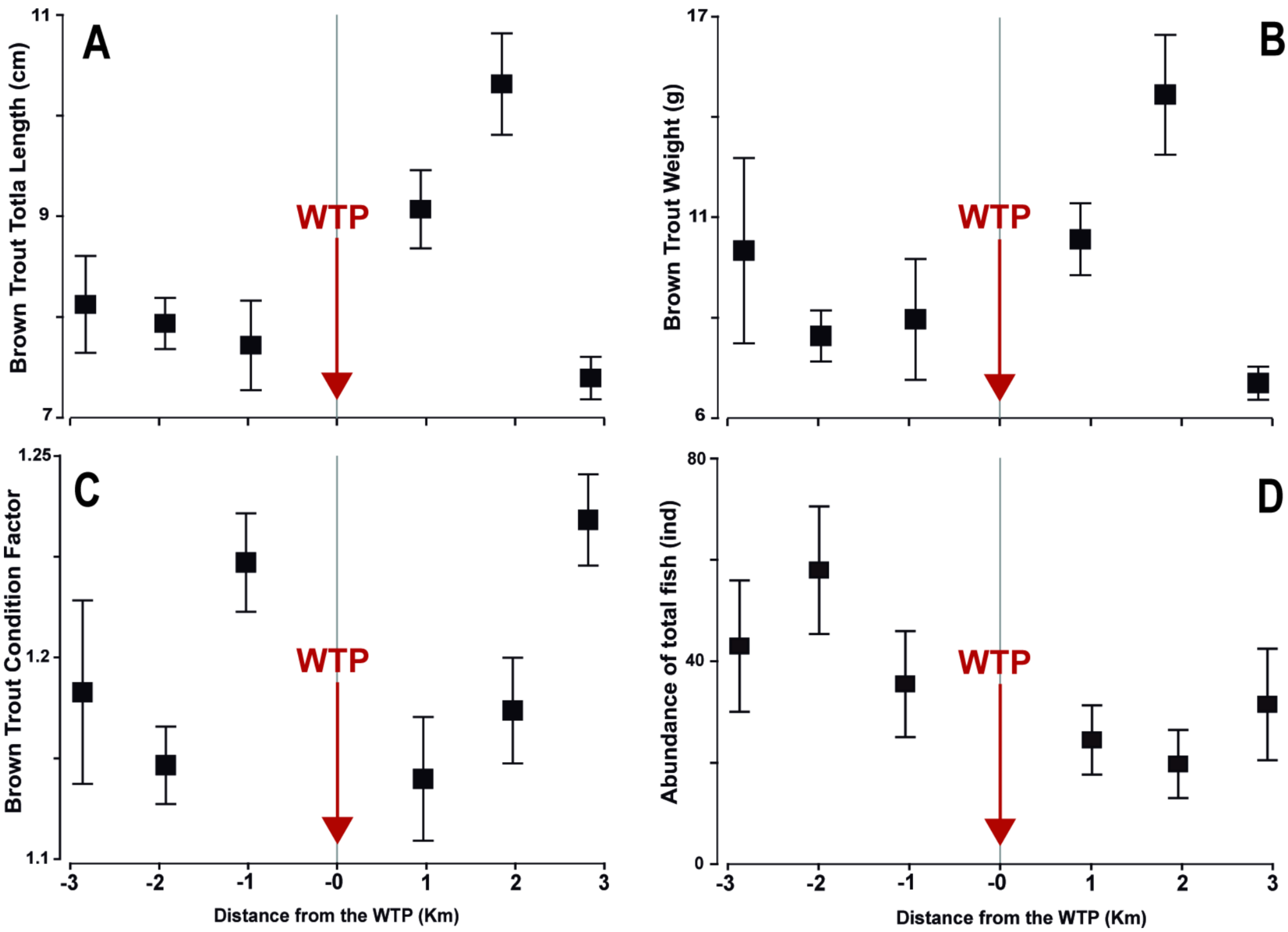

Fig. 4 Mean values of Brown trout (*Salmo trutta*) total length (A), weight (B), condition factor (C) and the total fish abundance (*Onchorynchus mykiss*, *S. trutta* and *Hatcheria macraei*) in pre- and post-WTP at different distances (1, 2, 3 km) on the River

WTP (3 km downstream) level of ammonium, as well as epilithic chlorophyll, declined markedly, whereas both nitrate plus nitrite nitrogen and phosphorus remained at high concentration values. In line with these observations, a study in the NE Iberian Peninsula showed that wastewater inputs into streams seriously impaired in-stream chemical features due to increased pollutant concentrations (Pereda et al. 2021). The high contribution of periphyton (Chl *a*) seen in our study was strongly associated with the availability of nutrients in the watercourse. Besides, a significant correlation between Chl *a* and nutrient values downstream from the WTP effluent discharge point was observed. Urrea-Clos et al. (2014) suggested that high periphyton production could be regarded as evidence of nutrient enrichment by wastewater in an Iberian watershed. In Patagonia, several studies have reported an increase of Chl *a* in streams associated with urbanized and agricultural areas (confined animal production, extensive pastureland) (Fierro et al. 2019; Horak et al. 2020). The increase in epilithic production could be due to urine and fecal excreta from cattle, combined with a higher solar exposure which results in the proliferation of primary producers (Miserendino et al. 2016). Consequently, this growth may directly or indirectly increase the carrying capacity of these sites via a "bottom-up" effect, creating an advantage for the top consumers because of the food resources available (McCallum et al. 2019). Here, we documented an increase in the concentrations of fecal coliform and *E. coli*, particularly downstream from the WTP outflow point. The best bacteriological condition was observed at the farthest point upstream (at 3 km) from the WTP outflow; nonetheless, this site showed the presence of coliform bacteria. Similarly, Paul and Meyer (2001) and Horak et al. (2020) found high bacterial activity in streams adjacent to urbanization and to agricultural areas, respectively. It is well known that bacteria from urban pollution might enter streams by runoff or through being directly deposited via wastewater effluent. Among the negative impacts that microorganisms produce are the observed decrease in dissolved oxygen concentration (Rizzo et al. 2012) and the possible effects on the health of recipient

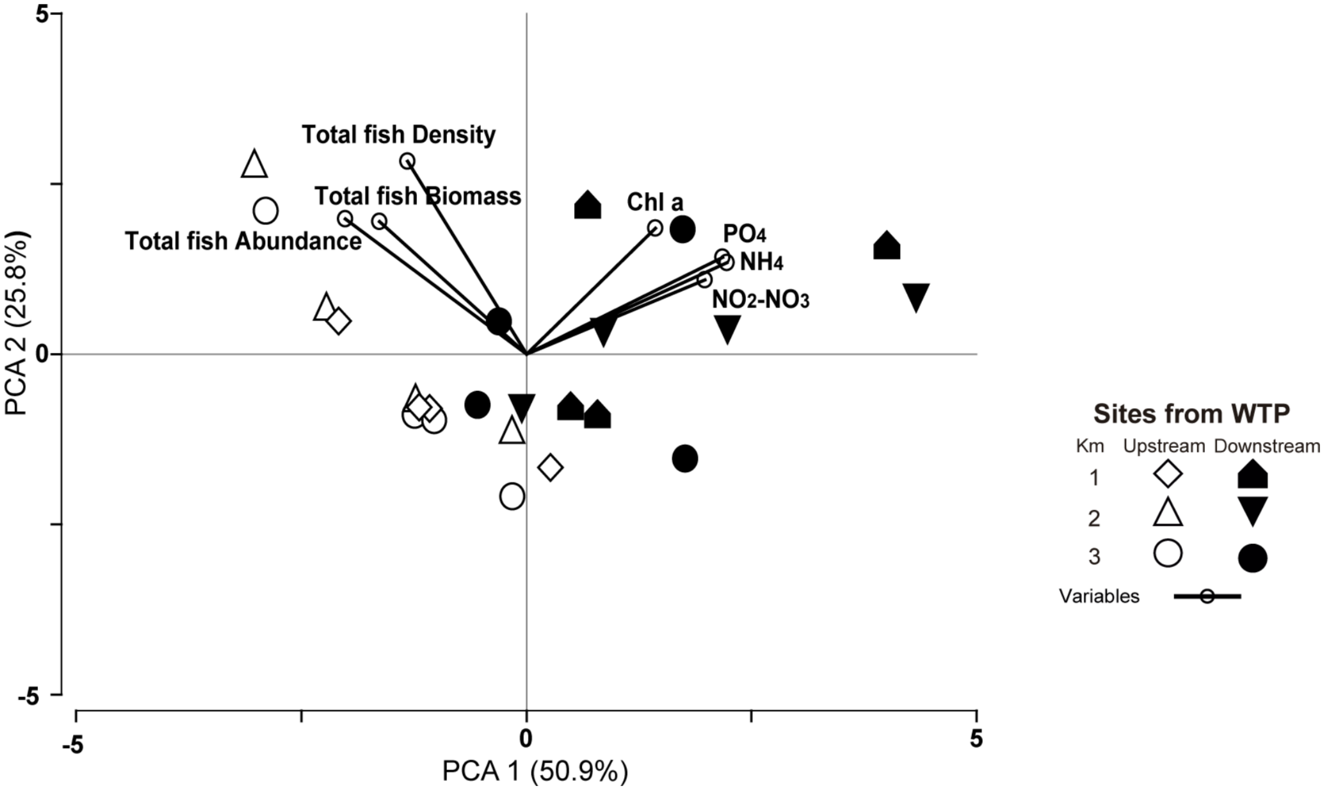

Fig. 5 Principal Component Analysis (PCA) based on total fish abundance, density, and biomass in relation pre and post-WTP sites as a function of wastewater effluent discharge distance and the nutrients as random effect variables. Pre-WTP symbols are in white colour, post-WTP symbols are in black colour

Table 4 Principal Component Analysis (PCA) axes weighted intraset correlation of environmental variables seasonally measured (n = 24) at pre- and post-WTP (wastewater treatment plant) sites in the Quemquemtreu River during 2018

Variables	PCA1	PCA2
Distance (km)	0.34	-0.03
Total fish abundance	-0.26	-0.29
Exotic fish	-0.21	-0.32
Native fish	0.03	-0.19
Water temperature (ºC)	-0.31	0.14
Conductivity ($\mu S.cm^{-1}$)	0.1	-0.38
Salinidad (‰)	0.28	-0.28
TDS ($mg.l^{-1}$)	0.13	-0.38
Dissolved oxygen ($mg.l^{-1}$)	-0.23	-0.19
Oxygen saturation (%)	-0.28	0.05
SRP ($\mu g.l^{-1}$)	0.35	0.01
NH_4^+ ($\mu g.l^{-1}$)	0.3	0.03
NO_3^--NO_2^- ($\mu g.l^{-1}$)	0.34	0.03
TSS ($mg.l^{-1}$)	0.33	0.13
Current velocity (m/s)	-0.06	0.37
Discharge (m^3/s)	0.02	0.44

habitats and the wildlife that reside in them (Holeton et al. 2011; Hamdhani et al. 2020). The fact that *E. coli* presence was recorded in the farthest sites, 3 km upstream from the WTP discharge point, is a wake-up call because the water would be suitable for recreational purposes only, and not for human consumption. A real prospect that this stretch of the watercourse will soon become unsuitable for recreational or cultural activities is raised. Especially serious are the conditions detected downstream from the WTP discharge point because exceeding the current standard recommendations (DPN 1540/16 Chubut Province). Therefore, it is crucial that any water for human drinking should first undergo a process of purification, irrespective of where in the watercourse it came from.

The present study also highlighted a significant impact on the richness and composition of the riparian forest between pre- and post-WTP stretches of the Quemquemtreu River. The increase in the richness of the exotic species downstream of the WTP revealed that urbanization produced a significant modification of the riparian vegetation. The presence of the exotic *Salix* spp. through the entire watercourse was not a surprising feature. Essentially, in riverine landscapes in Patagonia, willow invasion is a frequent phenomenon because these species are very competitive and successful in colonizing disturbed areas (Thomas and Leyer 2014; Miserendino et al. 2016). The invasion of riparian areas by exotic species; constitutes a threat to biodiversity and modifies the structural properties of the ecosystem, including nutrient cycles, primary productivity, and biotic interactions (Fuentes-Ramírez et al. 2011).

It would be fair to argue that those shifts in the increment of ammonium, nitrate plus nitrite nitrogen, and phosphorus downstream from the sewage input could play a role in the fish population alteration. These findings are similar to those reported for Canada and Catalonia (NE Spain), where scientists detected that water quality was the most significant environmental gradient for fish populations, which resulted in modification of fish composition at sites exposed to sewage (Figuerola et al. 2012; McCallum et al. 2019, Mehdi et al. 2018). The higher values of total fish abundance seen at the pre-WTP sites suggest a preference by the fish for the better stretch´s water quality as indicated by the low concentration of nutrients, chlorophyll, bacteria (total coliforms, *E. coli*), and the biological oxygen demand (BOD). This finding supports previous observations in rivers containing untreated wastewater whereby fish seemed to prefer sites with lower levels of organic matter, confirming the perception that fish in the wild have some capacity to avoid those impacted areas (Dyer et al. 2003; Nikel et al. 2021). It was notably observed in the present study and is reflected in the pattern for total fish abundance, which was lower in the sample point nearest to the WTP discharge point, and increased with distance from the WTP discharge point. This is in line with nutrient perturbations that may be displaced downstream generating an impact gradient because rivers flow unidirectional and nutrients exhibit a spiraling process; thus, fish can avoid it because of their mobility (Newbold et al. 1981). A particular trend in the distribution pattern of the exotic Brown Trout occurred. Individuals from this species were predominantly heavier and longer nearer to the WTP effluent discharge (at 1 km). However, the individuals with the best condition factor scores were detected at sites further away (3 km). Similar results were observed with exotic trout in an urban Patagonian stream, where fish biomass was higher at urban sites than at the reference sites further away from urbanization (Miserendino et al. 2008). Likewise, Nikel et al. (2021) have highlighted a divergence in thermal tolerance and somatic investment along a gradient of wastewater exposure as a greater abundance of fish near effluent outfalls. These observations suggest that despite the decrease in water quality caused by wastewater treatment plants, their contribution in terms of higher nutrient loading may allow many fish species to access the extra nutrients provided by such effluent outflows (Paul and Meyer 2001; Nickel et al. 2021). Indeed, several authors in North America, Europe, and South America have found that salmonid production was higher at urban sites than in other reference areas, as was the ability of salmonids to occupy and compete against other species in altered ecosystems (Habit et al. 2006; Di Prinzio et al. 2009; Hughes and Dunham 2014).

Our findings indicate that although Patagonian freshwater streams are pristine systems, urbanization leads to an increase in pollution, mainly from the discharge of domestic wastewater into rivers. Thus, wastewater discharge into surface water bodies may have an impact on fish communities, so it is critical to apply correct management and protect freshwater resources. There is arguably a need to change water management policies to improve the water quality of the effluents from wastewater treatment plants and guarantee the dilution capacity of Patagonian rivers. The consequences of chronic exposure to wastewater effluent on the wildlife residing in these impacted habitats are unknown. We consider that restoration actions are in time to be applied.

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Author´s contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by **Ph.D. student Alan Sebastian Andrade-Muñóz, Dr. Cecilia Di Prinzio, Dr. Yanina Assef, Dr. Adriana Kutchsker, MSc German Alday, Technical assistant Mauricio Dromaz, Dr. Pamela Quinteros and Dr. M Laura Miserendino**. The first draft of the manuscript was written by **Dr. Cecilia Di Prinzio** and **Ph.D. Student Alan Sebastian Andrade-Muñóz** and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Declarations

Conflicts of interest/competing interest Not applicable.

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