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RESEARCH ARTICLE

# Water quality and diarrhoeagenic *Escherichia coli* detection in surface Pampean aquatic systems

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## Abstract

Many surface water systems are impacted by point source pollution from sewage discharges and industrial wastes, as well as diffuse pollution from agriculture and livestock farming, inducing a potential biohazard to human, animal, and environmental health. This study aimed to determine the presence of diarrhoeagenic *Escherichia coli* (DEC) pathotypes and their antibiotic resistance, as well as the bacteriological, physical, and chemical water quality conditions in two Pampean peri-urban rivers (Rojas and Salado rivers, Buenos Aires, Argentina) used for recreation. Additionally, we explored the impact of the surrounding land use on the water quality. In the Rojas (R) and Salado (S) rivers, wastewater discharges from treatment plants increased nutrient content and coliform abundances at specific sampling sites (R2 and S3) and downstream (R3 and S4, respectively). Coliform abundances correlated with ammoniacal nitrogen concentrations, both exceeding recreational use guidelines. Out of 36 samples positive for DEC virulence factors, 11 DEC strains were isolated (5 enteroaggregative, 3 enteropathogenic, 1 shigatoxigenic-$stx_1$/$stx_2$, 1 shigatoxigenic-$stx_2$, 1 hybrid enteroaggregative-enterotoxigenic). Six strains were resistant to one or more antibiotics. Our results suggest that differences in *E. coli* pathotypes between the two rivers and the water quality of each sampling site are linked to the surrounding land use, evidencing both diffuse and point source pollution.

**Keywords** Aquatic systems · Physical parameters · Chemical parameters · Microbial quality · Diarrhoeagenic *Escherichia coli* · Antimicrobial susceptibility

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## Introduction

*Escherichia coli* is a Gram-negative bacterium which is part of the microbiota of warm-blooded animal gastrointestinal tracts, including humans (Kaper et al. 2004). While most *E. coli* strains are non-pathogenic and harmless, some have acquired mechanisms that make them pathogenic for humans (Barbau-Piednoir et al. 2018; Guo et al. 2019). The reservoirs of pathogenic *E. coli* include the intestinal tracts of ruminant animals, mainly bovines (Clements et al. 2012), as well as infected humans (CDC 2024).

*E. coli* is commonly used as an indicator of faecal contamination in water sources, indirectly pointing to the potential presence of pathogenic microorganisms (Blaustein et al. 2013). However, several studies reported that *E. coli* can survive and even reproduce also in secondary environments, such as marine waters and freshwaters (e.g., Berthe et al. 2013; Tymensen et al. 2015; Jang et al. 2017; Suzuki et al. 2019). Multiple environmental factors including

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temperature, pH, salinity, predation, and sunlight have been found to influence the survival of *E. coli* in water (Blaustein et al. 2013 and citations therein). This raises concerns regarding its reliability as a sole indicator of faecal contamination, highlighting the need for more field-based studies across diverse regions (Petersen and Hubbart 2020).

Evaluating water quality (physical, chemical, and microbiological characteristics) is a crucial first step towards disentangling and comprehending the effect of both natural and anthropogenic factors on aquatic ecosystems (Srebotnjak et al. 2012; Mamun et al. 2022). In particular, recreational waters and public water supplies contaminated by *E. coli* represent a significant health threat, since certain pathogenic strains can spread along with other harmful microorganisms such as *Cryptosporidium*, *Giardia*, *Shigella*, and norovirus (Orsi et al. 2007; Navab-Daneshmand et al. 2018), potentially exacerbating their impact. Major sources of *E. coli* contamination in water include human waste, untreated wastewater discharges, farms, livestock, waterfowl, wildlife, and domestic pets (Ishii et al. 2007).

Pathogenic *E. coli* are classified into two major groups: diarrhoeagenic *E. coli* (DEC) and extraintestinal *E. coli* (ExPEC), which can be further categorised into pathotypes, based on the virulence and the clinical syndrome that they produce (Barbau-Piednoir et al. 2018). The ExPEC group contains uropathogenic *E. coli*, neonatal meningitis *E. coli*, and sepsis-associated *E. coli*. Mainly six pathotypes are included in the DEC group: shigatoxigenic *E. coli* (STEC), enteropathogenic (EPEC), enterotoxigenic (ETEC), enteroinvasive (EIEC), enteroaggregative (EAEC), and the sixth diffusely adherent (DAEC) pathotype, which its association with diarrhoea continues under discussion (Croxen and Finlay 2009). Moreover, emergent pathotypes such as shigatoxigenic/enteroaggregative (EAEC-*stx*), ETEC/EPEC, and EPEC/STEC are also being considered new pathogens to be included in the classification (De Mello Santos et al. 2020). The first six DEC pathotypes mentioned have been implicated in waterborne outbreaks (Petit et al. 2017) and can be identified by specific virulence genes, making them detectable in distinct sources such as water (e.g., Sidhu et al. 2013).

Recently, *E. coli* has been identified as a high-priority pathogen for monitoring owing to its extensive antibiotic resistance (Tacconelli et al. 2018; Pellegrini et al. 2022). This concern is reflected in intensive global surveillance programmes that track outbreaks and include environmental monitoring; although, unfortunately, these programmes are often not completely implemented in developing countries (Croxen et al. 2013; Ayukekbong et al. 2017).

Argentina has one of the highest prevalence of STEC infections, predominantly associated with serotypes O157:H7 and O145:H28 (Rivas et al. 2014; Pianciola et al. 2016; Carbonari et al. 2022), and also holds the highest incidence of Hemolytic Uremic Syndrome (HUS) in the world (Rivas et al. 2006; Torti et al. 2021; BIV 2021). However, few works on surface aquatic systems from the Pampa Region (Argentina) have evaluated the presence of pathogenic *E. coli* strains surviving in this environmental matrix. For instance, Marucci et al. (2011) reported for the first time the STEC isolation from recreational waters in Sierra de la Ventana (southern Pampa Region) and described the streams and rivers from a beef-producing area of Argentina as potential reservoirs of these strains. Besides, Tanaro et al. (2010, 2014, 2018) detected the presence of STEC O157:H7 in surface waters, such as watercourses, lagoons, and streams inside and near beef cattle farms and feedlots from Entre Ríos province (northern Pampa Region). Other work published by Polifroni et al. (2014) reported the presence of STEC in different environmental matrices (ground, cattle drinking troughs, and feeders) from a Pampean dairy farm. Moreover, Díaz et al. (2024) found DEC at recreational sites on the Río de la Plata River. However, most studies have focused primarily on STEC, with limited research on other DEC pathotypes. Additionally, other works carried out in the Pampa Region also studied the presence of *E. coli*, their phylogroup structure and cryptic clades in a highly polluted urban stream network (Saraceno et al. 2021), as well as in environmental samples from horticultural farms, including *E. coli* antibiotic resistance (Pellegrini et al. 2022).

This study aimed to determine the presence of DEC pathotypes and their antibiotic resistance in two Pampean peri-urban rivers (Rojas and Salado rivers, Buenos Aires Province) during 2021, as well as to evaluate the bacteriological, physical and chemical quality of the water due to the fact that these rivers are usually used by the population for recreational purposes (e.g., fishing, canoeing). Besides, we explored the impact of the surrounding land use on the water quality and the association between the abundance of total coliforms, faecal coliforms, and *E. coli* with the local physical and chemical parameters measured. We posited the following hypotheses: (a) discharges from wastewater treatment plants (WWTP) and urban areas into the Salado and Rojas rivers not only alter the water's physical and chemical parameters but also increase the abundance of coliform bacteria; (b) both rivers possess self-purification capabilities, enabling them to gradually restore their water quality after flowing downstream from the WWTP; (c) the presence of DEC pathotypes, their antibiotic resistance, and virulence genes are present in both highly human-impacted rivers. This research represents the first investigation into the occurrence of DEC, their antibiotic resistance, and microbial quality (utilising both culture and molecular approaches) of these rivers.

## Materials and methods

### Study area

The two studied rivers are located in the Pampa Region of Argentina. This region is characterized by a marked thermal seasonality, variable precipitations (400–1000 mm/year), and alternating floods and droughts (Diovisalvi et al. 2015; Viglizzo and Frank 2006). The Rojas River has a channel width that ranges between 44 and 92 m, a depth that varies from 0.8 to 4.3 m, and a basin that occupies an area of 2050 $km^2$ (INA 2006). This river is located near the city of Rojas (34.12° S, 60.44° W; 69 m a.s.l.), with a population of 25,627 residents (INDEC 2022) (Fig. 1a). The primary economic activities in the area include agriculture, livestock breeding, and industry. The Salado River is a lowland lotic system with a length of approximately 600 km and a watershed of approximately 150,000 $km^2$ (Gabellone et al. 2005). Its basin is considered the second South American wetland in terms of rainfall accumulation and exhibits a high heterogeneity in land use, geomorphological features, soil types, and hydraulic modifications (Gabellone et al. 2005, 2008). This river receives nutrient inputs from agricultural activities and urban areas, impacting its trophic status (Neschuk et al. 2000, 2002; Gabellone et al. 2005). In the upper subbasin, the river flows through the vicinity of the city of Junín (34.59° S, 60.94° W; 76 m a.s.l.) with a population of around 103,787 inhabitants (INDEC 2022) (Fig. 1a).

### Sample collection

Triplicate water samples were collected seasonally over a period of 12 months (4 sampling campaigns: March, June, September, and December 2021) in the two rivers. We studied the course of the Salado River near the city of Junín and the course of the Rojas River adjacent to the city of Rojas. Sampling sites selected along each river were situated upstream of the city (S1 for Salado River, R1 for Rojas River), adjacent to the city (S2, R2) and downstream of the city (S3, R3, S4 and R4) (Fig. 1a). In the Rojas River, the R2 site was affected by the effluent from a WWTP, while in Salado River, this impact was observed at the S3 site.

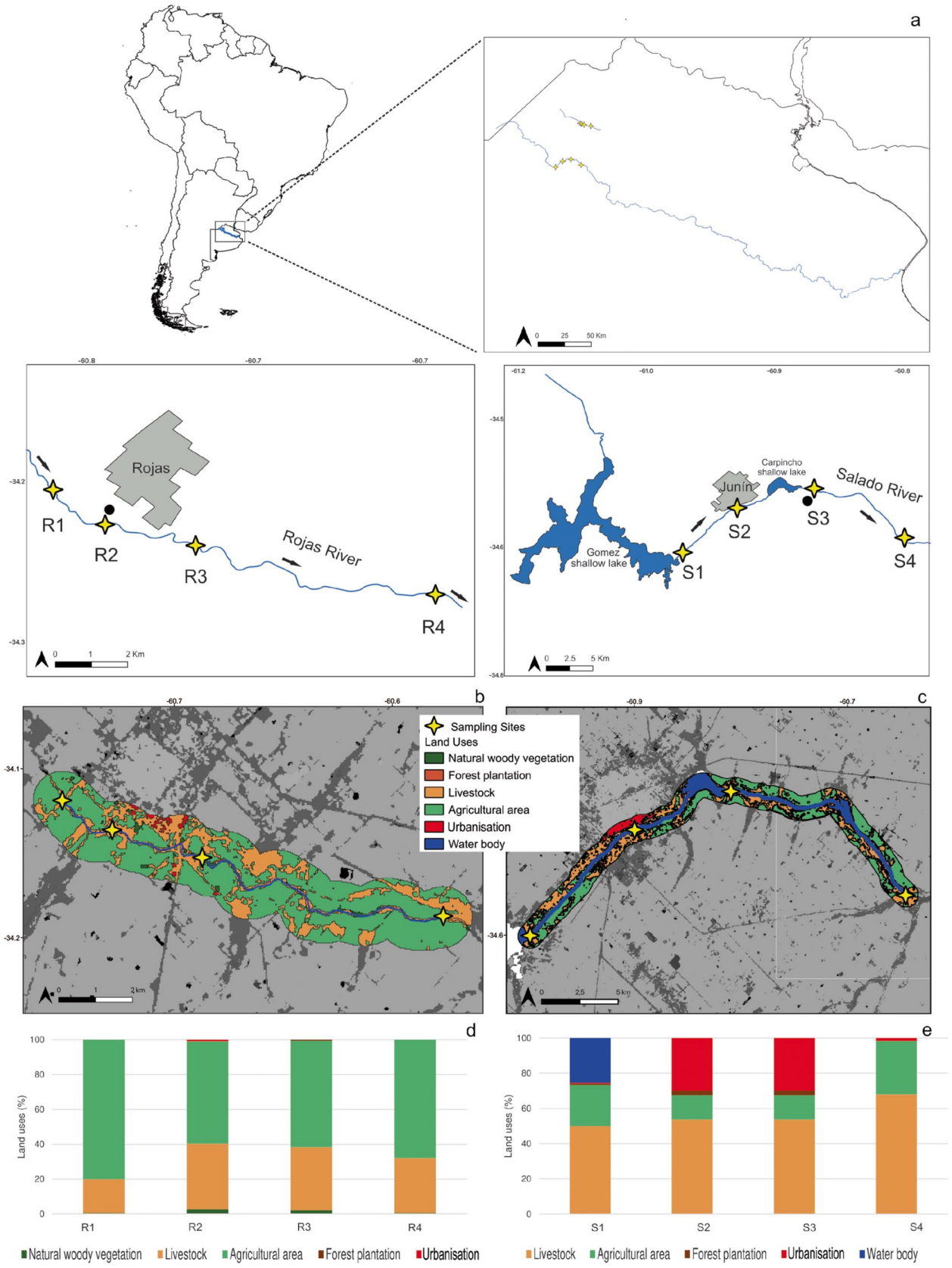
### Measurements of land use, physical, chemical, and biological parameters

Water temperature, pH, conductivity, total dissolved solids (TDS), dissolved oxygen (DO), and turbidity were registered in situ with portable meters (Hanna HI 991301, HACH HQ30D, Lutron TU-2016). Secchi depths (SD) were also measured in situ for the estimation of transparency. Samples were collected in sterile bottles from a depth of 20–30 cm below the surface in the centre of each river and transported to the laboratory in cold and dark conditions. Determinations of nutrients (ammoniacal nitrogen, $N\text{-}NH_3$, and soluble reactive phosphorus, SRP), chemical oxygen demand (COD), and chlorophyll a (Chl-a) were performed following the methods described by Schiaffino et al. (2019). Besides, chlorides and sulphates were performed by the Mohr (Belcher et al. 1957) and turbidimetric methods (ASTM 1995), respectively. Chromophoric dissolved organic matter (CDOM) was measured from filtered water samples (through GF/F filters) using quartz cuvettes and compared against ultrapure water blanks (Gerea et al. 2017). Absorbance at 440 nm, $a_g$ (440), was used as an optical parameter to describe water colour; absorbance at 254 nm, $a_g$ (254), as a proxy of the variability of CDOM quantity (CDOM $a_g$254) and the ratio $a_g$ (250)/ $a_g$ (365) as an indicator of molecular size index (Torremorell et al. 2015; Luculano et al. 2019).

For land use assessment, georeferenced Landsat images with a 30-m resolution were obtained from the trinational MapBiomas Pampa Initiative-Collection 3 (Proyecto MapBiomas Pampa Trinacional 2024). To this end, 800-m-wide buffer areas were constructed around each sampling point and along the riparian zone of both rivers. The selection of the 800-m buffer was based on its higher significance in relation to the measured environmental variables and land uses, as indicated by Xu et al. (2021). Area calculations were performed using QGIS v. 3.36 software (QGIS Development Team 2024), and land use categories were classified as natural woody vegetation, livestock, agricultural area, forest plantation, urbanisation, and water body according to the land use classification established by Baeza et al. (2022).

### Bacteriological analyses

Total coliforms (TC), faecal coliforms (FC), and *E. coli* were quantified using the most probable number (MPN) method in accordance with APHA (2005). In summary, 250 mL of water from each site and river were collected during the four sampling campaigns. These samples were then inoculated in lauryl sulphate broth (LS) in three dilutions (1/10, 1/100, 1/1000), by quintupling, respectively. Following incubation at 37 °C for 48 h, an aliquot of all the tubes was inoculated into the brilliant green lactose bile broth. The presence of TC was confirmed if gas formation occurred within 48 h after incubation at 37 °C. At the same time, FC were evaluated after inoculation of an aliquot of all LS broth tubes in EC broth. If turbidity and gas were produced 24 h after incubation at 44 °C, the presence of FC was confirmed. *E. coli* strains were analysed from each positive TC tube and confirmed by minimal biochemical tests: Triple Sugar Iron agar (TSI), Sulfide Indole Motility (SIM), and Simmons Citrate agar (Mossel et al. 2003).


a
0 25 50 Km
Rojas
R1
R2
R3
R4
Rojas River
0 1 2 Km
Carpincho shallow lake
Junín
Salado River
Gomez shallow lake
S1
S2
S3
S4
0 2.5 5 Km
b
c
Sampling Sites
Land Uses
Natural woody vegetation
Forest plantation
Livestock
Agricultural area
Urbanisation
Water body
0 1 2 km
0 2.5 5 km
d
e
Land uses (%)
R1
R2
R3
R4
S1
S2
S3
S4
Natural woody vegetation
Livestock
Agricultural area
Forest plantation
Urbanisation
Water body

◂**Fig. 1** **a** Map showing the location of Gómez shallow lake, Rojas and Salado rivers, the nearby cities, and the sampling sites in each river (Rojas River R1–R4 and Salado River S1–S4). The coordinates of the sampling sites were as follows: R1 (34°12′19.1″ S 60°45′32.0″ W), R2 (34°12′45.2″ S 60°44′38.2″ W), R3 (34°13′07.4″ S 60°43′11.1″ W), R4 (34°14′04.3″ S 60°38′48.7″ W) for Rojas River and S1 (34°39′53.5″ S 61°00′59.7″ W), S2 (34°36′12.0″ S 60°56′28.9″ W), S3 (34°34′52.8″ S 60°52′21.3″ W), S4 (34°38′35.3″ S 60°44′55.0″ W) for Salado River. Arrows indicate each river flow and black circles the location of the wastewater treatment plants. Land use maps of Rojas (**b**) and Salado (**c**) rivers, as well as the percentages of each type of land use around each sampling site in Rojas (**d**) and Salado (**e**) rivers. Land use categories were classified as natural woody vegetation, livestock, agricultural area, forest plantation, urbanisation, and water body

## *E. coli* quantification by digital PCR (ddPCR)

Since not all microorganisms can be cultivated in the laboratory, and some exist in a viable but non-culturable state (Tiwari et al. 2022), traditional bacteriological analyses may underestimate microbial abundance in environmental samples. To address this limitation, we adopted a molecular approach for quantifying *E. coli*, employing droplet digital PCR (ddPCR) at each sampling site. A volume of approximately 100 mL of water was filtered through 0.22 µm pore-size filters, followed by DNA extraction using the CTAB protocol (Fernández Zenoff et al. 2006). Purification was then performed with the DNeasy PowerClean Pro Cleanup Kit (MoBio, QIAGEN©). DNA integrity and concentration were subsequently evaluated by agarose gel electrophoresis and quantified using a Qubit 2.0 Fluorometer (Life Technologies) as per the method described by Sagua et al. (2023).

For ddPCR, we used a primer set (F: 5′GTCCAAAGC GGCGATTTG3′, R: 5′CAGGCCAGAAGTTCTTTT TCCA 3′) and a TaqMan® probe (Eco-PR) specific to *E. coli* designed by Lee et al. (2006). Eco-PR was labelled with a fluorescent reporter dye (FAM) at the 5′ end and a non-fluorescent quencher (BHQ1a) at the 3′ end, with the sequence [6-FAM]ACGGCAGAGAAGGTA[BHQ1a-A]. The expected PCR product was less than 100 bases in length.

The ddPCR reactions were outsourced to the High-Level Technological Service at IHEM-CCT-CONICET-Mendoza-UNCUYO. Each reaction included a No Template Control (NTC) and a positive control containing *E. coli* DNA at a known concentration of 200 ng/µL. The ddPCR setup employed the Eco-PR probe to target a conserved region of the uidA gene specific to *E. coli*. Samples were combined with the probes and reaction mix, then encapsulated into hydrophobic microdroplets using a microfluidic system, enabling an initial DNA concentration estimate of approximately one copy per droplet. Quantification of ddPCR products was conducted based on fluorescence intensity, with data analysed using QuantaSoft Analysis Software v1.96. To account for variations in initial DNA input, which varied according to the concentration of each sample, *E. coli* copy numbers were normalised to 100 mL of sample and reported as copies of *E. coli* per 100 mL of water. This approach ensured consistency and comparability across samples.

## Isolation, characterisation, and antimicrobial susceptibility testing of DEC

The workflow for DEC characterisation is summarised in Supplementary Fig. 1. Water samples from the last three sampling campaigns conducted in June, September, and December of 2021 were analysed. Aliquots from positive tubes of LS and EC broths by MPN quantification method were plated on MacConkey agar (MAC) plates. After incubation (37 °C, 18 h), DNA extracts from a small fraction of the confluent culture zone on MAC were obtained for further DEC molecular identification (Miliwebsky et al. 2019). Conversely, isolated *E. coli* colonies obtained in March 2021 from both rivers were analysed together with those obtained in October 2019 in previous research performed in the same rivers and in Gómez shallow lake (belonging to the Salado River basin and located upstream of site S1 at the Salado River) (Fig. 1a). These *E. coli* colonies were frozen at − 20 °C in nutrient broth with glycerol and grown in MAC before DNA extraction.

For screening purposes of all these samples, we performed DNA extractions. Bacteria grown in MAC were resuspended in 150 µl of 1X TE buffer with 1% Triton, boiled for 10 min, and centrifuged at 10,000 rpm for 5 min. The resulting supernatant was used as a DNA template for the detection of virulence genes through three multiplex PCRs (mPCR): STEC$_{stx1/stx2/rfb\text{O}157}$, EPEC/ETEC$_{eae/lt/stp/sth}$, and EAEC/EIEC$_{aggR/ipaH}$. (Miliwebsky et al. 2019). Samples that tested positive in any mPCR were plated on MAC to isolate DEC. The identification of these isolates as *E. coli* was confirmed through biochemical tests, while serotyping was conducted via agglutination tests utilising somatic and flagella antisera (provided by Denka Seiken Co. Ltd., Japan; the Staten Serum Institute, Denmark; and the Serum and Antigen Service of the INPB-ANLIS "Dr. Carlos G. Malbrán," Argentina). Virulence factor characterisation, including EIEC-*ipaH*, ETEC *lt*/*st*, EAEC *aggR*/*aaiC*, EPEC *eae*, STEC *stx*, *eae*, and *ehxA* genes, as well as *stx*/*eae* gene subtyping, was carried out using PCR, whereas clonal relationships were determined through subtyping using XbaI-PFGE, following previously described protocols (Ramachandran et al. 2003; CDC 2010; Scheutz et al. 2012; Miliwebsky et al. 2019). Antibiotic resistance testing of the *E. coli* strains was conducted using the agar diffusion method (Kirby-Bauer et al. 1959) with antimicrobial susceptibility test discs (BD BBL™, Sensi-disc™) in accordance with the guidelines outlined in the CLSI (2022). Ten antibiotics were tested, including amikacin (AMK), ampicillin (AMP), ciprofloxacin

(CIP), chloramphenicol (CHL), gentamicin (GEN), nalidixic acid (NAL), nitrofurantoin (NTF), streptomycin (STR), tetracycline (TET), and trimethoprim-sulfamethoxazole (SXT). These antimicrobials were selected because, in Argentina, some are used in food-producing animals (Prack McCormick et al. 2022), while others are commonly used in clinical practise (Valdés et al. 2020), but all have been detected in the environment (Prack McCormick et al. 2022; Valdés et al. 2020).

### Statistical analyses

To compare physical and chemical parameters, nutrient concentrations, and coliform abundances obtained from the different sampling sites and months, we performed the non-parametric Friedman test ($Q$) for non-independent samples and post hoc comparisons with Bonferroni-corrected $p$-values (Conover 1999), as data transformation failed to meet the assumptions of repeated-measures ANOVA. To study the association between the physical and chemical variables with TC, FC, *E. coli* abundances, and *E. coli* copy number/100 mL, we assessed pairwise Spearman non-parametric correlations. Correlation plots were performed to visualise these associations. To measure the relative importance of the bacteriological, physical, and chemical parameters (i.e., DO, nutrients, COD, TC, *E. coli*, water temperature, conductivity, pH, and CDOM) and the land use (i.e., natural woody vegetation, livestock, agricultural area, forest plantation, and urbanisation), we performed principal component analysis (PCA) for each river. Statistical analyses were performed in the R environment (R version 3.4.4; R Core Team 2018). Finally, we compared the densities of *E. coli* with the guideline levels for recreational moderate use (298 *E. coli* colonies/100 mL) for single water samples (EPA 1986; SRHN 2003). The N-$NH_3$ concentrations were compared with the guideline levels for the protection of surface freshwater aquatic life (1.37 mg $L^{-1}$) (Decree 831/93, P.E.N., Argentina).

## Results

### Land use, physical, chemical, and bacteriological quantification analyses

On average, the two studied rivers presented different land uses in the surrounding areas (Fig. 1b, c). Rojas River showed a higher fraction of its catchment dedicated to agriculture (between 58 and 80%; Fig. 1d), while the Salado River was more dominated by livestock activities (between 50 and 68%; Fig. 1e).

The results of the physical, chemical, and biological analyses are summarised in Supplementary Table 1. DO levels ranged between 1.83 and 13.28 mg $L^{-1}$, showing a wide range of variation together with very low levels in some instances (values < 5 mg $L^{-1}$ stress aquatic life). Accordingly, DO showed significant differences across sampling sites in Rojas ($Q = 13.50$, $p = 0.004$) and Salado rivers ($Q = 9.90$, $p = 0.019$). In Rojas River, R3 presented values of DO significantly lower than R1, R2, and R4 ($p < 0.05$), while in Salado River S4 showed values significantly lower than S1, S2, and S3 ($p < 0.018$). However, DO values remained fairly constant across sampling months in the Rojas ($Q = 4.90$, $p = 0.179$) and Salado ($Q = 2.75$, $p = 0.432$) rivers (Supplementary Table 1). Sampling sites affected by WWTP discharges (R2 and S3) and the sites downstream of the WWTP exhibited higher nutrient levels. N-$NH_3$ and SRP were significantly higher in R2 (N-$NH_3$ $Q = 27.50$, $p < 0.001$; Fig. 2a and SRP $Q = 24.58$, $p < 0.001$; Fig. 2c), and in S4 (N-$NH_3$ $Q = 18.80$, $p < 0.001$; Fig. 2b and SRP $Q = 11.26$, $p = 0.001$; Fig. 2d). These variables did not change amongst sampling months ($p > 0.05$), except for SRP (Rojas $Q = 32.90$ and $p < 0.001$; Salado $Q = 14.49$ and $p = 0.002$), with values in Rojas significantly lower in March than in June and September ($p < 0.012$) and values in Salado significantly lower in March than September and December ($p < 0.027$) (Supplementary Table 1). Abundances of TC were also significantly higher in R2 and R3 in comparison with R1 ($Q = 8.80$, $p = 0.032$; Fig. 2e). FC were also significantly higher in R2 in comparison with R1 and R4 ($Q = 10.08$, $p = 0.018$; Fig. 2g). The abundances of TC and FC were significantly higher in S3 and S4 (TC $Q = 10.80$, $p = 0.013$; Fig. 2f and FC $Q = 6.89$, $p = 0.05$; Fig. 2h) in comparison with S1. Additionally, *E. coli* abundances in MPN/100 mL did not show significant differences amongst sampling sites (Fig. 2i and j). None of these coliform values changed amongst sampling months in both rivers ($p > 0.05$) (Supplementary Table 1). However, it was observed that in each river, 19.0% of the samples exceeded the guideline levels of *E. coli* for moderate use for recreational purposes. These values were observed at the discharge points of each WWTP and downstream from these locations (Supplementary Table 1).

In the Rojas River, TC and FC correlated positively with N-$NH_3$ and SRP, whereas FC correlated negatively with pH (Fig. 3a). In the Salado River, coliforms also exhibited positive correlations with N-$NH_3$ but displayed negative correlations with chlorides, TDS, and CDOM $a_g254$ values (Fig. 3b).

The number of *E. coli* copies was significantly higher at the sites where the WWTP are located (Fig. 4a, b). Specifically, in the Rojas River, the samples collected at R2 were significantly higher than those taken at R1 ($Q = 9.30$, $p = 0.026$) (Fig. 4c). *E. coli* copies were positively correlated with the levels of TC ($r = 0.79$, $p = 0.0003$), FC ($r = 0.62$, $p = 0.0101$), and N-$NH_3$ ($r = 0.91$, $p = 0.0004$) (Fig. 3a). In the Salado River, the samples collected at S3 differed

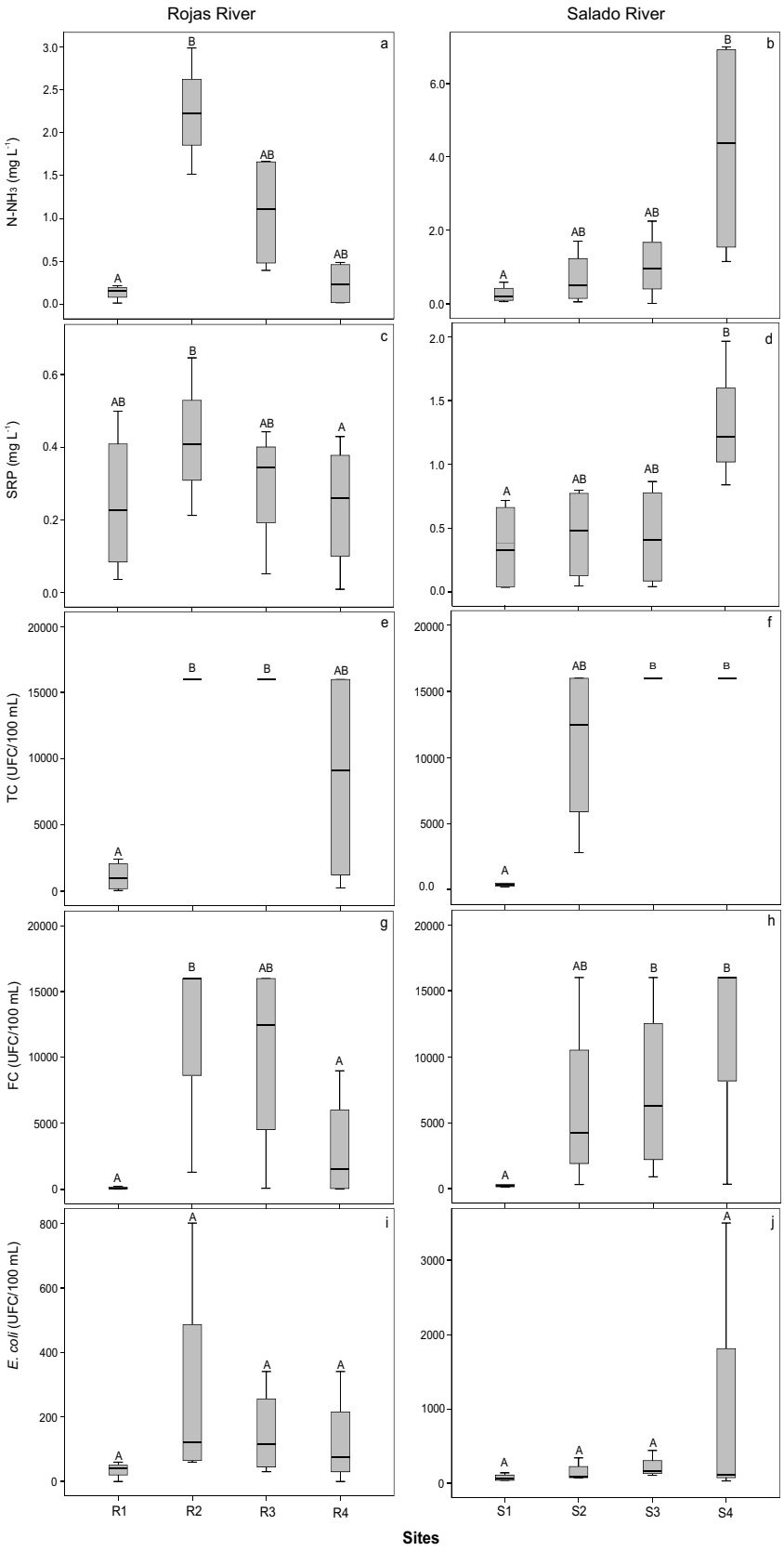



**Fig. 2** Box plot graphs of ammoniacal nitrogen (**a**, **b**), soluble reactive phosphorous (**c**, **d**), total coliforms (**e**, **f**), faecal coliforms (**g**, **h**), and *E. coli* (**i**, **j**) content per sampling sites in Rojas and Salado rivers, respectively. Different letters above box plots mean significant differences ($p < 0.05$) using post hoc comparisons with Bonferroni-corrected $p$-values

significantly from those taken at S1 and S2 ($Q = 10.20$, $p = 0.017$) (Fig. 4d), and the *E. coli* copies were also positively correlated with TC ($r = 0.79$, $p = 0.0003$), FC ($r = 0.56$, $p = 0.023$), N-$NH_3$ ($r = 0.58$, $p = 0.0241$), and negatively correlated with chlorides ($r = -0.77$, $p = 0.0027$) (Fig. 3b). These *E. coli* values did not change amongst sampling months ($p > 0.05$; Fig. 4a), except for Salado River, where values in September were significantly lower than in June ($Q = 8.10$ and $p = 0.044$; Fig. 4b).

When considering the bacteriological, physical, and chemical variables together with the land use, we principally observed in both rivers an ordination by sampling sites, mainly explained by water quality parameters and land use. In the Rojas River, samples from R2 and R3 were ordered together with higher values of nutrients, TC, and *E. coli* (ddPCR), urbanisation, natural woody vegetation, and livestock activities, while R1 and R4 were ordinated together with lower values of these parameters and higher values of agricultural activities (PC1: 50.1%; Fig. 5a). Besides, in this river, temperature was also an important variable. Samples from March and December were ordinated towards higher temperature values, while those from June and September were towards lower temperature values (PC2: 26.4%; Fig. 5a). In the Salado River, S2 and S3 were ordinated together with higher urbanisation, and mainly S3 towards higher values of TC and *E. coli* (ddPCR). S4 was ordinated together with higher values of livestock and agricultural activities, as well as nutrient concentrations, while samples from S1 were ordinated together with lower values of these parameters and higher values of conductivity, chlorides, and CDOM $a_g254$ (PC1: 57.0%; Fig. 5b).

## *E. coli* diarrheagenic detection, characterisation, and antimicrobial susceptibility

The isolated DEC pathotypes and positive samples for virulence gene factors without isolation were detected in different sites and sampling dates across both rivers (Table 1). A total of 11 DEC pathotypes were isolated. On the one hand, out of a total of 720 water samples obtained in June, September, and December 2021 cultured in LS and EC broth tubes for MPN quantification, 402 MPN-positive tubes were tested as previously described in materials and methods. The MAC agar cultures obtained from plating these 402 broth tubes were processed by the three mPCRs. This procedure led to the identification and characterisation of 9 different DEC pathotypes (comprising 2.2% of the total positive tubes). They were featured as follows: five (1.2%) typical EAEC *aggR*/*aaiC*, corresponding to O99:H10 (3), O153:H2 (1), and O78:H2 (1); two (0.5%) atypical EPEC $eae_\theta$ O127:H40 (aEPEC O127:H40); one (0.25%) STEC $stx_{1a}$ / $stx_{2a}$ / $eae_\varepsilon$ / *ehxA* O103:H2; and one (0.25%) hybrid pathotype EAEC-ETEC *aaiC*/*st*/*lt*

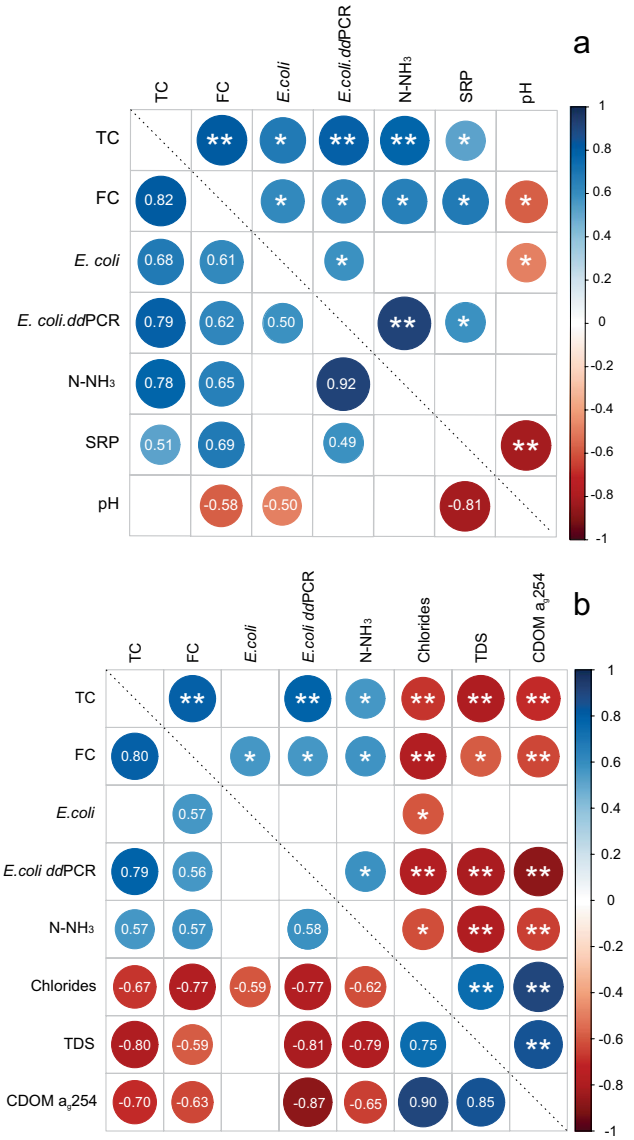



**Fig. 3** Correlation plots showing the Spearman Rho correlations between coliform abundances and different measured physical and chemical variables in Rojas (**a**) and Salado (**b**) rivers. Values below the diagonal are the correlation coefficient, and values above the diagonal are the *p*-values. $*p < 0.05$, $**p < 0.001$. $n = 48$ for chemical variables and $n = 16$ for microbiological variables in each river. TC, total coliforms; FC, faecal coliforms; N-$NH_3$, ammoniacal nitrogen; SRP, soluble reactive phosphorous; TDS, total dissolved solids; CDOM $a_g254$, chromophoric dissolved organic matter at 254 nm

O45:H11 (Table 1). In addition, in 36 confluent culture zones (8.9%) from these samplings, positive PCR signals for various virulence factors were detected. However, the positive colonies could not be isolated. Specifically, these positive samples without isolation were positive for *aggr* (12), *It* (8), *st* (8), $rfb_{O157}$ (6), $stx_2$ (1), and *eae* (1). On another hand, we also examined 61 *E. coli* colonies isolated from a sampling collection conducted in 2019 and in March 2021, which included samples from Gómez shallow

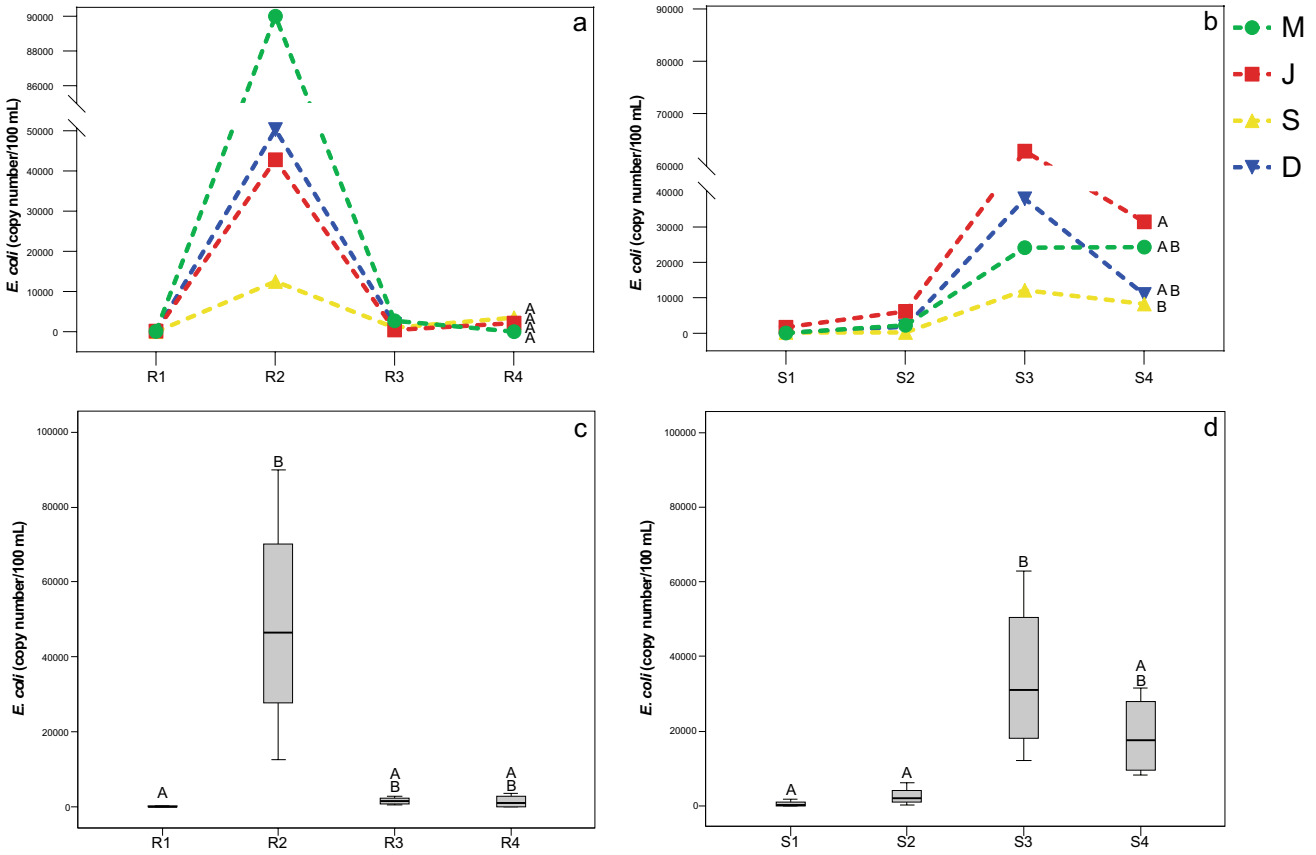
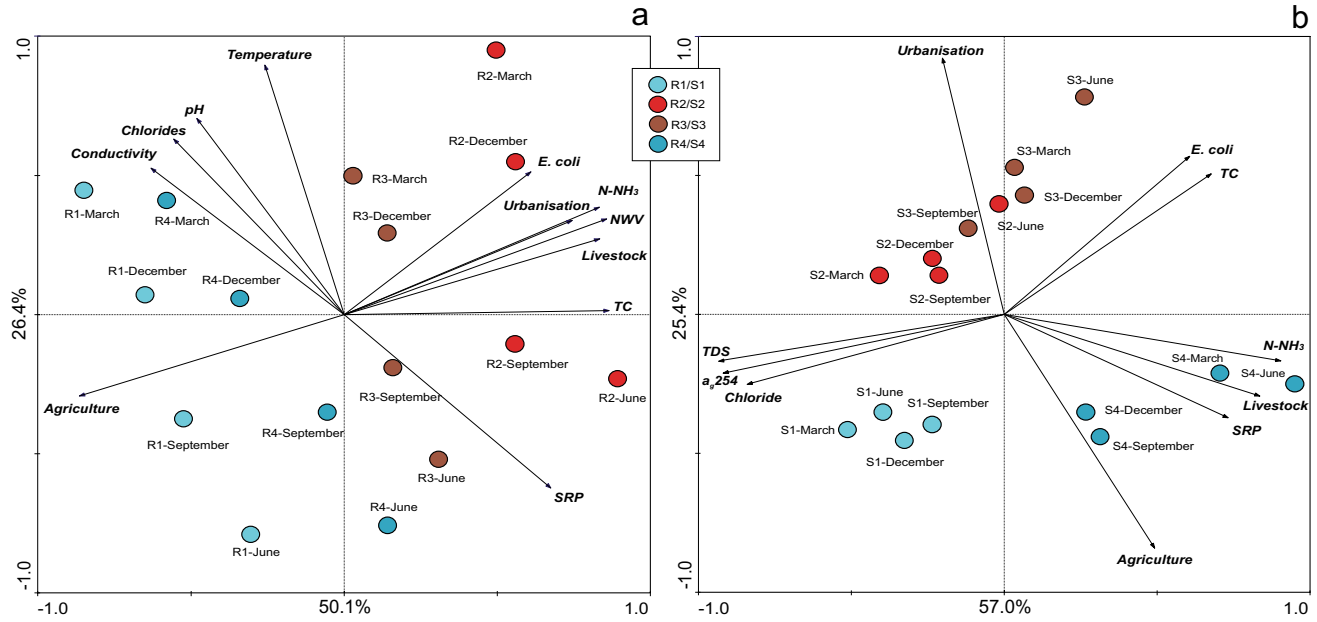



**Fig. 4** Line figure showing the values of *E. coli* measured by *dd*PCR per month and sampling sites in Rojas (**a**) and Salado (**b**) rivers. Box plot graphs of *E. coli* copy numbers obtained by ddPCR per sampling sites in Rojas (**c**) and Salado (**d**) rivers. Different letters in box plots and line figures mean significant differences ($p < 0.05$) using post hoc comparisons with Bonferroni-corrected *p*-values. M, March; J, June; S, September; D, December

lake, Rojas River, and Salado River. Amongst these colonies, two different DEC pathotypes were isolated: STEC O8:H7 $stx_{2a}$/*ehxA* and EPEC O174:H7 $eae_{\lambda}$. Both were identified upstream of the city of Junín, in proximity to Gómez shallow lake in 2019 (Table 1).

All the EAEC strains were recovered from site 2 (adjacent to the cities) in both rivers during the June and September samplings. Only aEPEC O127:H40 strains were obtained at S1 during June, while the hybrid pathotype EAEC-ETEC O45:H11 was isolated at S4 during December in the Salado River. Notably, the STEC O103:H2 strain was recovered from R1, upstream of Rojas city, an area primarily impacted by agricultural practises and to a lesser extent by livestock activities (Fig. 5a). However, we found differences in the *E. coli* pathotypes identified between the two rivers. The Salado River exhibited a greater number of pathotypes and positive genes linked to livestock activities, including one STEC isolation, two aEPEC findings, and five positive signals for $rfb_{O157}$. In contrast, the Rojas River showed fewer instances, with one STEC isolation, one $rfb_{O157}$ positive signal, and one $stx_2$ positive signal (Table 1).

It is noteworthy to mention that 6 out of 11 strains (54.5%) exhibited resistance to one or more of the antibiotics tested (Table 1). AMP (5 strains) and NAL (4 strains) were the antibiotics that demonstrated resistance in the largest number of strains. Two strains, EAEC-O99:H10 (AMP, NAL, STR, SXT, and TET) isolated in the Rojas River, and EPEC O174:H7 (AMP, STR, and SXT) isolated in the Salado River, displayed multiresistance to three or more antibiotics. It was observed that two of the EAEC-O99:H10 strains isolated from the Salado River were resistant to AMP and NAL, unlike the one detected in the Rojas River with multiresistance. All EAEC strains, including the hybrid pathotype, showed resistance to one or more of the tested antibiotics, except for the *E. coli* O78:H2-*aggR* strain isolated from Rojas River, which was found to be sensitive to all the antibiotics tested (Table 1). None of the STEC strains and two aEPEC (O127:H40) showed resistance. Strains with the same serotypes, aEPEC O127:H40 (2) and EAEC O99:H10 (3), isolated during the June sampling, exhibited indistinguishable *Xba*I-PFGE patterns between them, even though one of the O99:H10 strains was isolated




| Variable | PC1 | PC2 |
|---|---|---|
| N-$NH_3$ | 0.83 | 0.39 |
| SRP | 0.68 | -0.62 |
| Temperature | -0.26 | 0.90 |
| pH | -0.48 | 0.70 |
| Conductivity | -0.63 | 0.53 |
| Chlorides | -0.56 | 0.63 |
| TC (culture) | 0.87 | 0.01 |
| *E. coli* (ddPCR) | 0.61 | 0.51 |
| Livestock | 0.83 | 0.27 |
| Agriculture | -0.86 | -0.29 |
| Urbanisation | 0.74 | 0.34 |
| Natural woody vegetation (NWV) | 0.86 | 0.34 |

| Variable | PC1 | PC2 |
|---|---|---|
| N-$NH_3$ | 0.91 | -0.17 |
| SRP | 0.73 | -0.37 |
| TDS | -0.95 | -0.16 |
| Chlorides | -0.88 | -0.26 |
| a254 | -0.92 | -0.21 |
| TC (culture) | 0.68 | 0.51 |
| *E. coli* (ddPCR) | 0.61 | 0.57 |
| Livestock | 0.84 | -0.29 |
| Agriculture | 0.49 | -0.84 |
| Urbanisation | -0.20 | 0.92 |

**Fig. 5** Principal component analysis performed with the bacteriological, physical, and chemical parameters and the land use of the Rojas (**a**) and Salado (**b**) rivers. Arrows indicate the loading of each variable on the axes. Percentages are the variance values explained by each axis. TC, total coliforms; N-$NH_3$, ammoniacal nitrogen; SRP, soluble reactive phosphorous; TDS, total dissolved solids; CDOM ag254, chromophoric dissolved organic matter at 254 nm; NWV, natural woody vegetation. Tables below indicate the correlation of the original variables with each principal component selected

from the Rojas River and the other two from the Salado River (Table 1).

## Discussion

Our study suggests that the Rojas and Salado rivers are significantly impacted by sewage discharges from the cities, and their deficient WWTPs, together with the surrounding surface runoff coming from agriculture and livestock farming. We primarily observed an ordination by sampling sites in both rivers, which was mainly explained by water quality parameters (TC, *E. coli*, nutrients) and land use factors (livestock, urbanisation) (Fig. 5). Nutrient content and coliform abundances increased, and DO concentrations decreased at sampling sites located in the WWTPs and/or downstream. Municipal sewage is one of the largest sources of pollution discharged into rivers worldwide (EPA 2004; García-Aljaro et al. 2019; Pantanella et al. 2020), and together with livestock and farm sewage discharges, increases the inputs of organic material, nutrients, pathogens, and emerging pollutants into these aquatic systems (Mateo-Sagasta et al. 2017; Hassett et al. 2018; Bashir et al. 2020).

We detected higher values of SRP and N-$NH_3$ at the sampling sites affected by discharges of the WWTP from the urban areas and downstream of these sites (Fig. 2a–d). Furthermore, between 37.5 and 31.3% of the samples exceeded the guideline N-$NH_3$ levels for the protection of surface freshwater aquatic life (1.37 mg $L^{-1}$) in both rivers. Urine contains the largest fractions of nitrogen, phosphorus, and potassium released from the body (Larsen et al. 2013). The discharge of WWTP effluents with high levels of N-$NH_3$ into rivers and streams (e.g., Manzo et al. 2020) can have pervasive toxic effects on organisms (Horak et al. 2019). We also found positive associations between N-$NH_3$ levels and the abundance of coliform bacteria (TC, FC, and

**Table 1** Isolated strains and their antibiotic resistance, together with mPCR virulence gene factors without isolation from Gómez shallow lake and Rojas and Salado rivers sites and sampling months

| | Sites | 2019 | June 2021 | | September 2021 | | December 2021 | |
|---|---|---|---|---|---|---|---|---|
| | | DEC Isolation | mPCR signal without isolation (n° samples) | DEC Isolation | mPCR signal without isolation (n° samples) | DEC Isolation | mPCR signal without isolation (n° samples) | DEC Isolation |
| Rojas River | **R1** upstream the Rojas city | - | *lt* (3) | - | *aggR* (1)<br>$stx_2$ (1) | STEC O103:H2 $stx_{1a}$/$stx_{2a}$ $eae_{\text{Epsilon}}$ / *ehxA* Susceptible | - | - |
| | **R2** near the Rojas city at the mouth of the sewage treatment plant | - | *st* (1) | EAEC O99:H10 */aggR/ aai*C Resistant to: AMP-NAL-STR-SXT-TET | *aggR* (3) | EAEC O78:H2 */aggR/ aai*C Susceptible | *aggR* (2) | - |
| | **R3** downstream the Rojas city | - | - | - | $rfb_{\text{O157}}$ (1) | - | *lt* (1) | - |
| | **R4** downstream the Rojas city | - | - | - | *aggR* (1) | - | - | - |
| Gómez shallow lake | Shallow lake upstream **S1** | STEC O8:H7 $stx_{2a}$ / *ehxA* Susceptible | - | - | - | - | - | - |
| Salado River | **S1** upstream the Junín city, near Gómez shallow lake | EPEC O174:H7 $eae_{\text{lambda}}$ Resistant to: AMP-STR-SXT | *eae* (1) | aEPEC O127:H40 $eae_{\text{tita}}$ (2) Susceptible | - | - | - | - |
| | **S2** near the Junín city and Borchex park | - | - | EAEC O99:H10 */aggR/ aaiC* (2) Resistant to: NAL-AMP<br>EAEC O153:H2 / *aggR/ aaiC* Resistant to: AMP | $rfb_{\text{O157}}$ (1) | - | *st* (2) | - |
| | **S3** downstream the Junín city at the discharges of wastewater treatment plant | - | *st* (1) | - | *st* (2)<br>$rfb_{\text{O157}}$ (3) | - | *aggR* (2)<br>*lt* (2) | - |
| | **S4** downstream the Junín city and near Chacabuco city | - | *st* (1) | - | $rfb_{\text{O157}}$ (1) | - | *aggR* (3)<br>*lt* (2)<br>st (1) | EAEC-ETEC O45:H11 *aaiC / st / lt* Resistant to: NAL |

*AMP* ampicillin, *NAL* nalidixic acid, *STR* streptomycin, *SXT* trimethoprim-sulfamethoxazole, and *TET* tetracycline. Numbers between parentheses indicate the quantity of signals and isolations detected

number of *E. coli* copies obtained through ddPCR) in both rivers. The release of human faeces and urine into the river waters contributes to elevated concentrations of coliform bacteria and N-$NH_3$. Similar associations were reported by Cui et al. (2021), who found that the abundance of coliform bacteria was mainly correlated with the concentration of ammonium salt and nitrate. Besides, the input of organic matter and nutrients from municipal wastewater effluents can lead to eutrophication and a decrease in DO content (Chambers et al. 1997; Cooke 2006). These effluents also contain a variety of toxic contaminants such as chlorides, inorganic chloramines, and components that disrupt biota endocrine systems (e.g., Lishman et al. 2006; Metcalfe et al. 2009). We observed significantly low DO values, sometimes below 5 mg $L^{-1}$, at sites located downstream of the WWTP discharges (R3 and S4). This is likely due to oxygen consumption by bacterial degradation of the substantial input of organic materials. The impact of inadequate wastewater treatment on surface water is largely determined by the oxygen balance of the aquatic ecosystem, and its presence is essential for sustaining aquatic life within the system (e.g., Momba et al. 2006; Igbinosa and Okoh 2009). Interestingly, the lowest measured DO value (1.83 mg $L^{-1}$) was observed in Salado River, at the site upstream of WWTP during a cyanobacterial bloom and fish mortality event (personal observation). The rest of the sampling sites (S2, S3, and S4) exhibited cyanobacterial blooms without fish mortality, although reductions in DO values were observed at sites below WWTP in both rivers.

Thus, in relation to hypothesis (a), our results confirmed alterations in the water's physical and chemical parameters in both rivers, along with significantly higher coliform abundances at the discharge sites near the WWTP and downstream (R2, R3, S3, and S4). Consequently, in these sampling points, *E. coli* counts exceeded the recommended levels for moderate recreational use (EPA 1986; SRHN 2003). Furthermore, the abundance of *E. coli*, in copies/100 mL, was significantly higher at the sites where the WWTP are located. These results highlight the subpar sanitary quality of the waters in the studied rivers and the inadequacy of both, microbial and nutrient purification at the municipal WWTPs. These plants utilise percolating beds (trickling filters) for wastewater treatment. A 2023 study performed in Argentina reported that 63% of WWTP employing this method are in fair to poor condition, aligning with our findings. This deterioration is mainly attributed to inadequate secondary treatment, malfunctioning electromechanical equipment, and the ageing of both infrastructure and machinery (Katopodis et al. 2023).

Concerning hypothesis (b), the recovery of physical, chemical, and biological parameters examined in site R4 of the Rojas River (located 9.5 km downstream from the discharge of the WWTP) could be attributed to the intrinsic self-purification capacity of the river. On the other hand, in the Salado River, none of the parameters were recovered at site S4 localised 14.5 km from the discharge of the WWTP from the city of Junín. This may be attributed to the inability of the Salado River to achieve water restoration at this particular sampling location and/or to the high proportion of livestock activities (68%) in its surrounding (Fig. 1e, Fig. 5b). Additionally, the population of the nearby city of Junín is four times larger than that of Rojas (INDEC 2022), which may further contribute to the observed conditions. Water has an innate capability to neutralise contamination through dilution, driven by the hydrodynamics of fast-flowing rivers, which plays a key role in microbial fate and transport, alongside inactivation and adsorption effects (Díaz et al. 2024). However, when contamination becomes uncontrolled, water's inherent self-restoration potential can be diminished (Bashir et al. 2020). To corroborate this, forthcoming studies should include more sampling sites downstream S4.

In this study, we have isolated four distinct DEC pathotypes, specifically STEC, EAEC, EPEC (typical and atypical), and hybrid pathotype EAEC-ETEC *aai*C/*st*/*lt*, from the two rivers. It is important to note that pathogenic *E. coli* strains have been implicated in numerous waterborne outbreaks, with STEC and EPEC frequently reported as responsible for such outbreaks worldwide (Jang et al. 2017 and cites therein). In particular, domestic and wild animals, along with the environment, can act as reservoirs for atypical EPEC (aEPEC), a strain distinguished by lacking the adherence factor plasmid, causing human infections across various regions (Hernandes et al. 2009; Gomes et al. 2016). Ruminants are known to carry both aEPEC and ETEC (Nagy and Fekete 1999; Orden et al. 2002). The DEC pathotypes identified in this study have also been reported in other aquatic systems. In Argentina, Díaz et al. (2024) found EPEC, EAEC, ETEC, and EIEC strains in the Río de la Plata River, which receives different degrees of treated and undeclared untreated sewage. They attribute the presence of these DEC primarily to urban sewage discharge, although they do not dismiss the possibility of other sources, such as animal waste. Furthermore, Vinueza et al. (2021) identified the presence of EAEC, EPEC, and EIEC in the primary rivers of Ecuador. Petit et al. (2017) noted that the prevalence of pathogenic strains in water and sediment in a rural surface aquatic continuum in northwestern France reflected land use patterns. They detected STEC near the upstream pasture lands and aEPEC, EAEC, and DAEC in more urbanised downstream sites.

Regarding the serotypes identified in our study, EAEC O99:H10 was detected in more urbanised locations of a French aquatic continuum (Petit et al. 2017). In coincidence, in our study, EAEC O99:H10 was isolated from both rivers in proximity to urban sites (Table 1). However, Cabal et al.

(2015) detected *aggR* in cattle faecal samples, indicating that at least some EAEC-type strains are able to circulate in bovine hosts, implying that the paradigm that humans are the only EAEC reservoir needs to be revised. Although we found aEPEC O127:H40 $eae_{-\theta}$ upstream of Junín city, there is no reported evidence of clinical infection in Argentina. However, other countries such as England, Brazil, and Germany have linked this strain to gastroenteritis in young infants and children (Scotland et al. 1996; Ghilardi et al. 2003; Kozub-Witkowski et al. 2007). STEC O103:H2 has been linked to human infections and environmental samples. In Finland, for example, an investigation traced back an infection by STEC O103:H2 $eae_{-\varepsilon}$ from a child with bloody diarrhoea to faecal samples from cattle and the surrounding environment (Heinikainen et al. 2007). All of this evidence and events described cases of acute and bloody diarrhoea as well as HUS associated with DEC infections and have shown that the aquatic environment can be an important vehicle for the transmission of pathogenic *E. coli*.

Moreover, we identified six distinct gene-PCR-signals ($stx_2$, *lt*, *st*, *aggR*, $rfb_{O157}$, and *eae*) in samples where it was not possible to isolate the *E. coli* pathotypes harbouring those genes (Table 1). STEC pathotypes exhibit two distinct Shiga toxin types, with several subtypes: $stx_1$ (comprising $stx_{1a}$, $stx_{1c}$, and $stx_{1d}$) and $stx_2$ (comprising $stx_{2a}$, $stx_{2b}$, $stx_{2c}$, $stx_{2d}$, $stx_{2e}$, $stx_{2f}$, $stx_{2g}$, $stx_{2h}$, and $stx_{2i}$) (Scheutz et al. 2012; Lacher et al. 2016; Bai et al. 2018). These *stx* genes are encoded on lambdoid bacteriophages (e.g., Muniesa and Jofre 1998), and several studies have reported significant numbers of free $stx_2$-bearing bacteriophages in the environment, thereby increasing the potential for infecting new bacterial hosts (Muniesa et al. 1999; García-Aljaro et al. 2004). In addition, we isolated one STEC $stx_{2a}$ and one STEC $stx_{1a}$ / $stx_{2a}$ / $eae$-$_{\varepsilon}$. It was described that *eae* is one of the most frequently detected pathogen genes in the environment (Hamilton et al. 2010; Zhang et al. 2016). Several authors have underscored the strong association between the presence of the *eae* gene and the ability of STEC strains to cause severe human diseases (Paton and Paton 1998). Meichtri et al. (2004) reported that young steers from the main beef-producing area of Argentina are a significant reservoir of STEC strains, with 59% of these strains carrying the $stx_2$ gene. Tanaro et al. (2010) studied 288 faecal samples from a beef cattle farm in the Pampa Region and detected the $rfb_{O157}$ gene in 3.8% of the samples, besides the *stx* genes (mainly $stx_1$ and $stx_{2c}$) were detected in all $rfb_{O157}$-positive samples. In a study performed in puddles inside cattle pens, small water courses, and lagoons from feedlots areas in the Pampa Region, the isolated strains were found to carry the *eae*, *ehxA*, $rfb_{O157}$, and $fliC_{H7}$ genes, and 50.0% of the strains harboured the $stx_{2a}$/$stx_{2c}$ genes, 47.7% $stx_{2c}$, and 2.3% $stx_{1a}$/$stx_{2c}$ (Tanaro et al. 2018). Besides, Marucci et al. (2011) reported the isolation of STEC strains carrying $stx_2$ and *eae* factors in recreational waters (rivers and streams) within the Sierra de la Ventana Region of Buenos Aires Province.

Amongst the strains isolated from the Rojas and Salado rivers, typical EAEC *aggr*/*aaiC*, O99:H10 and O153:H2, and STEC $stx_{1a}$/$stx_{2a}$/$eae_{-\varepsilon}$/*ehxA* O103:H2 were also isolated from human cases in Argentina during the period 2017–2022 (National Reference Laboratory for STEC Database). The *E. coli* O99:H10 and O153:H2 variants presented 92.3% and 85.7% of pattern similarity by *Xba*I-PFGE, with strains isolated from five and three diarrhoea cases, respectively. Regarding the virulent STEC O103:H2 strain, it has been isolated in cases of diarrhoea ($n=2$), HUS (1), and even in food (1), showing 88.8% global pattern similarity by *Xba*I-PFGE between them (data not shown).

Surface water carries pathogenic and non-pathogenic bacteria with antibiotic-resistance genes from human and animal waste, as well as antibiotic residues from healthcare, livestock, and industry. These residues exert selective pressures that promote resistance. Aquatic environments further facilitate the exchange of resistance genes through horizontal gene transfer, thereby fostering the emergence and spread of multiresistance to these drugs (Cho et al. 2020). Moreover, some studies showed that WWTP effluents contribute to this process by releasing both resistance genes and antimicrobial residues, creating conditions that promote the development of resistance, as bacteria are continuously exposed to sub-inhibitory levels of antibiotics (Koczura et al. 2011; Rizzo et al. 2013). In this study, it was interesting that one of the multidrug-resistant strains, EAEC-O99:H10 (resistant to AMP, NAL, STR, SXT, and TET), was isolated at the mouth of the WWTP in the Rojas River. It is also important to note that all of the isolated EAEC strains, except for one, exhibited resistance to one or more antibiotics. In this sense, *E. coli* resistant to some of these antimicrobials have been reported in Argentina. However, they have been associated with intensive animal production practises and not with WWTP (Prack McCormick et al. 2022). This can be attributed to the remarkable ability of this pathotype to acquire mobile elements such as resistance or virulence genes. This aligns with the observations of Rasko et al. (2011), who suggested that horizontal genetic exchange allowed the emergence of the highly virulent, shigatoxigenic/enteroaggregative *E. coli* O104:H4 strain responsible for an outbreak in Germany. The isolation of the EAEC-ETEC hybrid strain in our research may similarly be explained by the same gene transfer mechanism. Therefore, it becomes evident that the aquatic environment investigated in this study possesses the optimal characteristics to serve as a reservoir for pathogenic strains such as EAEC. Coupled with the plasticity exhibited by bacterial genomes, it could potentially facilitate the emergence of novel, highly virulent pathogens.

Taken together, the presence of DEC pathotypes, their antibiotic resistance, and virulence genes were detected in all sites of both rivers, supporting hypothesis (c). The identification of the STEC and aEPEC pathotypes, along with the $rbf_{O157}$ gene in our study, suggests a potential link to livestock-related activities in the surrounding areas of both rivers. Notably, the two STEC strains identified in our study were isolated from sites upstream of the cities, where livestock activity is not predominant (Fig. 1b–e). However, the Salado River exhibited more pathotypes and positive genes associated with livestock activities than the Rojas River (Table 1), aligning with the higher percentage of such activities (ranging from 50 to 68%, as shown in Fig. 1e) in the Salado River's surroundings. There are about 28 million cattle heads in the Pampa Region, accounting for about 70% of the country's cattle population (INDEC 2020). Faecal pathogens may enter the environment through the direct deposition of faeces to the land or through overland runoff of faecal material deposited on soils, especially after heavy rainfall events (e.g., Marucci et al. 2011). The southern regions of the American continent remain a hot spot for infections and sequels associated with STEC infections (Torres et al. 2018). In a study performed in nine Argentinian beef-exporting abattoirs, the prevalence of STEC O157 was 4.1% in faecal matter, while the prevalence of non-O157 STEC was 22.3% (Masana et al. 2010, 2011). In the Pampa Region of Argentina, extensive evidence has confirmed that grazing cattle and fattening animals serve as sources of highly virulent STEC serotypes (Polifroni et al. 2009, 2014) and that these serotypes have also been identified in different environmental matrices (Tanaro et al. 2010, 2018; Marucci et al. 2011).

Although this is the first work to detect DEC and its antimicrobial resistance in these two Argentine rivers, limitations such as sampling frequency, the absence of samples from the WWTPs and nearby livestock areas, and the lack of epidemiological information prevent us from assessing human health risk and conducting effective source-tracking. On the other hand, as mentioned previously, *dd*PCR and culture methods are not two comparable techniques; *dd*PCR quantifies DNA copies of *E. coli*, including DNA from both viable and non-viable cells, while culture methods only detect viable and culturable cells. Consequently, the degree of agreement between the two methods will vary, particularly in samples with a high proportion of fresh faecal inputs where viable *E. coli* cells are more likely to be present (Moinet et al. 2024). In environmental water samples, however, faecal inputs often include a mix of fresh and non-recent contributions. This variability can introduce discrepancies, as *dd*PCR may detect *E. coli* DNA long after cell viability has declined, leading to potential overestimations when compared to culture-based counts. Thus, the age and composition of faecal contamination need to be carefully considered when interpreting and comparing results from these methods in environmental contexts. In addition, while chromogenic mediums have gained popularity for *E. coli* quantification, these methods rely on the bacterium's ability to metabolise specific substrates, which can impact both sensitivity and specificity (Moinet et al. 2024). As a result, some DEC strains lacking β-glucuronidase activity may not be detected, potentially leading to false negatives (Moinet et al. 2024). Additionally, false positives may arise when non-faecal indicator bacteria are present in high abundances, as these organisms can also enzymatically cleave chromogenic or fluorogenic substrates (Sercu et al. 2011).

## Conclusion

Our study revealed that elevated nutrient and coliform levels in the studied rivers are related to inadequately treated discharges from the WWTP and urban areas. We also observed a positive correlation between total coliform abundances and $N\text{-}NH_3$ concentrations, indicating a potential point source of contamination. Alarmingly, concentrations of *E. coli* and $N\text{-}NH_3$ exceeded guidelines established for recreational purposes. Furthermore, we identified several pathogenic strains of *E. coli* exhibiting antibiotic resistance. The presence of STEC, a-EPEC, and $rfb_{O157}$ positive genes suggests connections with livestock-related activities performed in the surrounding land areas of both rivers. The presence of such contaminants in water can potentially lead to the transmission of waterborne diseases when used for domestic purposes, recreation, irrigation, and other activities (MEA 2005; Chigor et al. 2013), posing significant threats to human, animal, and environmental health, as well as society at large (DWA 1999). Overall, our findings highlight the significant impact of both diffuse and point source pollution resulting from human activities on surface inland waters. It underscores the pressing need to ramp up efforts and enforce stricter controls to prevent irreversible water contamination and protect public health. Safeguarding the quality of aquatic ecosystems is one of the most arduous challenges facing global society in the twenty-first century (Hampel et al. 2015). Besides, urgent measures need to be implemented to stop the spread of antimicrobial resistance and mitigate the associated danger.

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**Data availability** The data that support the findings of this study are available from the corresponding author upon request.

## Declarations

**Ethical approval** Not applicable.

**Consent to participate** Not applicable.

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**Competing interests** The authors declare no competing interests.

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