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Ecological risk of rainfall-driven effluent pulses in agro-industrial streams of the Las Conchas basin (South America): *Rhinella arenarum* tadpoles as sentinels of acute ecotoxicity

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ABSTRACT

Heavy rainfall can trigger episodic severe contamination pulses in agro-industrial watersheds, frequently exceeding the temporal resolution of routine monitoring programs and threatening aquatic biota and public health. This study assessed physicochemical and microbiological shifts associated with post-rain effluent pulses and their acute ecotoxicological effects on Rhinella arenarum tadpoles across interconnected streams within the Las Conchas basin, a tributary network draining into the middle Paraná River (Argentina). Water samples from Crespo, Espinillo, and Las Conchas streams were collected 24 h post-rainfall to analyze physicochemical parameters, metals, microbial indicators, and cyanobacterial presence. Samples were used in a 48-h acute bioassay to assess larval mortality, enzymatic biomarkers, and swimming behavior. Samples from Crespo stream exhibited extreme oxygen depletion, elevated bacteriological loads, high metal concentrations, cyanobacterial presence, total larval mortality and pronounced sublethal impairments even at diluted exposures. Downstream sites exhibited attenuated but detectable biological effects, consistent with a longitudinal toxicity gradient linked to rainfall-driven wastewater and agro-industrial inputs. These findings are consistent with screening-level ecological risk assessment frameworks, which prioritize identification of high-risk conditions rather than precise exposure quantification. Ultimately, the detected fecal contamination in recreational waters underscores the necessity of a One Health perspective that integrates aquatic ecosystem integrity with public health safety. i

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Introduction

Heavy rainfall events can trigger hydrological surges that rapidly mobilize contaminants accumulated in agricultural soils, drainage networks, and wastewater treatment ponds, generating episodic contamination pulses in receiving water bodies (Smalling et al. 2021; Qiu et al. 2021). These first-flush events transport untreated effluents, agrochemical residues, pathogens, and sediment-bound pollutants that frequently exceed the temporal resolution of conventional monitoring programs (Beckers et al. 2025). In agro-industrial catchments, such episodic inputs often transiently exceed chronic exposure thresholds and trigger acute ecotoxicological effects within short timeframes, including increased mortality in aquatic invertebrates and elevated endocrine-disrupting activity downstream of wastewater treatment facilities during overflow conditions (Shuliakevich et al. 2022; Lin et al. 2025).

Storm-driven contamination pulses are increasingly recognized as major drivers of ecological degradation in regions characterized by intensive livestock production, food-processing industries, and deficient wastewater infrastructure (Pandey et al. 2014; Shrivastava et al. 2022). Rainfall-induced overflows from wastewater treatment ponds, slaughterhouse discharges, and unregulated effluents substantially increase pollutant loads, accelerating eutrophication, microbial proliferation, and chemical toxicity (Popick et al. 2022). This convergence of environmental degradation and pathogen dispersal underscores the necessity of a One Health approach, which recognizes that the health of aquatic ecosystems is inextricably linked to animal and human well-being. In mixed agricultural catchments, rainfall-driven runoff can also mobilize antibiotic residues, antimicrobial-resistant microorganisms, and associated resistance genes as well as metals, endocrine disruptors, and high organic matter loads—with potential cumulative impacts on biodiversity and ecosystem functioning (Giudice and Young 2011; Glaser et al. 2023).

In South American agricultural basins, the overflow of stabilization ponds and unlined canals during precipitation events leads to abrupt increases in microbial loads, nutrients, organic matter, and suspended solids, frequently resulting in oxygen depletion and severe physiological stress to aquatic fauna (Mateo-Sagasta et al. 2018; Andrade et al. 2022). The Las Conchas basin in Entre Ríos Province (Argentina)—a hydrologically connected tributary network that drains into the middle Paraná River—exemplifies these dynamics by integrating intensive agriculture, livestock production, and substandard effluent treatment within a single river-connected system. Recent ecotoxicological assessments in this basin have reported critical physicochemical, bacteriological, and pesticide contamination, including the highest glyphosate sediment concentration recorded in South America (5002 $\mu g\ kg^{-1}$), along with a broad spectrum of herbicides, insecticides, and fungicides in surface waters (Cuzziol Boccioni et al. 2025). These conditions induce severe biological effects in *Rhinella arenarum* tadpoles, acute lethality in streams receiving industrial and urban discharges (Crespo and Las Tunas streams) and pronounced sublethal responses—such as hormonal disruption, neurotoxicity, genotoxicity, and impaired growth—throughout the basin. Comparable studies in agro-industrial watersheds of the Pampas region indicate that dairy and meat-processing industries release complex mixtures of organic residues, surfactants, detergents, and metals that frequently exceed environmental quality standards and generate strong ecotoxicological effects on amphibian larvae (Peluso et al. 2025). Altogether, these pressures compromise the ecological integrity of the Las Conchas basin, even within legally protected areas such as the Parque Escolar Rural Enrique Berduc (PEREB) reserve.

Within this hydrological context, the Las Conchas basin forms part of the Paraná River system, one of the most significant fluvial systems globally (Orfeo et al. 2023). Its spatial configuration establishes a well-defined preservation gradient, extending from highly impacted urban and industrial headwaters to more conserved environments within protected areas, providing a unique scenario to evaluate the downstream propagation of ecotoxicological risks. During extreme rainfall events, tributaries act as rapid transfer pathways for water, sediments, nutrients, and contaminants toward major river corridors (Llamazares Vegh et al. 2023). Consequently, contamination pulses generated in small streams can propagate downstream and influence water quality at broader basin scales, highlighting the relevance of tributary-based studies for river basin management (Ronco et al. 2008, 2016).

Amphibians are widely used as bioindicators of environmental change due to their permeable skin and aquatic life stages. These vertebrates are globally threatened by a diverse array of anthropogenic stressors, including pesticides, metals, fertilizers and emerging contaminants such as pharmaceuticals and plastics (Costa et al. 2023). Their sensitivity varies depending on the type of contaminant and environmental conditions, but often exhibit sensitivities comparable to or exceeding those of standard aquatic test species (Peters et al. 2023; Azambuja et al. 2024). The limited mobility of tadpoles constrains their ability to avoid sudden contamination events, effectively coupling their exposure to the spatiotemporal dynamics of runoff-driven pulses. Continuous exchange with the surrounding water facilitates the uptake of dissolved contaminants, and suspended particles while exposing them to abrupt physicochemical changes.

Acute and sublethal effects in *Rhinella arenarum* exposed to agro-industrial effluents—including oxidative stress, acetylcholinesterase inhibition, and genotoxicity—have been consistently reported (Peltzer et al. 2024). Accordingly, the assessment of their acute responses during post-rain effluent pulses provides a valuable framework for characterizing real-time ecological risk in agro-industrial basins and for enhancing the efficacy of environmental monitoring and management strategies.

Empirical data describing post-rainfall contamination dynamics and associated biological impacts in South American agro-industrial watersheds remain scarce. Besides, most monitoring programs still fail to capture short-duration contamination events triggered by extreme rainfall, leaving a critical blind spot in watershed management. Building on previous research that already described the environmental status and the main sources of pollution in the Las Conchas basin (Cuzziol Boccioni et al. 2025), the present study specifically aims to evaluate the acute risk associated with rainfall-induced pulses, focusing on microbiological and physicochemical fluctuations linked to stormwater runoff in three representative streams—Crespo, Espinillo, and Las Conchas, the latter of which drains the PEREB natural reserve. Within this hydrological sequence, Crespo stream receives discharges from municipal wastewater treatment ponds and industrial stabilization ponds associated with the city of Crespo. These facilities frequently overflow during heavy rainfall events; consequently, it is expected these initial contamination pulses propagate downstream to the Espinillo stream and ultimately into the Las Conchas mainstem. Under this dynamic, we hypothesize that these post-rain effluent pulses generate rapid, high-intensity contamination events capable of significantly increasing acute toxicity in *Rhinella arenarum* tadpoles, exceeding background environmental conditions and exposing sensitive larval stages to complex mixtures of agro-industrial pollutants. We further anticipate that these effects may follow a longitudinal attenuation gradient, with maximum toxicity occurring at highly impacted upstream sites and decreasing toward relatively preserved downstream environments. To test this hypothesis, this study addresses two primary objectives: (i) quantifying microbiological and physicochemical changes associated with rainfall-induced runoff across these interconnected streams, (ii) evaluating the acute ecotoxicological responses of *R. arenarum* tadpoles during post-rain contamination pulses along a longitudinal gradient from highly impacted upstream conditions to relatively preserved downstream environments.

Event-based ecotoxicological studies are essential to capture lethal contamination peaks that elude routine monitoring, utilizing amphibian bioindicators to integrate the synergistic effects of complex chemical and microbial mixtures. By identifying these transient risks, this approach provides the necessary evidence to transition toward dynamic governance and One Health strategies that safeguard both biodiversity and environmental biosecurity (Hulme et al. 2025). Moreover, this study contributes directly to ecological risk assessment by quantifying acute biological responses and identifying episodic real exposure scenarios associated with rainfall-driven contamination pulses, rather than identifying individual toxicants or establishing causality. To translate such biological responses and environmental data into ecological risks, screening-level ecological risk assessment frameworks based on Risk Quotients (RQ) are widely used in freshwater ecosystems impacted by episodic contamination events (USEPA 1998; Stark and Banks 2021; Karki et al. 2024). This approach compares measured environmental concentrations of chemical stressors with regulatory or ecotoxicological values to estimate the potential environmental risk for the aquatic environment (Nika et al. 2020). Such screening-level frameworks are particularly relevant in rainfall-driven exposure scenarios, where contaminant pulses may produce acute exposure conditions capable of inducing acute biological effects.

Methods

Study area

The study area comprises the Las Conchas stream basin in Entre Ríos Province (Argentina), an area heavily impacted by agriculture and agro-industrial activity. Three interconnected tributaries were studied: 1) the Crespo stream, which originates in the city of the same name and is directly impacted by wastewater and industrial treatment plants in the area; 2) the Espinillo stream, which receives inflow from the previous watercourse; and 3) the Las Conchas stream (LC), which crosses PEREB reserve (recently proposed for Ramsar designation), and its buffer zone. This last stream receives tributaries not only from the Espinillo stream, but also from several other local streams at different geographical points (Bortoluzzi et al. 2007).

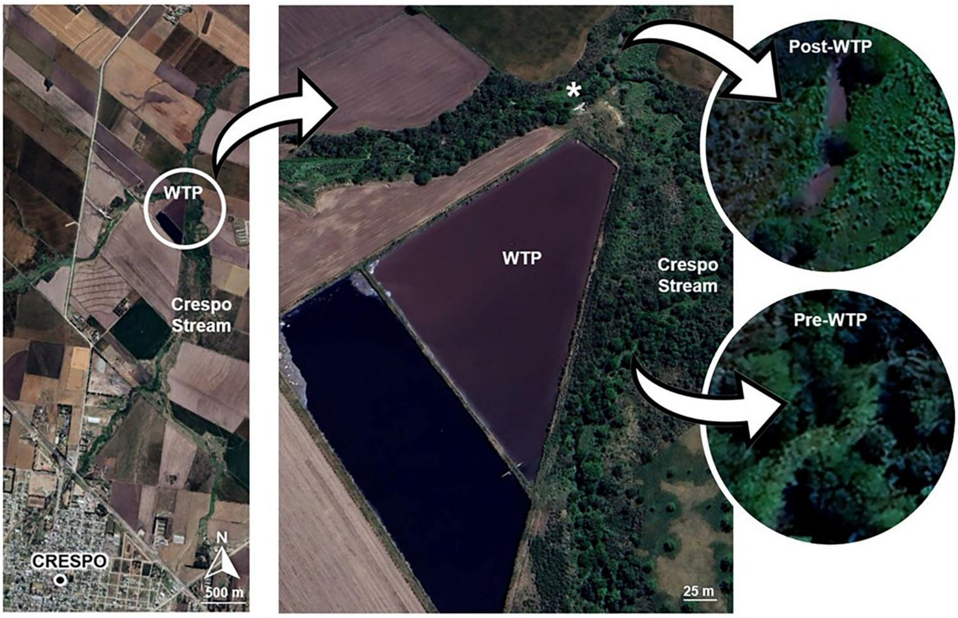

Figure 1. Satellite image of Crespo stream (Entre Ríos Province, Argentina) near wastewater treatment ponds (WTP) obtained from Google Earth (2023). Details show pre- and post-WTP stream coloration. (*) suggests a hydraulic connection between the WTP and the stream.

The connectivity between the wastewater treatment ponds (WTP) and the Crespo stream was assessed using available Google Earth satellite imagery. As illustrated in Figure 1, a distinct contrast in water coloration is visible upstream and downstream of the WTP discharge point. The presence of a reddish plume downstream suggests a direct hydraulic connection which drains part of the WTP's contents into the stream, as the optical characteristics of the stream shift resembling those of the effluent in the WTP. Historical images prior to the installation of the effluent treatment plant (up to 2019) show normal stream coloration along the entire stretch, with no obvious changes in coloration.

The field sampling was carried out in 26 October 2025 after a heavy rainfall event. According to official hydrological reports, precipitation ranged between 83 and 118 mm across the province within 24–25 October 2025 including 87 mm recorded in the Paraná Department (Dirección General de Hidráulica y Obras Sanitarias, Entre Ríos , 2025). Notably, this single event represented most of the total cumulative rainfall recorded for the entire month in the area (94 mm in October 2025; historical monthly average: 119 mm). The concentration of the monthly rainfall into a single event triggered a significant hydrological pulse, consistent with 'first flush' dynamics where accumulated contaminants are rapidly mobilized into the aquatic system.

Four sites were sampled across three streams 24 h after the rainfall event (Figure 2): P1) Crespo Stream approximately 10 km after its source in the city (31°57′11.2″S; 60°16′08.5″W); P2 and P3) at Espinillo stream next to the buffer zone of the reserve at two points: Calzada de Espinillo (P2, 31°47′52.8″S; 60°18′49.5″W) and Camping Espinillo (P3, 31°46′18.6″S 60°18′50.2″W); P4) at the Las Conchas stream crossing the PEREB reserve (31°43′22.2″S; 60°19′59.6″W).

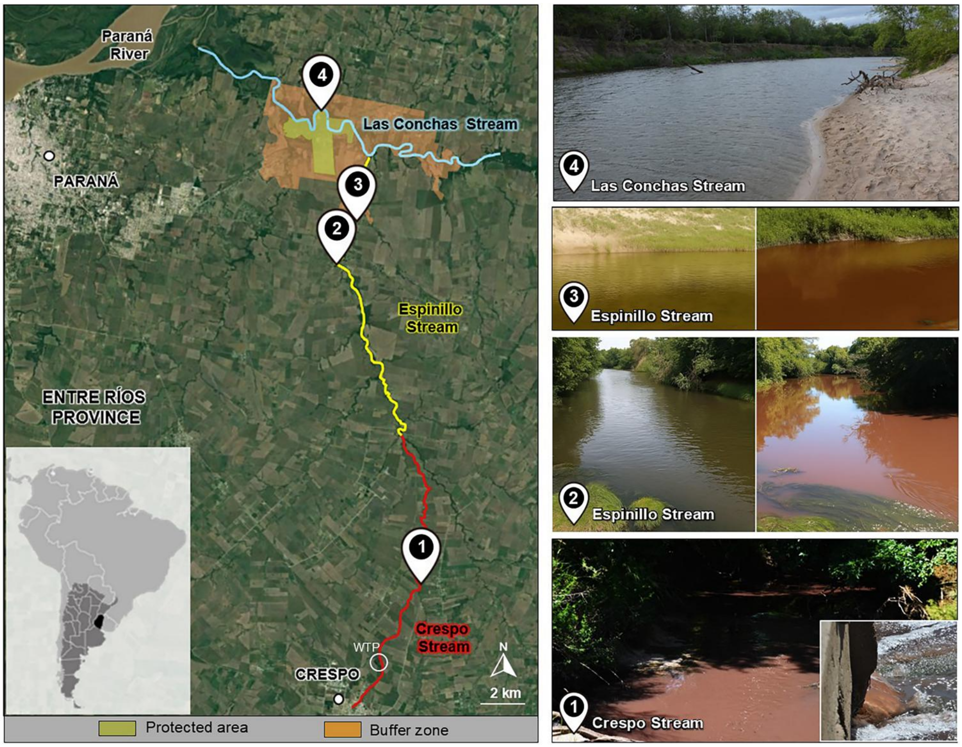

Figure 2. Location of sampling sites (P1–P4) in Entre Ríos province (Argentina). Photographs of Espinillo stream (P2–P3) show water condition pre-rainfall (left) and altered appearance 24 h after heavy rainfall event (right). WTP: Wastewater treatment ponds, indicating P1 is located downstream this point.

These sites represent a strategic spatial gradient of anthropogenic pressure and habitat conservation within the basin. P1 serves as the headwater impact point, directly influenced by urban and industrial discharges from the city of Crespo, as it is located after WTP insertion and directly receives effluent inputs during overflow conditions. Downstream, two closely located points (P2 and P3) were established along the Espinillo stream to capture short-range spatial variability in water quality and biological responses. These sites represent functionally distinct sections within the transition zone, including the buffer zone boundary and recreational areas, allowing for a high-resolution assessment of contaminant pulse propagation and attenuation at a local scale. Finally, P4 is situated within the PEREB natural reserve; although it integrates drainage from various upstream tributaries, it represents the site with the highest degree of riparian forest conservation and the least influence from direct point-source effluents in this study. This sequence allows for the evaluation of how contamination pulses are transported and modified as the stream moves toward areas with greater environmental protection and habitat complexity.

Water samples

At each sampling site, conductivity, dissolved oxygen, pH and water temperature, were measured *in situ* with standard previously calibrated digital devices (HANNA Dis2

HI98302; ETI 813-513; GIDIGI GX-DO01B). Other physicochemical parameters (nitrate, nitrite, phosphorus, and ammonia) and metal concentrations were assessed using commercial kits (API®, Carethetic® and Tetra® test). These semi-quantitative methods were used according to manufacturer specifications and follow established protocols for ecotoxicological field screening (Cuzziol Boccioni et al. 2025). Measurements were performed in duplicate at each sampling site. Deionized water was analyzed as blanks to verify absence of cross-contamination. Internal calibration verification was conducted using manufacturer-provided reference standards within the detection range of each kit. These methods are widely used for rapid environmental assessment and provide conservative estimates suitable for screening-level ecological risk assessment, particularly when integrated with biological endpoints (Lajmanovich et al. 2021).

Water odor and color were also characterized *in situ* according to Lebrero et al. (2011). In addition, water samples were collected in 10-L plastic containers for bioassays and 100-mL sterile plastic bottles for microbiological analysis, according to Alberro et al. (2011). Samples were transported to the laboratory in coolers on ice and used immediately for physicochemical profiling and bioassay treatments.

Microbiological analyses were determined according to the Standard Methods for the Examination of Water and Wastewater (SMEWW, Rice et al. 2012). For the enumeration of total aerobic mesophilic bacteria (SMEWW Section 9215), Pseudomonas spp. (SMEWW Section 9213), and Enterococci (SMEWW Section 9230 C), water samples were processed using the membrane filtration technique, where 100 mL of each sample and its corresponding dilutions were filtered through a 0.45 µm pore-size membrane. To determine mesophilic bacteria, membranes were placed on Plate Count Agar (PCA) and incubated at 37 °C for 48 h, with results expressed as colony-forming units per 100 milliliters (CFU/100 mL). For *Pseudomonas* spp., membranes were placed on Cetrimide Agar and incubated at 35 ± 0.5 °C for 48 h; presumptive colonies were identified by pyocyanin pigment production and fluorescence under UV light (CFU/100 mL). Enterococci were enumerated by placing membranes on KF Streptococcus Agar supplemented with 1% TTC (v/v) followed by incubation at 35 ± 0.5 °C for 48 h, counting all red-to-pink colonies as presumptive (CFU/100 mL). The Most Probable Number (MPN) of *Escherichia coli* was determined using the multiple-tube fermentation technique. Following a presumptive phase in Lauryl Tryptose Broth, positive tubes were confirmed by subculturing onto Chromogenic Agar (Chromagar, France) at 37 °C for 24 h. *E. coli* presence was identified by characteristic colony morphology, and the final density was calculated using standard probability tables and expressed as MPN/100 mL (Rice et al. 2012). Microbiological analyses were not performed at P3 due to logistical constraints during sample handling. However, given the close spatial proximity and hydrological continuity between P2 and P3 within the Espinillo stream, the lack of microbiological data at P3 does not preclude interpretation of the overall downstream contamination pattern.

Additional microscopic examinations of water samples were immediately conducted to assess the presence of cyanobacteria. For the qualitative assessment, a screening was performed by examining multiple drops (0.05 mL aliquots) randomly taken from the samples collected in the field after thorough manual homogenization, without any filtering technique. For each sampling site, 10 replicates ($n = 10$) were systematically analyzed using standard glass slides and coverslips under a light microscope (magnifications from 40X to 1000X). This approach, based on the direct examination of water drops for morphological characterization (Oliveira et al. 2019), allowed for a representative identification of dominant genera while preserving the integrity of colonial and filamentous structures. No pre-concentration or filtering techniques were applied, since no quantitative counts or biomass estimations were performed.

Experimental design

Tadpoles of *R. arenarum* were collected from the surroundings of a pristine forest area of the natural floodplain of the Paraná River (31° 11′ 31″ S, 60° 9′ 29″ W), where no pesticides are applied or detected (Cuzziol Boccioni et al. 2024). The collection was authorized by the Ministerio de Ambiente of the Province of Santa Fe (EXP. N° 02101-0026248-0) from Argentina. Tadpoles were immediately transported to the laboratory and acclimated for 48 h under controlled laboratory conditions prior to the start of the bioassays (24 ± 2 °C, 12 h light/dark cycle). During acclimation, tadpoles were maintained in tanks with dechlorinated tap water (DTW) obtained from the local drinking water supply. This type of water was also used for treatments dilutions, as recognized as a suitable medium for amphibians and applied to ecotoxicological bioassays (Pereyra et al. 2021; Cuzziol Boccioni et al. 2025; OECD 2025). The DTW was allowed to stand for at least 48 h under constant aeration to facilitate chlorine dissipation; no chemical dechlorinating agents were used to avoid potential chemical interferences. Before use, the absence of total chlorine was verified using commercial kits (API® Total Chlorine Test), resulting in concentrations of 0 mg L^{-1}. The physicochemical characteristics of the DTW after the aeration period were: pH 7.4 ± 0.05; conductivity 165 ± 12.5 μS cm^{-1}; dissolved oxygen concentration 6.5 ± 1.5 mg L^{-1}; hardness 50.6 mg L^{-1} $CaCO_3$).

A 48-h bioassay was performed in duplicate to evaluate the toxicity of water samples from the four sampling sites. The treatments were named according to the samples from each point (P1, P2, P3 and P4). Each treatment was tested at 100% concentration and at dilutions of 50% and 25%. Dilutions consisted of the initial 100% water for the sampling site, diluted with DTW. A treatment with 1 L of DTW was used as negative control, CO (-).

Prior to the exposure, visibly healthy individuals were selected from the acclimated pool based on uniform size and developmental stage (Gosner stage 28; Gosner 1960) to minimize biological variability. From this selection, ten tadpoles were randomly assigned to each experimental unit (tank).

During the exposure, temperature (24 ± 2 °C) and 12h light/dark cycle were constantly maintained. Although all dilution treatments were used to assess mortality, biochemical and behavioral analyses were conducted only on the highest concentration of each treatment that did not exceed 15% mortality.

The experiments followed the ASIH guidelines (2004) and tadpoles were treated according to the protocol of the Animal Euthanasia Guide proposed by the Institutional Animal Care and Use Committee (IACUC) and the committee of bioethics of the Facultad de Bioquímica y Ciencias Biológicas of the Universidad Nacional del Litoral (Santa Fe, Argentina) (Res. N° 388/06).

Biological endpoints

Mortality

Survival rate was evaluated at 24 and 48 h of exposure. Mortality was expressed as the percentage of dead individuals relative to the total in each treatment. Individuals were considered dead when they exhibited no movement after a gentle glass rod-stimulus (Peltzer et al. 2019).

Sublethal parameters were analyzed according to the following criteria: (i) for sites with no significant mortality, analyses were performed exclusively on the undiluted (100%) samples; (ii) for sites where mortality occurred, analyses were performed on the highest dilution with $\leq$15% mortality.

Enzyme activities

Enzyme activities (Glutathione reductase, GR; Glutathione-S-transferase, GST; and acetylcholinesterase, AChE) were determined at the end of the bioassay. To this end, $n = 8$ tadpoles per treatment were processed following standardized laboratory procedures for the biochemical analysis of amphibian samples (Attademo et al. 2023). Briefly, samples were weighed (g) to determine the appropriate buffer to use. Then, samples were homogenized (1:10, w/v) in ice-cold 25 mM sucrose, 20 mM Tris-HCl buffer (pH = 7.4) with 1 mM EDTA, using a polytron tissue grinder. Homogenates were then centrifuged at 10 000 rpm at 4 ± 1 °C for 15 min and stored at −80 °C until the analysis.

Enzymatic activities were measured at 22-25 °C using a JENWAY 6405 UV-VIS spectrophotometer. Non-enzymatic reactions were periodically confirmed in blanks (reaction mixture without the sample). Total protein concentration was determined using the Biuret method (Kingsley 1942; Attademo et al. 2021).

The GST activity was determined following the method described by Habig et al. (1974) and adapted to mammal serum GST activity by Habdous et al. (2002). The GST activity was expressed as nmol $min^{-1} mg^{-1}$ protein using a molar extinction coefficient of $9.6 \times 103\,M^{-1}\,cm^{-1}$. GR activity was assessed following the method of Ramos-Martinez et al. (1983), which measures the oxidation of NADPH at 340 nm in the presence of oxidized glutathione and 0.1 M Na-phosphate buffer (pH 7.0). The GR activity was calculated by means of a millimolar extinction coefficient for the NADPH oxidation of $6.22\,mM^{-1}\,cm^{-1}$. AChE activity was determined colorimetrically according to Ellman et al. (1961). The variation in optical density was recorded at 410 nm, and the activity was expressed as nmol $min^{-1} mg^{-1}$ protein using a molar extinction coefficient of $13.6 \times 103\,M^{-1}\,cm^{-1}$.

Swimming activity

Swimming activity was assessed by recording individual tadpoles ($n = 8$ per treatment) separately. Each tadpole was placed in an individual container (200 mL of the corresponding treatment), and its behavior was recorded for 1 min using a digital camera (Motic®, 10.0 M pixel). The videos were analyzed with the ToxTrac software version 2.96 (Rodriguez et al. 2018), adjusting the detection threshold for the recognition of individuals to obtain the swimming activity parameters: total distance moved (mm), mean swimming speed (mm sec^{-1}) and mobility rate (%).

Data analysis

Water parameters analysis

This study adopts a screening-level ecological risk assessment approach to evaluate the ecological implications of rainfall-driven contamination pulses. The Risk Quotient (RQ) was calculated for each physicochemical, microbiological and metal parameter obtained for each site as follows:

$$RQ = MEC/QO$$

where MEC represents the measured environmental concentration and QO the corresponding quality objective or regulatory benchmark for aquatic life protection or recreational exposure, as a close approach deriving from predicted no-effect concentrations (USEPA 2007; Gustavsson et al. 2017). When available, values for the protection of aquatic life defined by Argentine Law 13.577 (Res. 79.179/90) and Avila Pérez et al. (2011) were used. Microbiological risk quotients were calculated using WHO and USEPA recreational water safety criteria (WHO 2003; USEPA 2012)

Because dissolved oxygen (DO) represents an inverse stressor, RQ were calculated using inverse ratios:

$$RQ_{DO} = QO/DO$$

The obtained RQ interpretation followed a conservative screening framework (USEPA 1998; Suter 2007), considering RQ < 0.1 negligible risk, $0.1 \leq$ RQ < 1 low to moderate risk, $1 \leq$ RQ < 10 high ecological risk, and RQ ≥ 10 severe ecological risk.

Biological endpoints

Mortality data were analyzed using a one-tailed Fisher's exact test to compare the proportion of dead individuals in each treatment and its respective dilutions against the control group.

Normality (Shapiro test) and homogeneity of variances (Levene test) were assessed for statistical analysis of biomarker results. Since the assumptions were met for all datasets, one-way ANOVA and Dunnett's test for post-hoc comparisons were applied to determine significant differences in biomarkers in organisms exposed to treatments relative to the control (Lajmanovich et al. 2019).

Statistical analyses were performed using InfoStat/P version 1.1 (Grupo InfoStat Professional, FCA, Universidad Nacional de Córdoba, Argentina) and BioEstat software 5.0 (Ayres et al. 2008). A value of $p < 0.05$ was considered statistically significant.

Results

Water analysis

The results of the physicochemical, microbiological, and metal analyses of water samples from P1 to P4 are shown in Table 1. Water from P1 was characterized by a strong putrid odor and a deep red color, whereas water from the other sites exhibited no distinct odor, and coloration varied between ashen brown and clear. Some parameters, such as nitrites and phosphorus, were similar across the four sites, while others, such as

Table 1. Physicochemical parameters, metals and microbiological parameters, in water samples from the Crespo stream (P1), Espinillo stream (P2 and P3), and Las Conchas stream (P4) in mid-eastern Argentina (Entre Ríos Province) in South America.

	P1	P2	P3	P4
Physicochemical parameters				
Color	Deep red	Brown ash	Brown ash	Clear (no color)
Odor	Putrid	No odor	No odor	No odor
Water temperature (°C)	28.7	26.5	27.5	27.8
Dissolved oxygen (mg L^{-1})	$<$0.5	5 ± 1	7.5 ± 1	9 ± 1
pH	9 ± 0.5	8 ± 0.5	7.5 ± 0.5	8.5 ± 0.1
Conductivity (μ S cm^{-1})	636 ± 2	339 ± 3	834 ± 1.8	485 ± 3
Nitrite (NO_2^-) (mg L^{-1})	$<$0.5	0.5 ± 0.3	$<$0.5	$<$0.5
Nitrate (NO_3^-) (mg L^{-1})	12.5 ± 3	20 ± 3	15 ± 5	$<$0.5
Phosphorous (mg L^{-1})	1 ± 0.25	1 ± 0.25	1 ± 0.25	1 ± 0.25
Ammonia NH_4^+ (mg L^{-1})	0.5 ± 0.1	0.5 ± 0.1	25 ± 0.1	0.5 ± 0.1
Metals (mg L^{-1})				
Lead	2.5 ± 1.0	NDt	2.5 ± 1.5	NDt
Iron	0.15 ± 0.05	0.15 ± 0.1	0.3 ± 0.1	0.10 ± 0.05
Copper	5 ± 1.5	1.25 ± 0.5	2.5 ± 0.5	NDt
Mercury	0.002 ± 0.0005	NDt	$<$0.002	NDt
Zinc	2.5 ± 1.5	2.5 ± 0.5	2.5 ± 1	NDt
Microbiological parameters				
Total mesophilic aerobes bacteria (CFU[a] 100 mL^{-1})	7.6×10^7	5.4×10^5	NDm	1.8×10^5
Pseudomonas (CFU 100 mL^{-1})	1.1×10^6	1.2×10^3	NDm	5×10^2
Enterococci (CFU 100 mL^{-1})	3.4×10^5	NDt	NDm	2×10^4
Total coliform bacteria (MPN[b] 100 mL^{-1})	2×10^5	2.3×10^4	NDm	4×10^2
Escherichia coli [c] (MPN 100 mL^{-1})	2×10^5	4×10^3	NDm	4×10^2

Values are expressed as mean ± standard deviation (SD) based on duplicate measurements ($n = 2$).
[a]CFU: Colony-forming units.
[b]MPN: most probable number.
[c]*E. coli* was considered the main representative of total fecal coliform bacteria.
NDt: not detected; NDm: not determined.

conductivity and pH, were more dissimilar. Dissolved oxygen, nitrate, and ammonium were the physicochemical parameters that most fluctuated among sites and revealed spatial variability in ecological risk across sampling sites (Table 2).

At Crespo stream, hypoxic conditions (dissolved oxygen <0.5 mg L^{-1}) resulted in a high ecological risk (RQ = 9.4). At Espinillo Stream, nitrate levels exceeded the limit of the respective environmental objective at P2 (20 mg L^{-1}) and therefore a high risk was recorded for this parameter (RQ = 1.17). In contrast, severe ecological risk was associated with elevated ammonia concentrations (RQ = 19.38).

Bacteriological analysis showed fecal coliform levels (represented by *E. coli*) just above the QO and showed high ecological risk (RQ = 1.33) in P1. Microscopic analysis also revealed the presence of cyanobacteria in water from P1, primarily represented by *Microcystis sp*. colonies (Figure 3A) and *Oscillatoria sp.* (Figure 3B). Filaments of *Oscillatoria sp*. were also observed in water from P3.

Metal contamination represented the major ecological risk driver. Copper, lead, and zinc were found in high concentrations in sites P1 to P3 (Table 1). Consequently, RQ values (from 83 to 2500) resulted in severe ecological risk at these upstream sites (Table 2).

Biological endpoints

Mortality

Detailed mortality rates and statistical comparisons are summarized in Table 3. No mortality was recorded in larvae exposed to the control or to any dilution of the

Table 2. Risk quotient (RQ) values obtained for crespo stream (P1), Espinillo stream (P2 and P3), and Las Conchas stream (P4) in mid-eastern Argentina (Entre Ríos Province) in South America.

	QO	P1	P2	P3	P4
Dissolved oxygen	>4.7 mg L^{-1}	**9.4**	0.95	0.63	0.52
Conductivity	<1250 µS cm^{-1}	0.51	0.2	0.67	0.03
Nitrate (NO_3^-)	<17 mg L^{-1}	0.73	**1.17**	0.88	0.03
Ammonia NH_4^+	<1.29 mg L^{-1}	0.38	0.38	**19.38**	0.38
Lead	<0.028 mg L^{-1}	**89.28**	NC	**89.28**	NC
Copper	<0.002 mg L^{-1}	**2500**	**625**	**1250**	NC
Mercury	<0.0025 mg L^{-1}	0.8	NC	0.8	NC
Zinc	<0.03 mg L^{-1}	**83.33**	**83.33**	**83.33**	NC
Escherichia coli	$<1.5 \times 10^5$ MPN100 mL^{-1}	**1.33**	0.27	NC	0.003

For RQ calculation, quality objectives (QO) for aquatic life protection established by Avila Pérez et al. (2011), Argentine law 13.577 (res. 79.179/90) and USEPA (2007) were considered.
NC = not calculated because the parameter was not detected.
Bold RQ values indicate high (values between 1 and 10) to severe (>10) ecological risks.

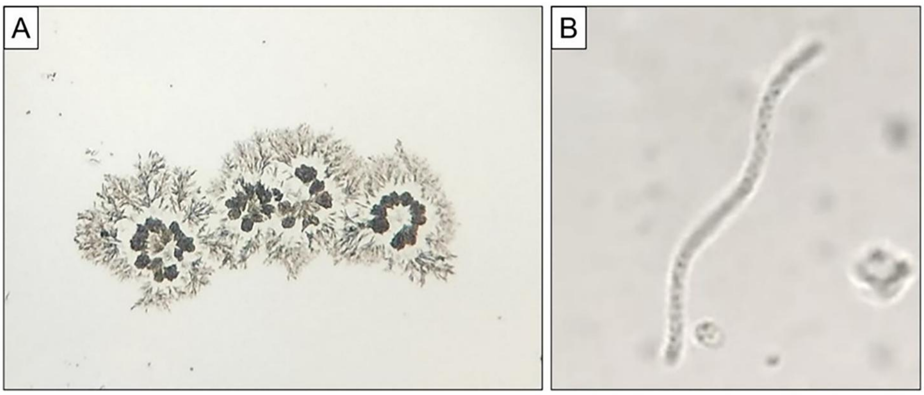

Figure 3. Cyanobacteria from water samples from Crespo stream and Espinillo stream were observed under light microscopy. A) *Microcystis sp.* colonies (magnification 40X). B) *Oscillatoria sp.* (magnification 1000X). Principio del formulario.

treatments from P2, P3, or P4 during the 48-h bioassay. In contrast, the undiluted sample (100%) from P1 caused 100% mortality within 24 h of exposure, representing a statistically significant difference compared to the control group (Fisher's exact test, $p < 0.0001$). At dilutions of P1 treatment, mortality decreased to 10% in the 50% dilution and 0% in the 25% dilution, showing no significant differences relative to the control ($p = 0.27$ and $p = 1$, respectively). Consequently, the 50% dilution for P1 and the 100% concentration (undiluted) for P2–P4 were used for sublethal analyses; hereafter, these will be referred to as P1, P2, P3, and P4.

Enzyme activities

GR activity was significantly reduced in the P1 and P2 treatments with respect to the CO, whereas it increased in P4 treatment ($F = 11.48$, $p < 0.01$; Figure 4A). The GR activity in CO tadpoles was 0.32 ± 0.07 nmol min^{-1} mg^{-1} of total protein; in comparison, it decreased to 0.14 ± 0.07 and 0.25 ± 0.09 nmol min^{-1} mg^{-1} total protein in P1 and P2 treated tadpoles, respectively (reduction of 56.25% and 21.87%).

Table 3. Mortality (% from total, $n = 8$ tadpoles) of *rhinella arenarum* from a control group (CO) and four treatments with water from the Crespo stream (P1), Espinillo stream (P2 and P3), and Las Conchas stream (P4) after 48 h.

Treatment	Dilution	Mortality	p value (Fisher test)
CO	–	0%	–
P1	**100%**	**100%**	$\mathbf{p < 0.0001}$
	50%	10%	$p = 0{,}27$
	25%	0%	$p = 1$
P2	100%	0%	$p = 1$
P3	100%	0%	$p = 1$
P4	100%	0%	$p = 1$

The p-values represent the comparison of each treatment with respect to CO using Fisher's exact test. Bold values indicate significant mortality ($p < 0.05$). For sites P2, P3, and P4, only the 100% concentration is shown as no mortality was observed in any dilution.

GST activity significantly increased in the P1 and P2 treatments with respect to the CO ($F = 2.84$, $p = 0.03$; Figure 4B). The GST activity in CO tadpoles was 194.25 ± 29.29 nmol min^{-1} mg^{-1} total protein; in contrast, it increased to 279.01 ± 62.23 and 263.72 ± 95.17 nmol min^{-1} mg^{-1} total protein in P1 and P2 treated tadpoles, respectively (representing inductions of 43.63% and 35.76%).

AChE activity did not vary significantly across treatments with respect to the CO ($F = 2.41$, $p > 0.05$; Figure 4C). The AChE activity in CO tadpoles was 23.08 ± 4.37 nmol min^{-1} mg^{-1} total protein, whereas in treatments, it ranged between 26.66 ± 4.03 and 21.58 ± 8.7 (P3 treated tadpoles) nmol min^{-1} mg^{-1} total protein.

Swimming activity

Swimming activity parameters were mainly affected by treatments with water from P1 and P3. The mean speed and total distance in the P1 and P3 treatments significantly decreased with respect to the CO ($F = 5.01$; $F = 5.91$ respectively; $p < 0.01$; Figure 5A,B.). Mean speed was 87.17 ± 21.29 mm sec^{-1} in CO tadpoles, decreasing to 33.59 ± 19.5 and 42.76 ± 23.58 mm sec^{-1} in P1 and P3 treated tadpoles, respectively. Similarly, total distance was 5671.44 ± 1357.7 mm in CO tadpoles, whereas it decreased to 2079.05 ± 1155.3 mm in P1 and 2730.51 ± 1708 mm in P3 treated tadpoles. Regarding mobility rate, a significant reduction was observed in the P1 treatment with respect to CO ($F = 2.23$; $p < 0.01$; Figure 5C). Mobility rate was $78.5 \pm 14.6\%$ in CO tadpoles, whereas it decreased to $54 \pm 17\%$ in P1 treated tadpoles.

Discussion

The present study demonstrates that heavy rainfall in agro-industrial tributaries of Las Conchas Basin within the Paraná River system is associated with acute contamination pulses that propagated downstream and caused severe effects in *R. arenarum* larvae. Our results suggest a longitudinal gradient of toxicity, where the sudden discharge of untreated or partially treated effluents—driven by first-flush dynamics of a rain event (87 mm) that accounted for the majority of the total cumulative rainfall recorded for that month—led to the degradation of physicochemical and bacteriological parameters, as well as chemical concentrations that exceed water quality

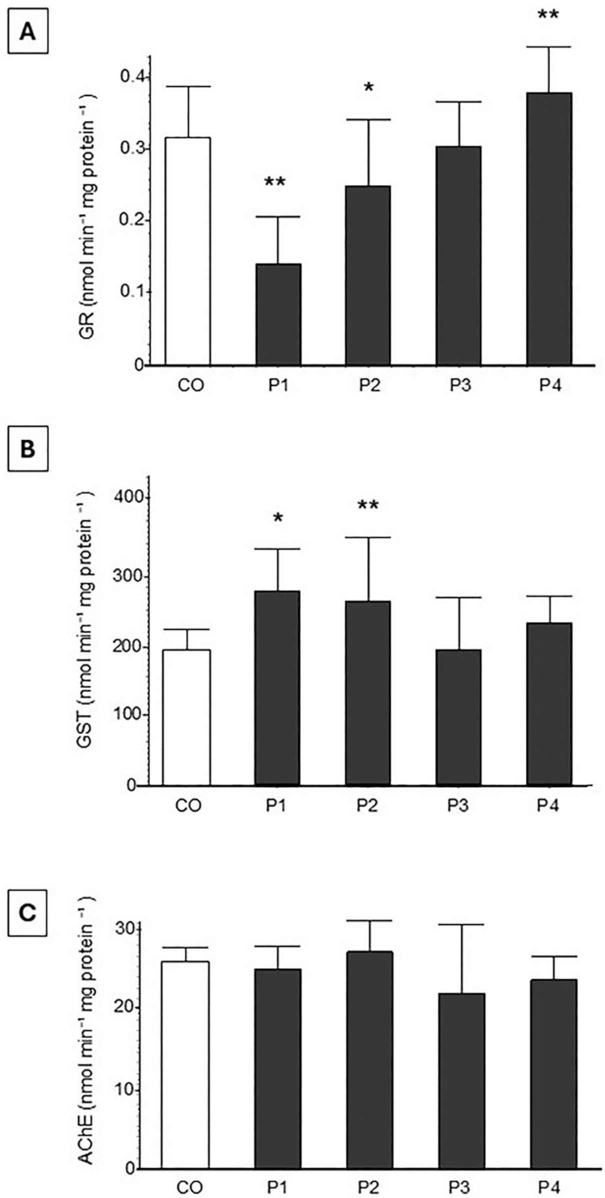

Figure 4. Activities of glutathione reductase, GR (a); glutathione-S-transferase, GST (B); and acetyl-cholinesterase, AChE (C) in *rhinella arenarum* tadpoles ($n = 8$) from the control (CO) and four treatments with water from the crespo stream (P1), Espinillo stream (P2 and P3), and Las Conchas stream (P4). Values are presented as the mean ± standard deviation. Significant differences respect to CO* $p<0.05$; ** $p<0.01$ (ANOVA and Dunnett's test).

guidelines for the protection of aquatic life. The convergence of chemical exceedances, acute toxicity responses, and biomarker alterations provides strong weight-of-evidence supporting ecological impairment. Overall, the observed biological responses followed the spatial gradient of river preservation; while urban-industrial pressure at

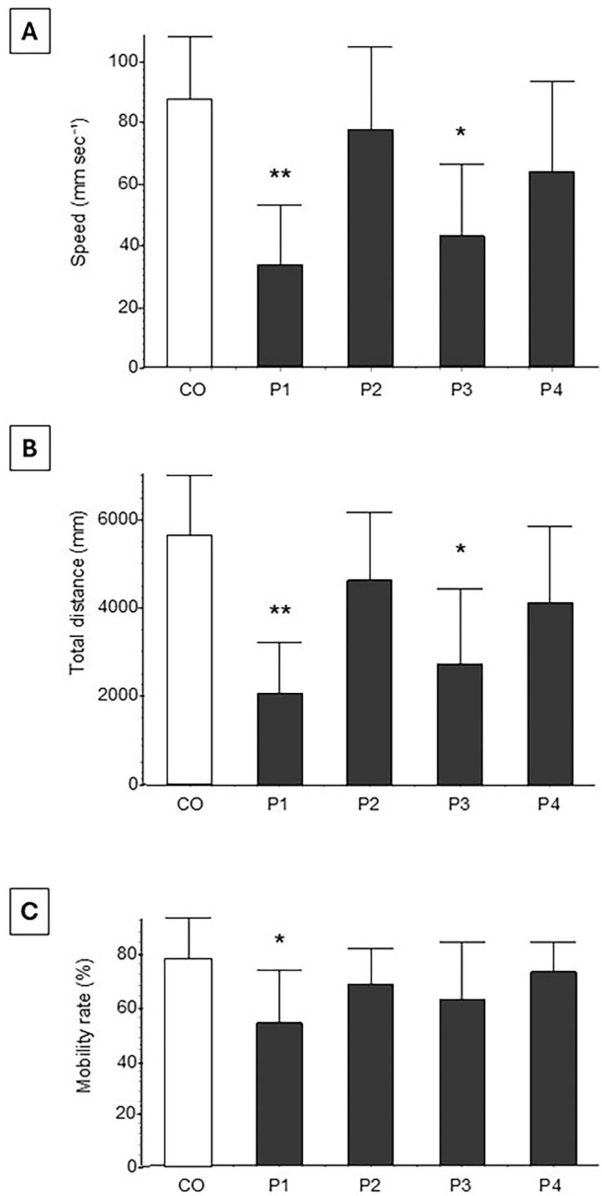

Figure 5. Mean speed (a), total distance traveled (B), and mobility rate (C) in *Rhinella arenarum* tadpoles ($n = 8$) from the control (CO) and four treatments with water from the Crespo stream (P1), Espinillo stream (P2 and P3), and Las Conchas stream (P4). Values are presented as the mean ± standard deviation. Significant differences with respect to CO. $^{*}p$ <0.05; $^{**}p$ <0.01 (ANOVA and Dunnett's test).

P1 generated maximum toxicity, it progressively decreased toward P4 at PEREB. This highlights the role of conserved riparian areas in mitigating downstream ecotoxicological risks within the basin. These findings have direct implications for regulatory frameworks, highlighting the need for event-based monitoring to improve ecological risk assessment accuracy.

Water analysis

The physicochemical and microbiological profile of the Crespo stream (P1) immediately following the rainfall event—characterized by a putrid odor, deep red coloration, and near-anoxic conditions ($<0.5\,\text{mg L}^{-1}$)—suggests a wastewater overflow rather than a natural phenomenon. The detection of cyanobacteria such as Microcystis and Oscillatoria following the rainfall event is unlikely to reflect *in situ* proliferation, given the short time frame between precipitation and sampling. Instead, their presence is more plausibly explained by the mobilization and downstream transport of preexisting populations associated with wastewater treatment systems or eutrophic upstream environments (Martins et al. 2011). Heavy rainfall events can resuspend and redistribute cyanobacterial biomass, facilitating its transfer into receiving streams.

The red coloration, extreme heterotrophic and fecal bacterial loads, and cyanobacterial filaments in Crespo stream (P1) suggest an anthropogenic contamination given by wastewater overflow events (Madoux-Humery et al. 2016). Metal contamination represented an additional and highly significant ecological risk factor. Extremely elevated RQ for copper, lead, and zinc were observed at upstream sites, exceeding ecological safety thresholds by several orders of magnitude incompatible with natural phototrophic processes (Alloway 2013; Ding et al. 2022). These stressors co-occurred temporally and spatially, generating a multi-stressor exposure scenario typical of rainfall-driven pulses (Sabater et al. 2014; Zheng et al. 2026).

Complementarily, satellite imagery shows a clear chromatic continuity between the WTP from Crespo city and the red-colored reach of the Crespo Stream. However, the lower ecological risk at P2 compared to P3 indicates that contaminant propagation is not strictly linear. This heterogeneity suggests the influence of local factors—such as diffuse runoff and tributary inputs—alongside hydrodynamic processes like dilution, sedimentation, and remobilization (Paton and Haacke 2021). While P1 is a critical contributor, the non-linear variability between P2 and P3 underscores that contamination pulses in agro-industrial basins are complex phenomena driven by both primary sources and local-scale processes.

Beyond the immediate risk to aquatic fauna, the persistence of fecal indicators and potentially toxin-producing cyanobacteria (e.g., *Microcystis*, *Oscillatoria*) poses a significant threat to human health, reinforcing the relevance of a One Health perspective (Gomes et al. 2024). While high ecological risk was mainly associated with P1, the downstream propagation of this plume impacts the Espinillo and Las Conchas streams, which serve as popular bathing resorts at Camping Espinillo and PEREB. When integrating current results with a previous assessment (Cuzziol Boccioni et al. 2025), the geometric mean for *E. coli* reached 871.17 MPN $100\,\text{mL}^{-1}$, exceeding international criteria recommended by the USEPA (2012) and the WHO (2003). Individual samples exceeding 500 MPN $100\,\text{mL}^{-1}$ surpass safe exposure thresholds and are associated with increased risks of gastrointestinal illness, otitis, and dermal infections. Collectively, these findings suggest sustained contamination levels incompatible with human health protection in recreational waters, highlighting the urgent need for early warning systems that bridge environmental and health data (Knecht et al. 2021).

Biological endpoints

In this study, acute mortality of *R. arenarum* was exclusive to the Crespo stream, correlating with peak microbial and metal loads, total oxygen depletion, and the highest RQ values. These results expand upon findings by Cuzziol Boccioni et al. (2025), where lethality was observed in water-sediment matrices from the same site. High microbial densities and nutrient influxes in the Crespo stream further indicate untreated effluent releases triggered by storm events, which can exert direct toxicity on larvae in systems with limited dilution (Pandey et al. 2014; Manini et al. 2022; Maphanga et al. 2022). Conversely, the lower microbial counts and lack of acute toxicity in the Espinillo and Las Conchas streams support a downstream gradient of dilution and self-purification (Hörchner et al. 2025; Kucharczyk et al. 2025). Ultimately, these findings reinforce the sentinel value of *R. arenarum* for detecting episodic contamination pulses and underscore the necessity of event-based monitoring to capture acute risk dynamics in agro-industrial watersheds (Pollard et al. 2019).

Our results regarding mortality in P1 are consistent with the 'toxic pulse' phenomena observed in other regional catchments (Arias et al. 2020), emphasizing the vulnerability of aquatic biota to runoff events. In this sense, episodic pollution pulses constitute a major ecological risk pathway in intensively cultivated basins (Kefford et al. 2012). In the present study, acute mortality of *R. arenarum* tadpoles was confined to the Crespo stream during a post-rain effluent pulse characterized by oxygen depletion and elevated microbial loads, illustrating a comparable mechanism of episodic contamination pulse within agro-industrial catchments of central Argentina.

Indeed, water from Crespo Stream (P1) showed an extremely low dissolved oxygen concentration ($<0.5\,mg\ L^{-1}$), representing near-anoxic conditions. Oxygen limitation acts as a primary stressor that may override or synergistically interact with chemical toxicity. While gill respiration is the main gas exchange pathway in early larval stages, individuals at Gosner stage 28 already possess developing lungs and can perform facultative air-breathing (Gosner 1960; West and Burggren 1982). Studies have shown that amphibian larvae exposed to aquatic hypoxia can adjust the relative importance of their respiratory surfaces, increasing their reliance on aerial respiration *via* air-gulping at the surface to compensate for the lack of dissolved oxygen (Burggren and Mwalukoma 1983; Feder 1983). However, this compensatory mechanism involves a high metabolic cost and increases the tadpoles vulnerability, as the demand for oxygen may outweigh their detoxication capacity. Therefore, the total mortality observed at P1 should be interpreted as the result of multiple interacting stressors exacerbated by hypoxia.

Beyond their immediate toxic effects, contaminant pulses can trigger broader ecological alterations that reshape aquatic community structure and function (Samuel et al. 2023; Bănăduc et al. 2024). Such transient disturbances may cause cascading trophic effects—including enhanced phytoplankton productivity or shifts in predator–prey relationships—leading to the decline of sensitive species and the dominance of more tolerant taxa (Mor et al. 2022). In agro-industrial catchments, these recurrent events act as chronic stressors, gradually altering nutrient cycling and biodiversity patterns (Wieczorek et al. 2018; Mattoo et al. 2025). Within the Las Conchas basin, this dynamic is especially critical for the PEREB Reserve. Despite its status as a natural sanctuary and a primary refuge for native aquatic fauna in central Argentina, the reserve remains hydrologically connected to the surrounding agricultural matrix. Consequently, its conservation value is increasingly threatened by episodic exposure to effluent-driven toxic pulses, underscoring that protected areas are not insulated from the systemic degradation of their broader landscapes

The enzymatic responses observed in *R. arenarum* tadpoles exposed to post-rain water samples indicate the activation of detoxification and redox-related pathways rather than a direct neurotoxic mechanism. On the one hand, significant changes were detected in glutathione-dependent enzymes, mostly in the Crespo and Espinillo streams treatments. Alterations in glutathione S-transferase (GST) activity indicate modulation of phase II biotransformation processes, reflecting the involvement of mixed contaminant loads and high organic inputs commonly associated with wastewater-derived effluents (Livingstone 2001; Attademo et al. 2021; Cardoso-Vera et al. 2024). Besides, the increase in glutathione reductase (GR) activity in the same treatments suggests a compensatory response aimed at maintaining intracellular redox balance and reduced glutathione availability under oxidative challenge (Mardirosian et al. 2017; Fernández et al. 2020).

On the other hand, AChE activity did not show significant inhibition across sites, suggesting that acute neurotoxicity was not the dominant biochemical response under the exposure conditions evaluated. This lack of AChE inhibition is consistent with previous studies reporting that short-term exposure to complex wastewater and agro-industrial mixtures may elicit pronounced behavioral alterations in the absence of measurable AChE responses, particularly when multiple stressors act simultaneously (Umar and Aisami 2020). The absence of significant AChE responses was not related to functional impairment, as behavioral endpoints have previously been shown to respond without biochemical disruption in amphibians (Santos et al. 2025).

Significant reductions in swimming activity in Crespo and Espinillo treatments align with reported declines in locomotor performance for amphibians exposed to wastewater pressure (Greenberg and Palen 2021; Tornabene 2021). These behavioral shifts likely stem from acute fluctuations in dissolved oxygen and conductivity, potentially compounded by cyanobacterial toxins that impair swimming frequency and speed while increasing disease susceptibility (Buss et al. 2019; Zeitler et al. 2021). While cyanobacteria and algae represent nutritional resources, their presence modulates larval responses to turbidity differently than inorganic solids Ultimately, such impairments in swimming performance compromise foraging efficiency and predator avoidance, increasing overall vulnerability and mortality risk. (Peltzer et al. 2019; Curi et al. 2022).

Therefore, the integration of physicochemical parameters, microbiological indicators, and biological responses—such as those observed in *R. arenarum* tadpoles—provides a robust framework for interpreting acute contamination episodes associated with rainfall. Moreover, the strong concordance between RQ exceedances meaning high to severe ecological risks and observed biological effects in amphibian bioassays provides robust support for causal linkage between contaminant exposure and ecological impact. These data also suggest a longitudinal pattern where urban-industrial pressure at P1 triggers maximum toxicity, which gradually attenuates as the water flows toward P4. The increased habitat complexity and riparian buffer zones within the PEREB may contribute to a partial recovery of water quality, but this protected area remains hydrologically connected to heavily impacted upstream zones. Despite the absence of acute mortality at P4, the continuous reception of water from degraded tributaries underscores that protected ecosystems are not isolated entities, but integrated components of larger watershed networks. In agreement with previous ecotoxicological studies conducted in the Pampas region (Cuzziol Boccioni et al. 2024), the persistence of elevated microbial loads following precipitation indicates that wastewater-derived inputs remain a dominant driver of short-term water-quality degradation in agro-industrial basins. These findings emphasize that rainfall acts as a major vector for pollutants in these systems.

Strengths and methodological limitations

A major strength of this study lies in the integration of biological responses, microbiological indicators, and physicochemical parameters within a rapid and cost-effective screening framework designed to characterize real-world post-rainfall contamination events in a resource-limited context. The methods applied were appropriate for rapid ecological risk screening during post-rainfall pulses, although semi-quantitative tools for metal analysis inherently provide lower analytical precision and higher detection limits than laboratory-based analytical techniques. Since the primary objective was not precise contaminant quantification, but rather the identification of acute ecological impairment under complex multi-stressor conditions, this study neither incorporated metal bioavailability modeling through biotic ligand approaches nor evaluated chronic exposure scenarios.

Some methodological constraints should also be considered. The absence of Biochemical Oxygen Demand (BOD) measurements limited further characterization of organic loads, while cyanobacterial assessment remained qualitative and did not include cyanotoxin confirmation. Additionally, the use of amphibians as a single taxonomic group does not allow direct extrapolation to the entire aquatic community. Likewise, although field-collected larvae may introduce some degree of biological variability compared to standardized laboratory cultures, they increase ecological realism and environmental relevance.

Conclusions

This study provides clear evidence that rainfall-driven effluent pulses represent high-risk ecological exposure scenarios in agro-industrial tributaries of the Paraná River basin. Post-rainfall contamination events were characterized by severe physicochemical degradation, including hypoxia, elevated microbial loads, and metal concentrations exceeding environmental quality thresholds, confirming the presence of hazardous exposure conditions.

The acute mortality and sublethal biomarker alterations observed in *R. arenarum* tadpoles provide strong evidence that these episodic contamination pulses can produce immediate and biologically significant toxic effects. These findings confirm the vulnerability of amphibian larvae to short-term, high-intensity contamination events and support their value as sentinel organisms for ecological risk assessment in freshwater ecosystems.

From a risk characterization perspective, the overall spatial gradient of toxicity observed along the hydrological continuum indicates that overflows from wastewater treatment ponds in Crespo City and agro-industrial discharges act as primary contamination sources capable of generating transient but severe ecological hazards within the Las Conchas Basin, which ultimately drains into the Paraná River. While the transition toward more conserved riparian areas (such as the PEREB reserve) contributed to a general reduction in acute toxicity, the persistence of detectable biological effects and non-linear fluctuations at intermediate sites highlight the complexity of these episodic exposure events at the basin scale.

Overall, these results demonstrate that conventional monitoring approaches based on routine sampling may substantially underestimate ecological risk, as they fail to capture short-duration contamination pulses associated with extreme rainfall. The incorporation of event-based monitoring into ecological risk assessment frameworks is therefore essential to accurately characterize exposure dynamics and protect aquatic biodiversity. The magnitude of the observed biological effects suggests that even accounting for potential uncertainty associated with field-based, semi-quantitative methods, contamination levels were sufficient to generate severe ecological risk. Furthermore, the detection of fecal contamination and toxic conditions in recreational waters underscores the interconnected nature of ecological and human health risks, reinforcing the relevance of a One Health approach. Strengthening wastewater infrastructure resilience and implementing adaptive monitoring strategies will be critical to mitigating ecological and public health risks under increasing climatic variability.

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Author contributions

CRediT: **Ana P. Cuzziol Boccioni**: Formal analysis, Investigation, Methodology, Software, Writing – original draft; **Andrés M. Attademo**: Formal analysis, Investigation, Methodology, Writing – original draft; **Karen Russell-White**: Methodology, Supervision; **María V. Lancelle**: Methodology; **Rafael C. Lajmanovich**: Conceptualization, Supervision, Writing – review & editing; **Paola M. Peltzer**: Funding acquisition, Supervision, Writing – review & editing.

Disclosure statement

No potential conflict of interest was reported by the authors.

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