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Attenuation of insecticide impact by a small wetland in a stream draining a horticultural basin in Argentina

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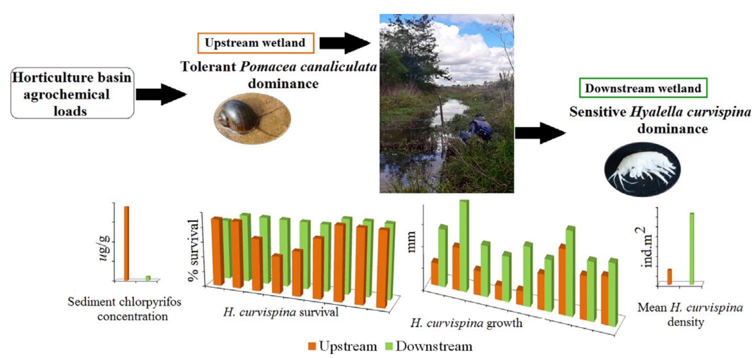
HIGHLIGHTS

- Agrochemicals from horticulture contaminate adjacent streams on the Argentine Pampas.
- Pesticide concentrations in sediments were lower downstream from a riparian wetland.
- Sediment toxicity to *Hyalella curvispina* decreased downstream from the wetland.
- Pesticide-sensitive *Hyalella curvispina* was dominant downstream from the wetland.
- Riparian marshes should be preserved and restored to attenuate stream contamination.

GRAPHICAL ABSTRACT



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ABSTRACT

Horticulture has greatly increased in Argentina in recent decades mainly due to increasing greenhouse utilization and agrochemical consumption, thus representing a threat to adjacent water bodies. Riparian wetlands, however, could attenuate agrochemical contamination. The present work therefore compared insecticide concentrations in bottom sediments in addition to sediment toxicity to the amphipod *Hyalella curvispina* and investigated the macroinvertebrate composition upstream and downstream from a natural wetland in a small stream draining a basin undergoing intense horticultural production. The wetland surface was covered by macrophytes, mainly *Thypha* sp., and the insecticide concentrations measured downstream from the wetland were significantly lower, at roughly 19% of the upstream values. The growth rates of *H. curvispina* were significantly higher when exposed to the sediments downstream from the wetland, while the macroinvertebrate-assemblage composition was significantly different upstream and downstream: the snail *Pomacea canaliculata* was the dominant species upstream while the amphipod *H. curvispina* was dominant downstream. *Pomacea canaliculata* is often the dominant species in the regional streams draining agriculture and horticultural basins. *Hyalella curvispina* is sensitive to pesticide toxicity and is often dominant in streams draining extensive livestock basins and within a biosphere reserve. We conclude that riparian wetlands effectively attenuate horticulture contamination in pampean streams and should therefore be preserved and restored.

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1. Introduction

Horticulture in Argentina is concentrated around large cities (Barsky, 2005). One of the main horticultural areas in the country is located around the city of La Plata in the province of Buenos Aires. The land in the area was traditionally used for the extensive breeding of livestock on natural pastures, but this was gradually replaced by crop cultivation, starting mainly with soybeans and transitioning to horticulture, which currently accounts for about 6000 ha (Ferraris and Ferrero, 2018). The expanded use of greenhouses in the 1990s enabled vegetable production in seasons during which farming had previously not been feasible. The high productivity of horticultural crops depends on the intensive application of agrochemicals (Strassera et al., 2009); therefore, agrochemical utilization has also gradually increased. Horticulture employs larger amounts of agrochemical products per unit of area than conventional agriculture (Sarandón et al., 2015). For example, up to 62 different agrochemical products are registered for tomato production alone (Adlercreutz et al., 2014). Pesticides applied to crops may be transported by runoff to adjacent streams (Mugni et al., 2011; Stehle and Schulz, 2015), thus threatening the non-target resident fauna (Liess and Von der Ohe, 2005). Large-scale agrochemical applications therefore represent an environmental risk for the adjacent water bodies.

Wetlands in agricultural watersheds perform major ecological functions, providing diverse habitats and refuge for organisms such as macroinvertebrates, birds, and fish (Detenbeck et al., 1996). Riparian wetlands represent an interphase between terrestrial and aquatic environments by retaining eroded soil material (Lizotte et al., 2012) and associated contaminants (Johnson, 1986) coming from adjacent plots. Pesticide retention by wetlands in agricultural basins has been reported in the literature; Table 1 summarizes certain informative data.

Maillard et al. (2011) registered an overall pesticide retention ranging from 39 (simazine) to 100% (cymoxanil, glufosinate, kresoxim methyl, and terbuthylazine) in a storm-water constructed wetland within a vineyard catchment during the period of pesticide application. Milam et al. (2004) assessed methyl-parathion removal in a 40 m long constructed wetland during simulated storms and concluded that pesticides can be reduced to no effect concentrations even with short distances and residence times. Vymazal and Březinová (2015) reviewed the results from 47 studies on pesticide retention in constructed wetlands and concluded that constructed wetlands remove pesticide loads in agricultural areas, and, therefore, attenuate the agrochemical impact from surrounding crops. The authors, however, pointed out that retention efficiency varied widely among different wetlands.

The Argentine pampas is a vast plain occupying the central part of the country. The mild climate and fertile soils enabled its early development into the main agricultural region of the country. The abundant stream wetlands in the pampas have scarcely been studied. Consequently, a thorough evaluation of the multiple contributions of such areas to the ecosystem has not yet been achieved. The aim of this study was, therefore, to evaluate the attenuation of agrochemical impact by a natural wetland along a small first-order stream in a basin undergoing intense horticultural production. To that purpose, the insecticide concentrations in the sediment, the toxicity of the sediment and water, and the condition of the macroinvertebrate assemblages were compared both upstream and downstream from the wetland.

2. Materials and methods

2.1. Study site

The first-order Sauce Stream is located at the headwaters of the Pescado Stream basin (35° 1′ 31.87″ S; 57° 59′ 39.6″ W), within the horticultural belt around the city of La Plata (Fig. 1). The stream has formed a natural wetland roughly 150 m long and 2–10 m wide, surrounded by intensively cropped plots.

The wetland surface was completely covered by conspicuous vegetation. The upstream part of the wetland, occupying roughly 40% of the total surface, was covered by dense stands of the emergent macrophyte *Typha* sp. and had a depth of roughly 20 cm. The remaining part was slightly deeper (30 cm) and covered by a heterogeneous mixture of emergent (*Thypha* sp., *Scirpus* sp.), submerged (*Potamogeton* sp., *Ceratophyllum*, and *Egeria*), and floating (*Eichhornia crassipes*) vegetation. The bottom sediments were roughly 5–10 cm deep, with abundant organic-matter accumulation. Within the sediments, the silt fraction was dominant (44%), while clay and sand accounted for 32% and 24%, respectively. The stream was sampled during basal-flow conditions. The discharge was low (10–20 l/s, Mugni et al., 2011), the water volume roughly 300 m^3, and the estimated water-residence time 3.5 h.

The climate is mild and humid, with mean monthly temperatures ranging from 9.9 °C in July to 22.4 °C in January. The mean annual precipitation is roughly 1000 mm with the highest monthly rainfall in March (111 mm) and the lowest in June (63 mm; Hurtado et al., 2006).

Nine samplings were performed upstream and downstream from the wetland during three successive years (2017–2019), in summer, the period of largest agrochemical applications. The toxicity of the water and the bottom sediments, as well as the invertebrate composition were assessed on each sampling date. The pesticides in the sediments were analyzed during the second and the third years studied (2018–2019).

2.2. Environmental samples

The dissolved-oxygen concentration and temperature (YSI 51B), the conductivity (Hanna instruments 8733), and the pH (HI98108 HANNA) in the stream water were measured in situ upstream and downstream from the wetland. Water samples were collected in glass bottles and filtered through 1 μ pore-size glass-fiber Whatman GF/C filters and then carried in coolers to the laboratory.

Sediment samples were taken with a stainless-steel scoop from the top 2 cm and roughly 200 g wet weight placed in pesticide-free amber

Table 1
Pesticide retention in wetlands.

Wetland type	Length (m)	Pesticide assessed	Retention (%)	Reference
CW[a]	59–70	Chlorpyrifos	47–65	Moore et al., 2002
CW	50	Parathion	100	Schulz et al., 2003
Hydrologically managed floodplain	500	Atrazine, S-metolachlor, and Permethrin	85	Lizotte et al., 2012
Field-scale CW	134	Chlorpyfifos	100	Moore et al., 2002
Storm-water CW	26	Simazine	39	Maillard et al., 2011
		Cymoxanil, Glufosinate, Terbuthylazine	100	
CW	134	Chlorpyrifos; Endosulfan	100	Schulz and Peall, 2001
Field-scale CW	250	Pyretroids	52–94	Budd et al., 2009
		Chlorpyrifos	52–61	
CW	180	λ-Cyhalothrin; cyfluthrin	100	Moore et al., 2009
CW	40	Dimethoate, dicamba	91	Elsaesser et al., 2011

[a] CW, constructed wetland.

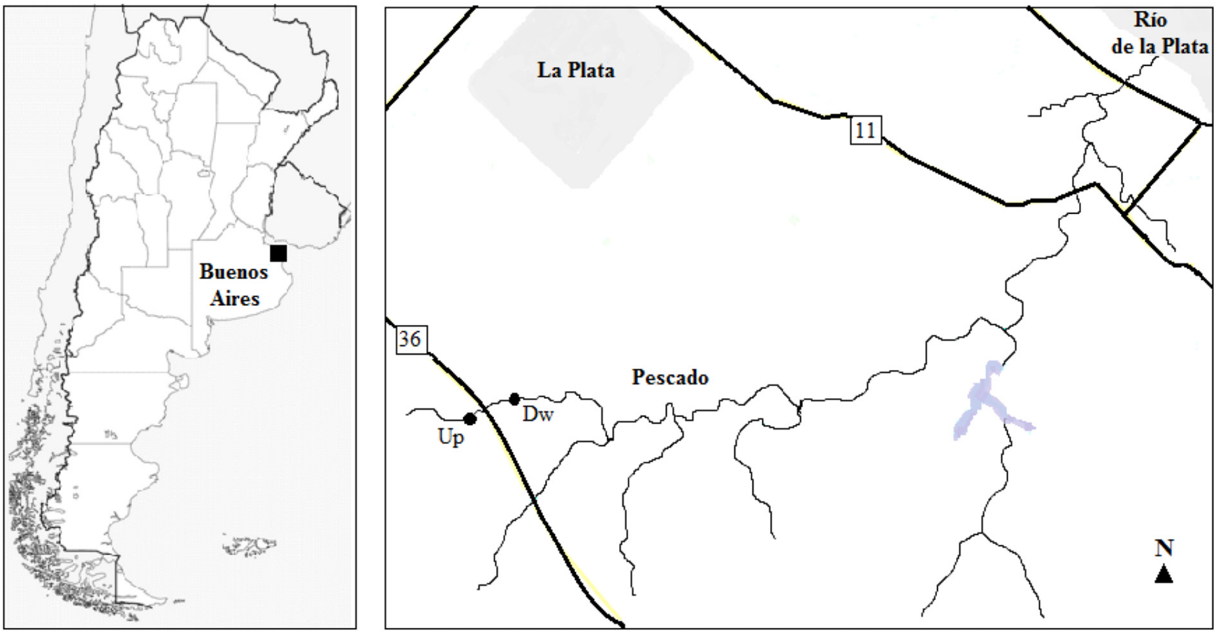

Fig. 1. Study area and sampling sites. The map to the left indicates the location of Buenos Aires within Argentina. In the map to the right depicting the Pescado Stream basin, Up and Dw indicate the sampling sites upstream and downstream, respectively, from the wetland.

glass jars with Teflon lids. The samples were kept on ice, within coolers, until arrival at the laboratory and thereafter stored under refrigeration until extraction (a maximum of five days later). The sediment samples were homogenized and divided to assay for moisture (24 h; 100 °C), organic-matter content (as total organic carbon; ignition loss at 400 °C), and pesticide. Sediment subsamples of about 20 g were extracted for pesticide analysis with a mixture of acetone and methylene chloride after You et al. (2004). A cleanup treatment was performed on the extracts by passage through Florisil solid-phase-extraction cartridges (EPA, 2014; Solis et al., 2017).

2.3. Pesticide analysis

The pesticides assessed were those previously reported at higher detection frequencies in the region (Hunt et al., 2016; Solis et al., 2017; Arias et al., 2020). Chlorpyrifos, endosulfan I, II, and sulfate; permethrin; λ-cyalothrin; cypermethrin; and deltamethrin were quantified by high resolution gas chromatography (HRGC HP 6890) with 30 m HP1 columns and a ^{63}Ni electron-capture detector. Two microliters were injected in splitless mode, with the detector being kept at 290 °C. The oven-temperature program started at 190 °C, followed by an increase of 10 °C min^{-1} up to 260 °C (maintained for 13 min), then 10 °C min^{-1} up to 290 °C (maintained for 7 min). Quantification was performed with pure standard pesticides (Accustandard; 4-point calibration curve) and tetrachloro-m-xylene (Ultra Scientific) as an internal standard. The detection limits (3:1 signal-versus-noise value) ranged from 0.35 ng/g sediment dry weight for chlorpyrifos, endosulfan I, II, and sulfate, to 1.0–3.0 ng/g for pyrethroids. The quantitation limits (10:1 signal-versus-noise value) ranged from 1.2 ng/g sediment dry weight for chlorpyrifos, endosulfan I, II, and sulfate, to 3.3–9.5 ng/g for pyrethroids. The procedural and instrumental blanks (one for every batch of twelve samples) had concentrations below the detection limits. The pesticide concentrations below the detection levels were treated as half the detection limits in the statistical analysis. The pesticide reduction downstream from the wetland was calculated as the mean concentration throughout the study at the downstream site expressed as a percent of the mean concentration at the upstream site (Budd et al., 2009).

2.4. Quality assurance and quality control

Method blanks and matrix spikes were assessed in triplicate for quality-control purposes. No pesticides were detected in any of the blank samples (with sodium sulfate being used as the blank matrix). Three 20 g wet-sediment samples were spiked with 1250 ng of chlorpyrifos, endosulfan, and pyrethroid insecticides and subsequently analyzed. The triplicate matrix spike had a minimum recovery of 70% and a maximum of 130% for the entire study, averaging a recovery of 93 to 110% for each of the pesticides analyzed (Table S1). Standard-deviation values lower than 21% were observed among the replicates of each pesticide. Therefore, the analytical method attained a complete recovery of the analytes at an acceptable level of precision (EPA, 2007).

2.5. Toxicity tests

The amphipods for toxicity testing were obtained from a stream located within the Parque Costero Sur biosphere reserve (Athor, 2009), roughly 70 km south of the wetland under study (35° 8′ 17.54″ S; 57° 23′ 57.74″; W). The stream had previously been studied by Solis et al. (2017), and the insecticide concentrations fell below the detection limits. The crustaceans were raised in the laboratory in glass aquaria containing stream water. Lettuce leaves were supplied as food to the aquaria ad libitum. The *Hyalella curvispina* were maintained at 20–22 °C under a natural photoperiod.

Toxicity tests were performed on water and sediment samples taken upstream and downstream from the wetland, following the method prescribed by USEPA (2000) and by Jergentz et al. (2004). The acute toxicity (mortality) in water and sediments and the sublethal toxicity (growth inhibition) in sediments were evaluated with the test organisms. For the water tests, ten specimens were exposed to 200 ml of stream water for 96 h without feeding. For the sediment tests, ten specimens were exposed, for ten days, to 20 g of sediment and 200 mL of dechlorinated tap water and fed with algae (*Chlorella* sp.) every two days. All the treatments were performed in triplicate under a natural photoperiod. A mortality lower than 10% in water and 20% in sediment were considered to indicate no effect (USEPA, 2000). The water-quality measurements were performed at the beginning and the end of the test.

The pH, temperature, conductivity, and dissolved-oxygen concentration recorded were 7.8 ± 0.3, 22 ± 2 °C, 1285 ± 5 μS/cm and 7.5 ± 0.6 mg/L, respectively. The length of the specimens—measured between the eye and the last segment of the urosome—was determined with the ImageJ 1.48v program at the beginning and end of each test. The organisms were previously anesthetized by exposure to a minimum amount CO_2 for easier manipulation, without causing any subsequent harm. The assays were performed with specimens selected by passing aquarium water containing *H. curvispina* through a 750 μm pore-size mesh. The amphipods that were retained in the mesh were used in the assays.

2.6. Macroinvertebrate sampling

Macroinvertebrates were sampled from the emergent vegetation by means of a D-net of 500-μm pore size and 30-cm diameter. At each site, three sweeps were collected, covering an area of 1 m^2 per sample. The samples were taken in triplicate and preserved with 70% aqueous ethanol containing erythrosine B and transported to the laboratory for sorting. All macroinvertebrates were later identified under a stereoscopic microscope. Taxa were identified down to the family or genus according to Dominguez and Fernandez (2009) and Merritt et al. (2008).

2.7. Statistical analysis

To evaluate differences in the insecticide concentrations measured upstream and downstream of the wetland, a paired Student *t*-test was performed. The assumption of normality was previously tested. In instances of noncompliance with the assumptions, the nonparametric equivalent test (Wilcoxon Signed Rank Test) was performed with the SigmaStat 3.2 program at a significance level of $p < 0.05$.

The growth of *H. curvispina* exposed to sediments sampled upstream and downstream in the wetland was compared by means of an analysis of covariance (ANCOVA). The data were previously transformed to log $(x + 1)$, the normality assessed by the Shapiro-Wilk test (Shapiro and Wilk, 1965) and the homogeneity of variances tested by Cochran's C test (Cochran, 1951). Eta^2 (η^2) was computed as a measurement of the effect size (Cohen, 1992), with a small effect being defined as $\eta^2 \leq 0.20$, a moderate effect as $0.20 < \eta^2 \leq 0.80$, and a large effect as $\eta^2 \geq 0.80$. The Student-Newman-Keuls (SNK) test was used as a *post-hoc* analysis when significant differences were found. The factors considered were Treatment (two levels: upstream and downstream) and Sampling Date (with nine levels); the dependent variable was Growth (mm). The initial length for each treatment was used as the covariate.

To assess differences in the acute-toxicity tests in water and sediment samples, a two-way ANOVA was performed as previously described. The factors were: Treatment (two levels: upstream and downstream) and Sampling Date (with nine levels). All analyses were conducted in R (3.5.2) in RStudio (1.2.5033) with the rstatix package.

To evaluate differences in the mean macroinvertebrate density upstream and downstream from the wetland, a paired Student *t*-test was performed as previously described. Macroinvertebrate-assemblage data were analyzed with the PRIMER version 5 multivariate statistical package (Clarke and Gorley, 2001). The data were transformed by a square-root function to reduce the contribution of the dominant groups and analyzed by means of the similarity index of Bray and Curtis (1957). The differences between the samples taken upstream and downstream from the wetland and between the 3 sampling periods (2017-2018-2019) were determined by means of the two-way similarity analysis (ANOSIM). The percentage of similarity (SIMPER) was calculated to assess the taxa that contributed the most to the differences in the assemblages among the sites (Clarke and Warwick 2001).

3. Results

3.1. Environmental variables

Table 2 summarizes the environmental variables measured in the stream. The dissolved oxygen concentrations ranged from 1.5 to 8.8 mg/l and was inversely correlated with the temperature (-0.76, $p < 0.05$). The conductivity ranged from 126 to 545 μS/cm and was inversely correlated with the amount of rainfall the week before each sampling (-0.62, $p < 0.1$). The pH of the water varied from slightly on the acidic side of neutrality to well within the alkaline range.

3.2. Insecticides

Table 3 lists the pesticide concentrations in the bottom sediments along with the wetland retention on each sampling date. Similar to that of the environmental parameters, a large variability was observed among samplings. The highest chlorpyrifos concentration upstream from the wetland (1605 ng/g dw) was measured on 22/03/2018, four days after a heavy rainfall of 42 mm, while the highest cypermethrin concentration (202 ng/g dw) was recorded on 17/01/2018, five days after rainfalls amounting 44 mm. Except endosulfan on 17/01/2018, the concentrations measured downstream from the wetland were always lower than those upstream. Chlorpyrifos and cypermethrin retention within the wetland were correlated with the amount of rainfall before each sampling (0.7, $p < 0.05$ and 0.56, $p < 0.1$, respectively). Chlorpyrifos and deltamethrin sediment concentrations were correlated with total organic carbon in the sediments (0.79, $p < 0.05$ and 0.62, $p < 0.1$).

Seven of the eight pesticides analyzed were detected in the bottom sediments upstream of the wetland, while only five downstream: λ-cyhalothrin and endosulfan II fell below the detection limits downstream (Table 4). The mean detection frequency of the pesticides assessed was also significantly lower downstream ($40 \pm 30\%$) than upstream ($60 \pm 29\%$).

Table 2
Environmental variables measured in the stream on each sampling date.

Date	Rainfall (mm)	Temp. (°C)	pH	DO (mg/l)	EC (μS/cm)	TOC (%)	Temp. (°C)	pH	DO (mg/l)	EC (μS/cm)	TOC (%)
24/1/2017	5	21.5	7.0	1.5	263	NA[a]	23.0	7.5	1.3	197	NA
26/10/2017	6	23.0	7.6	4.8	282	NA	21	7.5	5.8	269	NA
19/12/2017	40	19.0	7.1	2.0	185	NA	19.0	7.3	3.5	191	NA
17/1/2018	44	23.0	7.1	1.3	190	5.8	23.0	7.2	1.3	240	6.8
20/2/2018	7	23.0	7.4	1.8	545	7.1	22.5	7.3	2.8	344	7.2
22/3/2018	42	21.0	7.6	3.4	192	16.6	19.5	7.8	3.2	217	9.2
15/2/2019	29	20.5	8.3	8.8	260	12.3	20.5	7.35	8.8	417	14.9
14/3/2019	27	21.5	7.6	4.0	138	21.3	22.0	6.7	4.39	124	5.09
24/4/2019	1	14.5	8.9	3.5	410	NA	16.5	7.9	1.6	361	NA

[a] NA: not analyzed; DO: dissolved-oxygen concentration; TOC: total organic carbon; EC: electrical conductivity.

Table 3
Pesticide concentrations in sediment samples upstream (Up) and downstream (Dw) from the wetland and the estimated percent retention (R%) on each sampling date.

Date	17/1/2018			20/2/2018			22/3/2018			15/2/2019			14/3/2019			24/4/2019		
Pesticide (ng/g dry w)	Up	Dw	R (%)	Up	Dw	R (%)	Up	Dw	R (%)	Up	Dw	R (%)	Up	Dw	R (%)	Up	Dw	R (%)
Chlorpyrifos	ND[a]	ND	–	ND	ND	–	1605	23.0	98.6	63.5	14.5	77.1	24.0	5.00	79.2	16.2	11.2	30.6
Endosulfan I	ND	7.90	–	ND	ND	–	ND	ND	–	34.4	2.90	91.6	20.5	2.00	90.2	7.50	2.00	73.2
Endosulfan II	ND	ND	–	12.8	ND	100	21.6	ND	100	ND	ND	–	ND	ND	–	ND	ND	–
Endosulfan Sulphate	ND	ND	–	10.5	ND	100	81.5	ND	100	ND	ND	–	7.00	3.75	54	12.3	1.5	87
λ-Cyhalothrin	ND	ND	–	ND	ND	–	ND	ND	–	ND	ND	–	13.5	ND	100	ND	ND	–
Cypermethrin	202	ND	100	99.2	80	19.5	ND	ND	–	49.0	32.1	34.6	27.0	8.35	69.1	29.5	10.9	62.7
Deltamethrin	36	ND	100	47.0	ND	100	65,3	ND	100	170	55	67.7	54.0	18.3	66.2	65.9	8.60	86.9

[a] ND, not detected.

The concentrations of chlorpyrifos, endosulfan I, endosulfan sulfate, cypermethrin, and deltamethrin were significantly lower ($p < 0.05$) downstream than upstream from the wetland. The mean chlorpyrifos concentrations measured downstream represented 5% of the upstream concentrations, while total endosulfan and pyrethroids downstream were reduced to 15 and 33% of the respective upstream concentrations. Upon pooling all the pesticides together, the overall marsh retention was 81% of the upstream load. Only one of the pesticides assessed, the pyrethroid permethrin, was not detected at any time throughout the study. Endosulfan sulfate, a degradation product of endosulfan, was present upstream from the wetland in 4 out of 6 samplings. On two occasions endosulfan was not measured, while in the other 2, concentrations of endosulfan and endosulfan sulfate were similar. Endosulfan II was not detected at any time downstream of the wetland (Table 3). No significant differences among the various pesticide retentions were found.

3.3. Toxicity tests

Sublethal toxicity was detected in the sediments assayed (Fig. 2). No interaction was observed between the factors Treatment and Sampling Date (ANCOVA: $F = 0.519$, $df = 8$, $p = 0.834$), whereas significant differences were found between Treatments (ANCOVA: $F = 20.710$, $df = 1$, $p < 0.01$), with higher growth occurring downstream than upstream from the wetland. No significant differences were found in the growth between the sampling dates (ANCOVA: $F = 1.380$, $df = 8$, $p = 0.239$). A comparison of the Eta^2 values revealed a moderate effect of the factors Treatment and Sampling Date (37.2% and 24%, respectively) along with a small effect between the factors (10.6%).

A significant interaction between the factors Position (upstream and downstream) and the Sampling Date were observed in the acute-toxicity test in both the water and the sediment samples (ANOVA: $F = 5$, $df = 8$, $p < 0.05$; Fig. 3). The mortality upon exposure to both the sediment and the water was significantly lower downstream of the wetland ($p < 0.05$) in December 2017 and January and February 2018 (ANOVA: sediment $F = 4.39$, $df = 8$, $p = 0.001$; water $F = 4.52$, $df = 8$, $p = 0.001$). For January and February 2018, the mortality in the upstream site was coincident with high cypermethrin and deltamethrin concentrations, whereas a decreased mortality in the downstream site was corresponded to lower pyrethroid concentrations (Table 3).

3.4. Macroinvertebrate assemblage

A total of 3858 specimens were identified belonging to 32 taxa. The mean macroinvertebrate density was significantly higher downstream (738 ind/m^2) than upstream (561 ind/m^2) from the wetland. *Pomacea canaliculata* was the dominant taxon upstream, with its density being significantly higher there (182 ind/m^2) than downstream (27 ind/m^2; $p < 0.05$). *Hyalella curvispina* was the dominant taxon downstream from the wetland, likewise at a density significantly higher downstream (354 ind/m^2) than upstream (71 ind/m^2; $p < 0.05$). In the samplings in which acute toxicity was detected in the water and sediment upstream from the wetland (Fig. 3), *H. curvispina* was either absent at the site (February 18) or present only at low densities (12 and 3 ind/m^2 on Dec 17 and January 18, respectively).

The ANOSIM indicated that the macroinvertebrate composition was significantly different upstream and downstream from the wetland (Global $R = 0.519$, $p < 0.006$), with no significant differences being detected among the various sampling periods. The SIMPER analysis identified the taxa with the highest contribution to the assemblage composition for each sampling site (Table 5).

3.5. Discussion

The measured parameters exhibited an extended range of variation, for example the dissolved-oxygen concentration and conductivity readings. There are two explanations that clarify the origins of these high variations. First, the dominance of emergent macrophytes attaining a high biomass resulted in a large production of organic matter; increased temperatures enhanced organic-matter decomposition, causing the oxygen depletion that was measured in coincidence with the higher temperatures. Second, the inverse correlation between conductivity and the

Table 4
Detection frequency (Freq %), mean and standard deviation (SD), range of detectable values, and downstream percent reduction R (%) in the sediment insecticide concentrations (ng/g) upstream and downstream in the wetland.

	Upstream			Downstream			
	Freq %	Mean (SD)	Range	Freq%	Mean (SD)	Range	R (%)
Chlorpyrifos	70	187 (499)	13–1605	67	9.9 (9.5)	2.5–23	94.7
Endosulfan I	50	11.5 (13.5)	7.5–34.4	67	2.1 (2.5)	2.0–7.9	81.7
Endosulfan II	33	5.0 (7.3)	12.8–21.6	–	ND[a]	–	–
Endosulfan sulfate	67	17.0 (28.8)	5–81.5	30	1.57 (2.21)	1.0–6.5	90.7
λ-Cyhalothrin	17	4.7 (5,6)	6.8–20.2	–	ND	–	–
Cypermethrin	83	56.2 (57.5)	26–202	67	21.7 (25.7)	6.8–80	61.3
Deltamethrin	100	89.9 (68)	36–261	50	22.3 (31.9)	3.8–86	75.2

[a] ND: not detectable. Detection limits, 1.0–0.3 ng/g sediment dry weight for pyrethroids; 0.35 ng/g sediment dry weight for chlorpyrifos and endosulfan I, II, and sulfate.

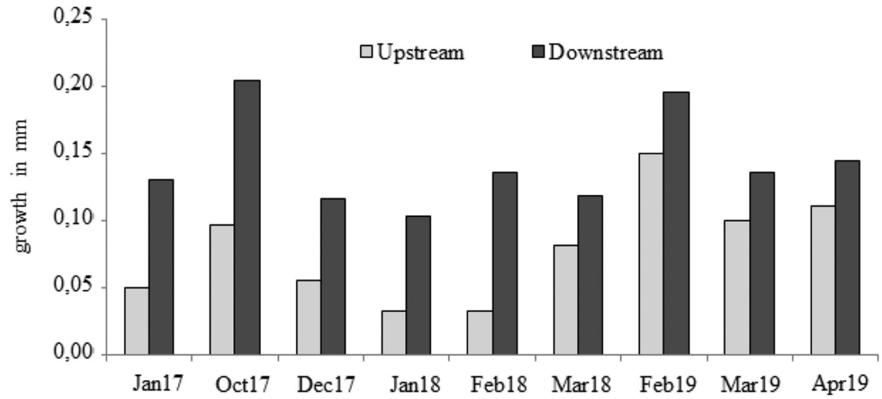

Fig. 2. Growth of *Hyalella curvispina* exposed to bottom sediments sampled upstream and downstream from the wetland.

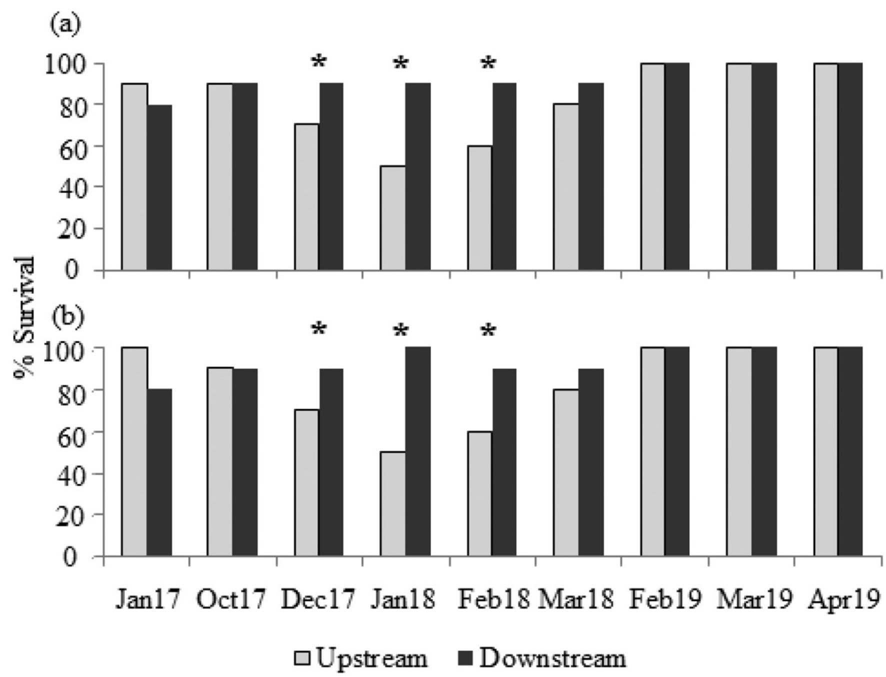

Fig. 3. *Hyalella curvispina* survival upon exposure to sediments (Panel a) and water (Panel b) upstream and downstream from the wetland. The asterisks represent significant differences between the upstream and the downstream values.

extent of rainfall the week before each sampling was produced by a dilution of the stream's ionic content by less mineralized runoff water.

The pesticides detected in the bottom sediments of the stream were those most commonly measured in streams draining horticulture basins in the area (Mac Loughlin et al., 2017; Arias et al., 2020), suggesting a contribution from the adjacent crops. The substantial variability in the pesticides measured upstream from the wetland reflects the variety of chemical applications to different crops in the farms within the basin.

Table 5
Taxa identified by the SIMPER analysis with the highest contribution to the assemblage composition at each sampling site.

	Upstream			Downstream	
	Contribution %	Cumulative %		Contribution %	Cumulative %
P. canaliculata	23.5	23.5	*H. curvispina*	33.8	33.8
H. curvispina	10.7	34.2	Dugesiidae	16.8	50.6
Belostoma	10.6	44.7	*P. Canaliculata*	10.0	60.6
Enochrus	10.2	54.9	Curculionidae	9.5	70.2
Hirudinea	9.4	64.3	Enochrus	8.2	78.3
Bidessini	8.3	72.6	*Psidium musculium*	8.0	86.4
Curculionidae	8.1	80.7	Hirudinea	4.1	90.4
Coenagrionidae	5.9	86.5			
Gundlachia	4.9	91.4			

Moreover, high peak concentrations were reported by Mugni et al. (2011) in coincidence with the first rain after a pesticide application to an adjacent plot in the same stream. Similarly, the correlation between cypermethrin and chlorpyrifos retention and the amount of rain previous to each sampling suggests an increased loading through runoff from adjacent crops. The concentrations of the pesticides measured were significantly lower downstream from the wetland, suggesting marsh removal.

Several environmental variables affected pesticide concentrations and retention within the wetland. The correlation of deltamethrin and chlorpyrifos with total organic carbon suggests an enhanced sorption of hydrophobic compounds when the organic-matter content in the bottom sediments increased. Accordingly, Budd et al. (2011) reported that the distribution of pyrethroids in 2 constructed wetlands in California (USA) was found to mimic the organic carbon distribution and became enriched in large particles that consisted of partially decomposed plant materials and in clay-sized particles.

Pesticide retention in wetlands has repeatedly been reported in the literature. Moore et al. (2002) assessed chlorpyrifos retention in constructed wetlands following a simulated runoff. The wetland cells measured 14 m × 59–73 m, and were vegetated with *Juncus effusus*, *Leersia* sp., and *Ludwigia* sp. Roughly 47–65% of the added chlorpyrifos was retained within the first 30–36 m of wetland. Vymazal and Březinová (2015) reviewed the literature on pesticide removal by constructed wetlands. They recognized that pesticide removal was highly variable; the highest removal was achieved for strongly hydrophobic pesticides, increasing as the K_{OC} increased. The authors also emphasized that dense vegetation enhanced the effectiveness of pesticide removal. Likewise, Tournebize et al. (2017) in a review of the literature on wetland retention reported that the efficiency usually ranged between 20 and 90% retention. The key parameters affecting retention capacity were the vegetation cover, water-resident time, and the K_{oc} of assessed pesticides.

In the present study, the reduced pesticide concentrations in the sediments downstream from the wetland coincided with a significant reduction in toxicity: roughly a fifth (19%) of the total pesticide concentrations in the bottom sediments (Table 4) resulted in almost a doubling of *H. curvispina* growth (Fig. 2) downstream from the wetland. A reduced toxicity by wetlands has also been reported in the literature. Lizotte et al. (2012) assessed the aqueous toxicity-mitigation capability of a hydrologically managed floodplain wetland following an experimental runoff spiked with a mixture of atrazine, S-metolachlor, and permethrin, using *Hyalella azteca* assays for toxicity assessment. The dominant vegetation was grasses (*Leersia* sp.), sedges (*Cyperus* sp., *Carex* sp.) and duckweed (*Lemnaceae* sp.). At 500 m from the inlet, the pesticides peaked within 48 h at <15% of the inlet peak concentrations. *Hyalella azteca* survival significantly decreased within 48 h of the input up to 300 m from the inlet in association with the permethrin concentrations.

In a similar work, Schulz et al. (2003) compared methylparathion toxicity to *H. azteca* in a simulated runoff in vegetated (90% cover of *Juncus effuses*) and unvegetated constructed wetland units (50 × 5 × 0.2 m). Methylparathion was detected throughout the non-vegetated wetland, whereas the pesticide was only transported halfway through the vegetated wetland. Consistently, *H. azteca* mortality was significantly lower in the vegetated wetland, decreasing with increasing distance from the inlet. Significant linear regressions of mortality versus distance from the pesticide inlet indicated that 44 m of vegetated and 111 m of nonvegetated wetland would reduce *H. azteca* mortality to lower than 5% of the initial value.

Additionally, Moore et al. (2002) studied the effect of a field-scale constructed wetland over a small affluent of the Lourens River, South Africa, in coincidence with a runoff. The wetland was 36 m wide and 134 m in length; 75% of the surface was covered, with 60% being *Typha capensis*, 10% *Juncus kraussii*, and 5% *Cyperus* sp. The chlorpyrifos concentration at the runoff peak decreased from 89 μg/kg at the inlet to undetectable at the outlet, while *Chironomus* sp. mortality was reduced from 46% at the inlet to 6% at the outlet. The wetland studied by Moore et al. (2002) had a similar length to that of the wetland described in the present study and the vegetation of both was comprised mainly of *Typha* sp. Likewise, chlorpyrifos was completely removed in the wetland studied by Moore et al. (2002) while attaining 95% retention in our study. Present evidence suggests that the wetland in our study reached a comparatively high toxicity reduction because the main pesticides utilized by the local farmers are highly hydrophobic and the wetland developed dense stands of the conspicuous emergent macrophyte *Typha* sp.; whose presence, in turn, resulted in increased organic matter abundance and sorption capability.

Amphipods are sensitive to pesticide exposure and have often been used as ecotoxicological models for risk assessment to nontarget invertebrate fauna (Borgmann et al., 1989). The amphipod *H. azteca,* commonly used for toxicity assessment in the USA (Lizotte et al., 2012; Schulz et al., 2003), is not present in South America (Gonzalez and Watling, 2002). Instead, *H. curvispina* has a wide area of distribution, covering vast agricultural areas in the southern countries of South America (Argentina, Brazil, and Uruguay; García et al., 2010). Commonly found at high densities in shallow vegetated watercourses, *H. curvispina* is sensitive to pesticides and has often been used as a test organism (Mugni et al., 2013; Solis et al., 2019). The present results demonstrating a growth impairment together with a lower abundance of *H. curvispina* upstream from the wetland, where pesticide concentrations were higher, pointed to the chronic effect of toxicity on *H. curvipina* abundance in the stream. Solis et al. (2018) compared the macroinvertebrate assemblage composition in streams draining intensively cultivated and extensive-livestock basins along with streams located within a biological reserve. In a manner similar to the present results, *P. canaliculata,* considered to be comparatively tolerant to pesticide exposure (Liess and Von der Ohe, 2005), was often the dominant taxon in the agricultural streams, where pesticide contamination was also recorded, whereas *H. curvispina*, being dominant in the reserve and livestock streams, was either absent or present only at low densities in the agricultural streams. The higher growth rates together with the dominance of *H. curvispina* downstream from the wetland emphasized the attenuation effect that pampean wetlands exert on the toxicity of upstream incoming pesticides.

4. Conclusion

The studied wetland removed pesticides, decreased toxicity, and enabled the downstream dominance of the sensitive invertebrate *H. curvispina*, commonly prevalent in the less impacted streams. Therefore, riparian environments in the pampean streams represent a valuable resource for attenuating horticulture contamination.

The Argentine pampean streams contain abundant riparian and floodplain habitats, but in recent decades those environments have been progressively drained to increase the surface available for crop cultivation, causing a deterioration in water quality. Current research emphasizes the usefulness of wetlands for the mitigation of environmental contamination from pesticides and points to the need for both the preservation and restoration of riparian habitats. Landowners and government agencies should incorporate this information into water-management strategies for improving the attenuation of contaminants in runoff water from the surrounding cultivated fields.

CRediT authorship contribution statement

M. Solis: Investigation, Formal analysis, Writing – original draft. **N. Cappelletti:** Investigation, Formal analysis. **C. Bonetto:** Conceptualization, Writing – review & editing, Funding acquisition, Supervision. **M. Franco:** Investigation. **S. Fanelli:** Investigation. **J. Amalvy:** Resources. **H. Mugni:** Investigation, Writing – review & editing, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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