

Sublethal effects on *Simocephalus vetulus* (Cladocera: Daphnidae) of pulse exposures of cypermethrin

Marina Arias*, Carlos Bonetto, Hernán Mugni

Instituto de Limnología "Dr. Raúl A. Ringuet" (ILPLA), UNLP-CONICET-FCNyM, Boulevard 120 y 62, 1900, La Plata, Buenos Aires, Argentina

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ABSTRACT

Pyrethroids are among the most widely applied insecticides worldwide and cypermethrin is the pyrethroid most used in Argentina. Pesticides used in crops can reach adjacent watercourses through runoff and may lead to non-target fauna receiving toxic pulse exposures. The aim of this study was to determine the effects of cypermethrin pulse exposures on the widely distributed crustacean *Simocephalus vetulus*. The 48h-LC50 of cypermethrin for *S. vetulus* was determined at $0.18 \pm 0.09 \mu\text{g/L}$. To assess the effects of cypermethrin under environmentally realistic exposures, two experiments were performed. In the first one, specimens were exposed for 90 min to cypermethrin at 0.02 (T1), 0.2 (T2) and 1 $\mu\text{g/L}$ (T3), transferred to clean water and monitored for 24 h as regards survival and feeding rates; specimens exposed to T2 and T3 concentrations showed significant lower feeding rates than those in the control group. In the second experiment, specimens were exposed for 90 min every 7 days and monitored over 25 days; *S. vetulus* showed lower cumulative fecundity and reproduction rates at all concentrations tested, and lower population growth at the highest concentration. All exposure concentrations lay within reported environmental concentrations and risk assessment indicated risk ($RQ > 1$), suggesting that sensitive species would be affected by such pulse exposures of cypermethrin. The present study thus suggests that ongoing agricultural practices affect the non-target invertebrates in streams adjacent to crops.

1. Introduction

South America is the main soybean farming region in the world, Brazil and Argentina together being responsible for the 49% of global production (Oliveira and Hecht, 2016). The implementation of an intensive system based on monoculture, genetically modified seeds, no-till farming and intense agrochemicals usage led to a rise in crop production from 32 to 100 million tons between the 1970s and 2018 (MA, 2019). Correspondingly, insecticide consumption rose from 39,000 tons in 1991 (Moltoni, 2012) to 336,000 tn in 2011 (CASAFE, 2013).

Pyrethroids have gradually replaced the highly persistent organochlorine and organophosphate pesticides for growing and stored crops, in veterinary medicine, and in vector control, becoming one of the most used types of insecticides worldwide (Xiao et al., 2012). In Argentina, cypermethrin is the most widely used pyrethroid (CASAFE, 2013). Being highly hydrophobic ($K_{ow} = 6.3$) it is largely adsorbed to sediments (Maund et al., 2002; Yang et al., 2006), resulting in its fast disappearance from the water (Knauer et al., 2017; Mugni et al., 2011). Despite its low persistence in the environment (Schäfer et al., 2011), there is rising concern about contamination by pyrethroids in freshwater systems, particularly its toxicity to non-target fauna (Loetti and

Belloq, 2017; Macagnan et al., 2017; Subrero et al., 2019) and its bioaccumulation risks (Arisekar et al., 2019; Corcellas et al., 2015; Riaz et al., 2018).

Insecticides reach watercourses by surface runoff produced by rains following applications in adjacent plots (Jergentz et al., 2005; Mugni et al., 2011; Schulz, 2004). A wide range of cypermethrin concentrations in the regional aquatic systems have been reported, ranging from 0.05 $\mu\text{g/L}$ (Jergentz et al., 2005) to 6.6 $\mu\text{g/L}$ (Etchegoyen et al., 2013) in water, and from 0.57 to 221 $\mu\text{g/kg}$ in sediments (Etchegoyen et al., 2013). Insecticide non-point sources reaching streams might represent a risk to non-target fauna.

The toxic effects of pyrethroids have mainly been studied as regards continuous exposures (Day and Kaushik, 1987; Kim et al., 2008; Martínez-Jerónimo et al., 2013; Toumi et al., 2013). However, it has been proposed that pesticides enter into streams in pulses (Richards and Baker, 1993; Liess et al., 1999) resulting in ephemeral peak exposure concentrations reaching non-target species. Thus, setting up pulse exposures followed by non-exposure periods along the species' life cycles could be an environmentally realistic experimental design.

Daphnids (Cladocera: Daphniidae) are common organisms used in toxicity tests to assess the effects of pesticides on non-target aquatic

* Corresponding author. 5 N1672 2D, 1900, La Plata, Buenos Aires, Argentina.
E-mail address: arias@ilpla.edu.ar (M. Arias).

fauna. Among them, *Daphnia magna* Straus 1820 is the most extensively used species in international water quality protocols (OECD, 2000; ISO, 2012; USEPA, 2016). Nevertheless, it does not occur naturally in South America (Hebert, 1978). On the other hand, the cladoceran *Simocephalus vetulus* (Müller 1776) is commonly present in Argentina (Paggi, 1995); it has been used as a model organism in toxicity tests (Chen et al., 2004; Olvera-Hernández et al., 2004; Schroer et al., 2004; Willis et al., 1995; Wu et al., 2007). Juárez and Villagra de Gamundi (2007) and Reno et al. (2014) used *S. vetulus* as model organism in Argentina to assess the toxicity of lindane and glyphosate, respectively. However, experiments assessing the sublethal effects of pyrethroids on *S. vetulus* after brief exposures to reported field concentrations haven't previously been reported in the country.

The aim of the present study was to determine the effects of pulse exposures of cypermethrin on *S. vetulus* in order to improve the assessment of the impact of insecticides on non-target freshwater fauna under realistic environmental scenarios.

2. Materials and methods

2.1. Test chemical

Cypermethrin (C₂₂H₁₉Cl₂NO₃) or (RS)-alpha-cyano-3-phenoxylbenzyl-(1RS,3RS,1RS,3SR)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate (IUPAC) is the active substance in the Galgotrin® (25%) formulation marketed by Chemotécnica S. A. (<https://www.chemotecnica.com/>). Stock solutions were prepared by dissolving the insecticide in 100 ml analytical grade acetone (Baker) to achieve a 1000 µg/L concentration stock solution.

2.2. Test organism

Simocephalus vetulus was obtained from the Sin Nombre stream (35°02'26"S; 57°42'39.5"W) and grown in the laboratory. The sampling site is surrounded by extensive livestock-raising fields over natural pastures. Arias et al. (2020) compared the invertebrate fauna in four streams draining horticultural basins with another four regarded as less disturbed: two of the latter are located in a Biosphere Reserve and the other two, including the Sin Nombre stream, in extensive livestock-raising basins. The less-disturbed streams exhibited significantly higher taxa richness and density. *Simocephalus vetulus* and *Hyaella sp.* were dominant in the less-disturbed streams while being absent or rare in the horticultural streams.

Specimens were raised in 3 L glass beakers with dechlorinated tap water, at 22 °C ± 1 °C and with a natural photoperiod. The medium was renewed weekly. The specimens were fed *ad libitum* with *Chlorella vulgaris* grown at 22 °C ± 2 °C in a Bold medium with a 24:0 light/dark photoperiod and constant aeration to avoid precipitation. The neonates utilized in the experiments belong to the F3 or F4 cultured generations from the wild-caught *S. vetulus*.

2.3. Acute toxicity

Acute tests were conducted following the test guidelines for daphnids (USEPA, 2016) with slight modifications. The suggested reconstituted water was replaced by dechlorinated tap water, the same as used in growing the specimens. Test volume was 30 ml, following Chen et al. (2004). Ten laboratory-grown neonates < 24 h were exposed to five different cypermethrin concentrations and a control solution, in triplicate, in 100 ml-glass beakers. Exposure concentrations (0.05, 0.1, 0.2, 0.4 and 0.8 µg/L) were prepared by diluting the stock solution in dechlorinated tap water. A range-finding test was previously carried out to assay an appropriate concentration range. Controls were prepared by adding acetone at 0.08% (v/v), the same as added in the highest exposure concentration. Toxicity to acetone was further assessed to ensure the survival of *S. vetulus* in exposure solutions; five neonates were

exposed to solutions containing 1, 10 and 100 µg/L of technical grade acetone (Baker) for 48 h. No mortality was registered.

The test was repeated on three independent occasions. Mortality was recorded after 48 h of exposure. Specimens were considered dead when no movement was observed after being gently stirred with a Pasteur pipette for 10 s. Experimental conditions were temperature 22 °C ± 2 °C and natural photoperiod (13:11 light/dark). Individuals were not fed during the exposure.

2.4. Pulse exposures

Two experimental designs were assayed using 3 and 5 day-old specimens. The exposure period was 90 min and three concentrations were established in proportion to the estimated 48h-LC50: 0.1, 1 and 5 times the LC50 of cypermethrin. All exposures were carried out in glass beakers with 30 ml of dechlorinated tap water and controls were conducted by exposing specimens to acetone at 0.33% (v/v), the same as used in the highest exposure concentration. Water samples from the two highest concentrations were taken at the beginning of the exposure for analytical determinations. After the 90 min exposure, specimens were transferred first to petri dishes with dechlorinated tap water, then to clean beakers, using a Pasteur pipette, and monitored during the post-exposure period. Experimental conditions were: temperature 22 °C ± 2 °C, natural photoperiod (13:11 light/dark).

2.4.1. Effect on individuals: feeding rate

Five *S. vetulus* specimens, aged 5 days, were exposed to each assay concentration, with four replicates. After exposure, the specimens were transferred to 30 ml of dechlorinated tap water supplied with 0.5 × 10⁶ cel/ml of *C. vulgaris*. Additional blank treatment (without *S. vetulus* specimens) was performed in order to assess algae concentration along the experiment (Barata et al., 2008). Survival of specimens was monitored over the course of 24 h. Algae concentration was determined at 0 h (initial concentration), 3 and 24 h following exposure, in order to determine the effect of a single brief exposure on post-exposure feeding depression (Mc William and Baird, 2002). Algal cells were counted in a 1 ml aliquot in a Neubauer counting chamber. Individual feeding rates were calculated as the number of algal cells ingested per specimen per hour at 3 and 24 h following exposure.

2.4.2. Effect on populations: life table parameters

Ten 3-day-old *S. vetulus* specimens were exposed during 90 min to the above-mentioned cypermethrin pulse exposure concentrations, in triplicate. After exposure, the specimens were transferred to 30 ml of dechlorinated tap water supplied with 0.5 × 10⁶ cel/ml of *C. vulgaris*. The number of adult females (n) and neonates (m) was recorded daily and neonates were removed after being counted to maintain the initial number of adults (n = 10). Specimens were exposed every 7 days and a count always preceded an exposure. The medium with dechlorinated tap water plus *C. vulgaris* was renewed daily after counting, to ensure the oxygen and food supply. Recorded numbers of adults and neonates were used to obtain the proportion of specimens that survived each day (i.e. survival) and the mean number of neonates per surviving specimen (i.e. age-specific fecundity). These data were used to calculate the life expectancy (ex), gross and net reproduction rate (GR and R0, respectively), and generation time (G) (Pianka, 1988; Begon et al., 2006). Cumulative fecundity was calculated as follows: $CF = \sum_{x=0}^{\infty} m(x) + m(x-1)$ where m(x) is the number of neonates and m(x-1) the neonates of the previous brood. Finally, population growth was estimated by the equation (Lotka 1913) $1 = \sum_{x=0}^{\infty} l_x \cdot m_x \cdot e^{-rx}$ where e is the base of the natural logarithm and r is the intrinsic rate of population increase.

2.5. Analytical determinations

Water samples were extracted following You et al. (2004) with

acetone and methylene chloride. The extracts were dried under a stream of nitrogen and suspended to 0.5 mL in hexane. A Hewlett Packard HP 6890 gas chromatograph, equipped with a microelectron-capture detector (μ ECD) and an HP1 analytical column (30 m, 0.25 mm inner diameter, 0.25 μ m film thickness), was used for analytical determination of cypermethrin (detector temperature: 320 °C, oven temperature program: 190–250 °C; injector temperature 250 °C; injection volume: 2 μ L; gas carrier: N_2). The injection was in pulsed splitless mode. Reference standards were from Accustandard® (purity > 99%) and were prepared in methanol. The quantitation limit was based at the lowest concentration in the calibration standard and the detection limit was 0.025 μ g/L.

2.6. Data analysis

The median lethal concentration (LC50) and the corresponding 95% confidence limit were calculated by the Probit Analysis program, version 1.5 (USEPA, 1999). The sensitivity of *S. vetulus* to cypermethrin was compared with that of other crustacean species by means of the Species Sensitivity Distribution (SSD), by fitting the 48h-LC50-laboratory toxicity test for crustaceans in a linearized log-normal distribution using the CADDIS Species Sensitivity Distribution Generator v.1 (USEPA, 2015). The LC50s of different crustacean species were obtained from the ECOTOX database (ECOTOX, 2000) and from reported values in published articles. A mean value was calculated when more than one lethal concentration was reported for the same species (USEPA, 2015).

In the feeding experiment, the algae concentration in the controls and in the blanks after 3 and 24 h were compared by means of Student T-tests to assess the feeding rates of *S. vetulus*. Estimated feeding rates were analyzed by One-Way ANOVA or Kruskal-Wallis whenever normality or homocedasticity were not achieved. Dunnett's *post hoc* test was then applied when significant differences were found. Survival and fecundity were assessed by Repeated Measures ANOVA to analyze the effect of pulse exposures (factor: treatments) over time (factor: time) and the interaction. Sphericity was analyzed with the Mauchly test and the Bonferroni *post hoc* test was carried out when significant differences were observed. Life table parameters were analyzed with One-Way ANOVA and Dunnett's *post hoc* test. All statistical analyses were performed with SigmaPlot 1.2, except RM-ANOVA, performed with STATISTICA version 7. Significant differences were established at a significance level of $p < 0.05$.

The environmental risk of cypermethrin for *S. vetulus* was estimated by the Risk Quotient (RQ) (USEPA, 1998). The risk quotient relates an environmental exposure concentration of a chemical to the toxicity test effect level (e.g. LC₅₀). RQs were calculated for acute risk dividing the highest measured concentrations in regional streams to the obtained 48h-LC50 of cypermethrin for *S. vetulus*, referred to as RQ_{ac} hereinafter. The Risk Quotient was also calculated using the lowest concentration at which effects were recorded in the sublethal pulse experiments, and termed RQ_{sub} hereinafter; whenever $RQ > 1$, toxic effects are expected. Moreover, the frequencies at which reported cypermethrin concentrations exceeded the 48h-LC50 for *S. vetulus* were also calculated.

3. Results and discussion

3.1. Acute toxicity and sensitivity comparison

Mean registered mortalities of *S. vetulus* after 48 h cypermethrin exposure are shown in Fig. 1. The mean determined 48h-LC50 of cypermethrin for *S. vetulus* in three independent assays was 0.18 ± 0.09 μ g/L. Lethal concentrations of several insecticides for *S. vetulus* have been reported, such as chlorpyrifos (van Wijngaarden et al., 1993), carbaryl, methomyl (Mano et al., 2010), malathion (Olvera-Hernández et al., 2004) and lambda-cyhalothrin (Schroer et al.,

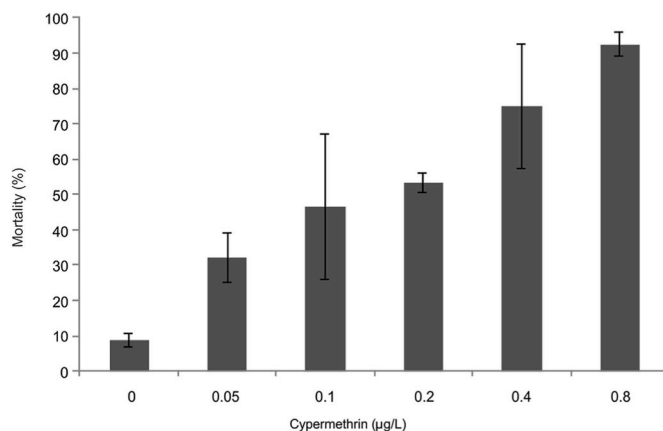


Fig. 1. Mean mortalities (%) and standard deviation of *S. vetulus* 48 h after exposure to different cypermethrin concentrations.

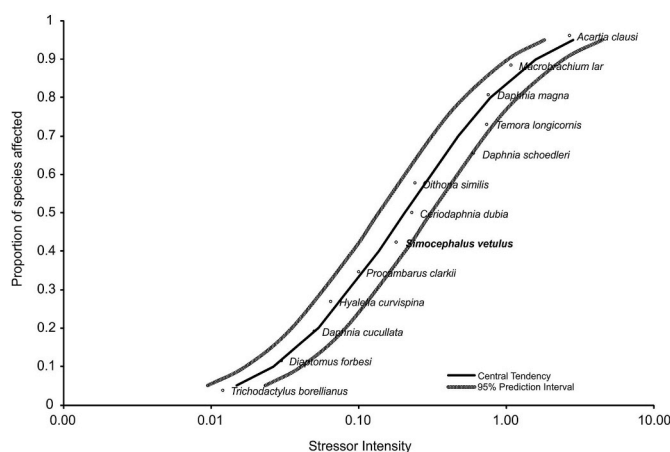


Fig. 2. Sensitivity distribution of crustaceans to cypermethrin obtained with the CADDIS species Sensitivity Distribution Generator v.1. Only data on the 48h-LC50 laboratory toxicity test were compared. Reported data are available in the Supplementary Material.

2004). To our knowledge, the lethal concentration of cypermethrin for *S. vetulus* had not been previously reported.

The sensitivity distribution of crustaceans to cypermethrin is shown in Fig. 2. Selected data from the ECOTOX database and published articles on different crustacean species are detailed in the Supplementary Material. Twelve species were reported under the same experimental conditions. Present results show that *S. vetulus* is sensitive to cypermethrin; the latter's lethal concentration lies roughly at midpoint in the reported range for other crustacean species and *S. vetulus* could thus be useful as a sentinel organism for cypermethrin contamination in streams.

3.2. Feeding rates

Nominal concentrations of cypermethrin in the pulse exposure experiments were 0.02, 0.2 and 1 μ g/L. Measured cypermethrin concentrations of test solutions T2 and T3 were 0.19 μ g/L and 0.86 μ g/L, respectively. The initial algae concentration was determined at 4.35×10^6 cell/ml. *Simocephalus vetulus* survival was 100% for all treatments and the control. Algae concentrations in the controls after 3 and 24 h were significantly lower than in the blanks ($p = 0.003$ and $p < 0.001$, respectively), indicating the effect of active *S. vetulus* feeding and allowing calculation of the feeding rates. Significant differences were determined in the individual feeding rate 3 and 24 h following exposure ($p = 0.009$ and $p < 0.001$, respectively). Three

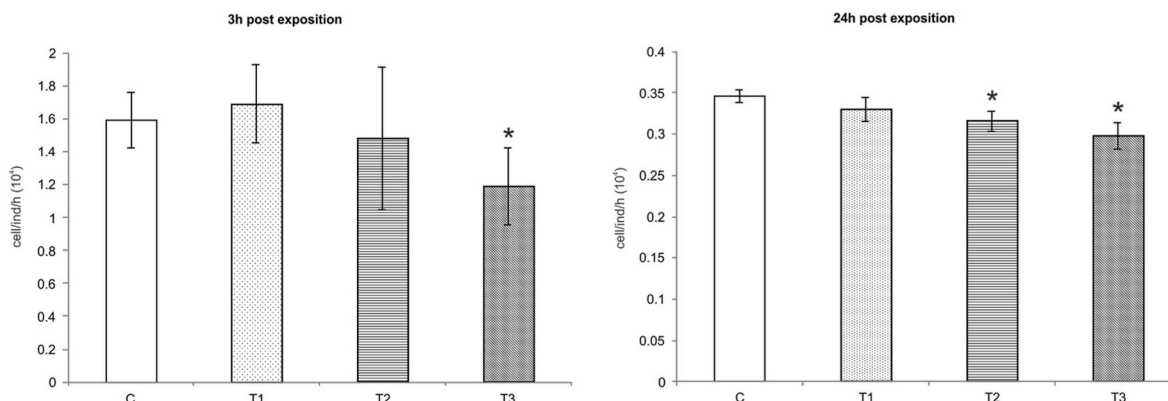


Fig. 3. Feeding rates of individuals per treatment at 3 and 24 h post-exposure. Mean values are shown with bars with their correspondent standard deviation. C: Control (acetone 30 µg/L); T1: Treatment 1 (0.01 µg/L); T2: Treatment 2 (0.2 µg/L); T3: Treatment 3 (1 µg/L). (*) indicates significant differences with respect to control (Dunnet's test, $\alpha < 0.05$).

hours after exposure, specimens in T3 showed a 25% reduction in the feeding rate compared to control; 24 h following exposure, T2 showed a 9% and T3 a 14% reduction in the feeding rate compared to control (Fig. 3).

Simocephalus vetulus tolerates ephemeral exposures to comparatively high cypermethrin concentrations without mortality, higher than the 48h-LC50. However, sublethal effects were observed after a 90 min pulse exposure. Decreased feeding activity after pyrethroid exposure has been reported for other invertebrate species (Rasmussen et al., 2013). Twenty-four hour exposure of *D. magna* at concentrations 0.1–0.5 times the LC50 of permethrin and 0.35–0.75 times the LC50 of lambda-cyhalothrin resulted in reduced feeding rates (Mc William and Baird, 2002). Christensen et al. (2005) reported reduced content of chlorophyll pigments ingested by *D. magna* exposed to cypermethrin concentrations of 0.1–1 µg/L. Barata et al. (2008) proposed the 24 h feeding inhibition bioassay as a rapid and sensitive biomonitoring tool for assessing pesticide contamination.

3.3. Life table parameters

The effects on the different measured life table parameters at the end of the experiment are reported in Table 2. From an overall perspective, all measured parameters decreased at the higher pulse exposure concentrations. Survival showed significant differences as regards time and the interaction between factors (RM-ANOVA: $p < 0.001$, Table 1). The Bonferroni *post hoc* test indicated that survival for all treatments was significantly different from the control from day 17 onward; that is, after the third pulse exposure. Fecundity showed significant differences in interaction and both factors (RM-ANOVA: $p < 0.001$, Table 1), and the Bonferroni test indicated that all treatments resulted in differences with control throughout the experiment.

One-Way ANOVA showed significant differences with respect to

Table 1
Statistical analysis of survival and fecundity by Repeated Measures ANOVA.

	DF	Mean square	F-ratio	p-value
Survival				
Treatments (A)	3	0.22	1.53	0.28
Time (B)	25	1.01	118.76	< 0.001
A*B	75	0.05	6.31	< 0.001
Fecundity				
Treatments (A)	3	57.81	21.31	< 0.001
Time (B)	20	37.53	5.86	< 0.001
A*B	60	17.39	2.72	< 0.001

DF: degrees of freedom.

control in cumulative fecundity ($p = 0.001$), net reproduction rate ($p = 0.005$) and population growth ($p = 0.032$) (Table 2). Dunnet's *post hoc* test showed all treatments were different from control in cumulative fecundity and net reproduction rate, while population growth was different from control only at the highest concentration.

Fig. 4 shows the cumulative fecundity per treatment over the course of the experiment. During the first 15 days, control and treatments showed a similar trend. However, after the third exposure, the number of neonates per treatment became asymptotic while continuing to increase in the control. Also, the number of neonates was lower when the pulse exposure concentration was higher. The net reproduction rate showed the same trend, being lower when the concentration of cypermethrin was higher.

Repeated 90 min pulse exposures to 0.02–1 µg/L of cypermethrin (0.1–5 times the 48h-LC50) resulted in sublethal effects on the reproductive ability of *S. vetulus*. Toxicity pulses reduced the *S. vetulus* net reproduction rate at all tested concentrations, while population growth was significantly reduced at the highest concentration. Kim et al. (2008) reported delayed offspring release and smaller brood size and number in *D. magna* neonates and juveniles exposed to 0.0002, 0.002 and 0.2 µg/L of cypermethrin for 21 days (48h-LC50 being 0.1 µg/L). Shen et al. (2012) reported reduced reproduction and growth of *C. dubia* exposed 8 days to 0.1 and 0.25 µg/L, representing 0.12 and 0.25 of the 48h-LC50 of cypermethrin, respectively. Continuous exposure of *D. schoedleri* for 21 days to 5.4 and 54 ng/L of cypermethrin (48h-LC50 being 600 ng/L) caused decreased lifespan, life expectancy, generation time and net reproduction, and at the highest concentration also decreased population growth (Martínez-Jerónimo et al., 2013). These studies reported the effect of long continuous exposures that are not likely to happen in the field. The present study determined sublethal effects of more realistic short pulse exposures. Similarly to this study, Cold and Forbes (2004) reported effects on reproductive parameters when *Gammarus pulex* was exposed to a single 60-min pulse exposure to the pyrethroid esfenvalerate at concentrations of 0.05–2 µg/L; disruption of reproducing pairs, release of eggs and, similarly to our results, a decreased number of offspring per female were reported. Rasmussen et al. (2012) determined that 60-min exposures to 0.1 and 1 µg/L of cypermethrin did not produce mortality but reduced the shredding activity of *Gammarus pulex* (24h-LC50: 0.1 µg/L; Stephenson, 1982) and *Halesus radiatus* (LC50 not reported). Pyrethroids exposure implies a redistribution of metabolic energy towards detoxification (Friberg-Jensen et al., 2010; Maltby, 1999), leading to lower energy availability for feeding and reproduction (Tripathi and Singh, 2004; Zubrod et al., 2011). The reduction in feeding efficiency alone also has consequences for the energy balance and for reproduction (Agatz et al., 2013; Barata et al., 2002; Guisande and Gliwicz, 1992; Urabe, 1991). Both mechanisms might explain the observed reduction in the fecundity of

Table 2
Life table parameters and one-way ANOVA results.

	Life expectancy	Gross reproduction rate	Net reproduction rate (R0)	Generation time	Cumulated fecundity	Population growth (r)
Control	16.8 ± 2.5	105.4 ± 10.9	57.3 ± 9.8	16.4 ± 0.4	565 ± 98	0.35 ± 0.02
T1	15.3 ± 0.4	85.3 ± 31.3	33.4 ± 5 (*)	13.9 ± 1.8	297 ± 20 (*)	0.32 ± 0.01
T2	14.3 ± 2.3	82.6 ± 34.1	28.8 ± 7.5 (*)	14.1 ± 2.8	253 ± 32 (*)	0.30 ± 0.02
T3	14.3 ± 2.7	86.3 ± 35.1	25.1 ± 8.7 (*)	14.5 ± 2.3	220 ± 61 (*)	0.28 ± 0.03 (*)
DF	3	3	3	3	3	3
MS	4.19	328.91	631.99	3.83	247622	0.002
F	0.87	0.38	9.92	0.92	67.16	4.93
p	0.49	0.77	0.005	0.47	< 0.001	0.032

Bold letters indicate parameters showing significant differences ($p < 0.05$). (*) indicates significant differences to control.

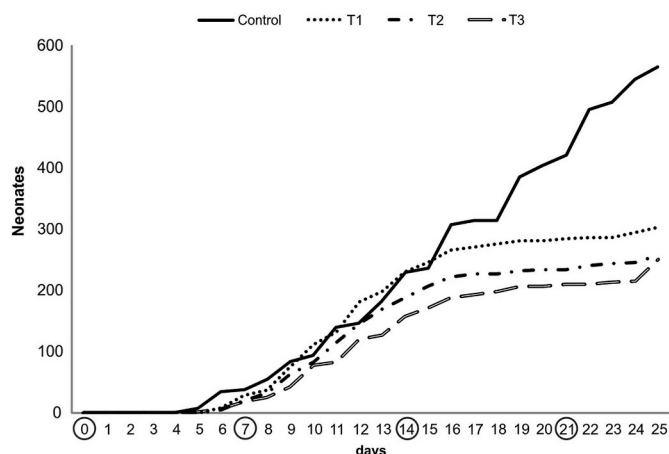


Fig. 4. Cumulative fecundity (aggregate of neonates per day) of *S. vetulus* for each treatment over the course of the experiment. Circles show the successive pulse exposures. C: Control; T1: Treatment 1 (0.01 µg/L); T2: Treatment 2 (0.2 µg/L); T3: Treatment 3 (1 µg/L).

exposed organisms.

3.4. Environmental risk assessment

Cypermethrin is frequently detected in streams draining intensively cultivated basins within the Pampas, the main agricultural region of Argentina. Jergentz et al. (2005) reported 0.05–0.7 µg/L in a first order stream running through soybean plots. Demetrio (2012) reported 0.3–1.2 µg/L at El Sauce stream, a first order stream close to La Plata city running through a large cultivated plot. Etchegoyen et al. (2013) reported pesticide concentrations in the tributaries of the Paraná River, the second largest basin in South America, an intensively cultivated area; cypermethrin was detected in 100% of water samples at concentrations in the 0.01–6.6 µg/L range.

The risk quotients for acute (RQ_{ac}) and sublethal (RQ_{sub}) toxicity were calculated considering the highest reported concentrations of cypermethrin in streams draining agricultural basins in Buenos Aires province, Argentina (Table 3). The obtained RQs were always higher than 1, ranging from 3.9 to 37 for acute and 35–330 for sublethal

Table 3
Risk assessment by Risk Quotients.

Reference	Stream	HMC (µg/L)	RQ_{ac} (HMC/LC50)	RQ_{sub} (HMC/LCP)	Samples > DL	Samples > LC	Frequency of samples > LC50 (%)
Jergentz et al. (2005)	Brown	0.7	3.9	35	10	3	30
Demetrio (2012)	El Sauce	1.2	7	60	23	6	26
Etchegoyen et al. (2013)	Paraná	6.6	37	330	37	14	38

HMC: Highest measured concentration; RQ_{ac} : risk quotient based on the 48h-LC50 for *S. vetulus*; RQ_{sub} : risk quotient based on the lowest concentration at which effects were determined in pulse exposure experiments (LCP) (i.e.: 0.02 µg/L); Samples > DL: number of samples with concentrations above the detection limit; Samples > LC: number of samples with concentrations exceeding the 48h-LC50 for *S. vetulus*; Frequency of samples > LC50 (%): percentage of samples with cypermethrin concentrations above the 48h-LC50 of *S. vetulus*.

effects. Furthermore, 26–38% of the reported concentrations exceeded the 48h-LC50 of *S. vetulus* and almost all reported concentrations exceeded the concentrations causing sublethal toxicity determined in the present study.

High calculated risk quotients indicate a hazard to *S. vetulus* and other sensitive non-target fauna. Roughly 30% of reported concentrations exceeded the 48h-LC50 for *S. vetulus* in the reviewed research, suggesting that acute effects are likely to happen in the studied streams. Furthermore, even brief exposures at concentrations lower than 48h-LC50 impaired reproduction, with a potential effect on the population level; risk estimations for the lowest sublethal-effect concentration determined in the present study (0.02 µg/L) resulted in remarkably high RQ values (35–330), thus indicating that cypermethrin exposure has substantial effects on the resident invertebrate fauna.

Cladocerans are non-selective filterers (De Bernardi et al., 1987) and play an important role in the food web, feeding on phytoplankton (Balayla and Moss, 2004; Lair, 1991) as well as being prey for macro-invertebrates (Gonzalez Sagrario et al., 2009; Lancaster and Robertson, 1995) and fish (Brooks and Dodson, 1965; Northcote, 1988). Pulse exposures likely impair sensitive cladocerans, as reported here, by reducing their filtering capacity and fecundity, probably resulting in lower population growth and, finally, declining cladoceran density in freshwater systems. This might cause both top-down effects, increasing phytoplankton biomass (Wendt-Rasch et al., 2003; van Wijngaarden et al., 2005) and bottom-up effects, altering the energy transfer along the food web (Hanazato, 2001). Present results suggest that pulse exposures also result in lower abundances of sensitive species (Heckmann and Friberg, 2005; Jergentz et al., 2005; Schulz and Liess, 1999; Wiczeorek et al., 2018), altering community composition (Wendt-Rasch et al., 2003; Medina et al., 2004). Arias et al. (2020) studied the invertebrate composition in streams draining horticultural basins compared with less impacted streams surrounded by pastures, in Buenos Aires province, Argentina; invertebrate assemblages were significantly different in the horticultural streams than in the less disturbed streams: sensitive taxa such as *S. vetulus*, *Hyalella* sp. and *Caenis* sp. were abundant in the less disturbed and rare or absent in the horticultural streams, where Entomobryoidae, Dugessidae and Glossiphoniidae were dominant. Particularly, *S. vetulus* showed significantly lower density in horticultural streams, suggesting pesticide effects.

The present research assessed toxicity in laboratory assays

simulating brief runoff exposures. Pyrethroid toxicity may be affected by several environmental parameters, such as, among others, temperature (Weston et al., 2009), salinity (Hall and Anderson, 1995; Heugens et al., 2001), suspended and organic matter (Knauer et al., 2017). Further research is needed to improve our understanding of the effects of pulse exposure on the resident non-target fauna. Carriquiriborde et al. (2007) reported lower toxicity of cypermethrin to the fish *Cnesterodon decemmaculatus* in streams than in dechlorinated tap water and interpreted the results as being mainly occasioned by the insecticide's interaction with organic matter. Yang et al. (2006) reported decreased toxicity of pyrethroids to *Ceriodaphnia dubia* when suspended matter in the water was experimentally increased. Future experimental designs could be implemented using stream instead of tap water, thus assessing the effect on toxicity of environmentally realistic scenarios. Furthermore, the use of stream mesocosms might be suitable for assessing other environmental features, such as hydrological variables (Heckmann and Friberg, 2005).

4. Conclusions

The reported 48 h-LC50 of cypermethrin for *S. vetulus* indicated that the latter is a sensitive species and could be a reliable indicator of cypermethrin toxicity in the environment. Experiments showed that a single 90 min-pulse exposure at environmentally relevant concentrations inhibits feeding, and repeated pulse exposures reduce fecundity and population growth. Present results suggest that a non-target population exposed to pulses of cypermethrin will decrease its feeding rate, fecundity and population growth, reducing its density. Ultimately, pulse exposures could change the assemblage structure because of different species sensitivity.

CRediT authorship contribution statement

Marina Arias: Methodology, Formal analysis, Investigation, Writing - original draft. **Carlos Bonetto:** Conceptualization, Writing - review & editing, Supervision, Funding acquisition. **Hernán Mugni:** Conceptualization, Supervision.

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

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ecoenv.2020.110546>.

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