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Effects of glyphosate formulations on the population dynamics of two freshwater cladoceran species

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Abstract
The general objective of this work is to experimentally assess the effects of acute glyphosate pollution on two freshwater cladoceran species (*Daphnia magna* and *Ceriodaphnia dubia*) and to use this information to predict the population dynamics and the potential for recovery of exposed organisms. Five to six concentrations of four formulations of glyphosate (4-Gly) (Eskoba®, Panzer Gold®, Roundup Ultramax® and Sulfosato Touchdown®) were evaluated in both cladoceran species through acute tests and 15-day recovery tests in order to estimate the population dynamics of microcrustaceans. The endpoints of the recovery test were: survival, growth (number of molts), fecundity, and the intrinsic population growth rate (r). A matrix population model (MPM) was applied to r of the survivor individuals of the acute tests, followed by a Monte Carlo simulation study. Among the 4-Gly tested, Sulfosato Touchdown® was the one that showed higher toxicity, and *C. dubia* was the most sensitive species. The Monte Carlo simulation study showed an average value of λ always <1 for *D. magna*, indicating that its populations would not be able to survive under natural environmental conditions after an acute Gly exposure between 0.25 and 35 a.e. mg L^{-1}. The average value of λ for *C. dubia* was also <1 after exposure to Roundup Ultramax®: 1.30 and 1.20 for 1.21 and 2.5 mg a.e. L^{-1},respectively. The combined methodology—recovery tests and the later analysis through MPM with a Monte Carlo simulation study—is proposed to integrate key demographic parameters and predict the possible fate of microcrustacean populations after being exposed to acute 4-Gly contamination events.

Keywords Glyphosate formulations · *Daphnia magna* · *Ceriodaphnia dubia* · Recovery tests · Matrix population models

Introduction

The use of agrochemicals has increased over the last decades in response to the higher food demands of a growing world population (Salazar 2010; Heinemann et al. 2013). Despite the benefits of agrochemicals, their intensive use poses serious concerns to humans, wildlife and overall environmental compartments. Agriculture is a significant diffuse source of contaminants, which might end up in adjacent freshwater courses, hence compromising water quality and generating toxicity to the aquatic biota (Lupi et al. 2015; Ronco et al. 2016). Nevertheless, the effect of agricultural pollution at a population level and the potential recovery capacity of the exposed organisms remain poorly studied, partly because of methodological constraints.

The expansion of genetically modified (GM) seeds resistant to the herbicide glyphosate (*N*-(phosphonomethyl) glycine) and its wide use in forestry, home orchards and urban environment have produced a constant increase of the production and consumption of glyphosate-based formulations (Santadino et al. 2014). Glyphosate is commercialized in more than 130 formulations that are applied to at least 100 different crops (Monsanto 2009; World Health Organization 2015). Often, only a small amount of the applied products reaches the target pests, while the greatest part is spread throughout the surrounding environment. The presence of glyphosate in surface water bodies due to direct spraying over rivers, streams, volatilization, water flows, or drift has been reported by many authors (Cerejeira et al. 2003; Gilliom and Hamilton 2006; Peruzzo et al. 2008; Pereira et al. 2009; Grube et al. 2011).

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Argentina is the third producer of GM crops in the world, surpassed only by the United States and Brazil (James 2014). The constant increase of the volumes used, together with the frequently negligent mode of application, has led to scenarios of acute or diffuse contamination (Aparicio et al. 2013; Ronco et al. 2016).

The reported effects of glyphosate or its formulations are mainly acute toxicity (Tsui and Chu 2003; Cox 2004; Kitulagodage et al. 2008; Pereira et al. 2009; Piola et al. 2013) but little is known about the effects on organisms after being exposed to glyphosate in natural environments or even under simulations of that situation. Furthermore, due to their bioactive nature, the agrochemical compounds tend to be toxic to non-target organisms. This is frequently associated with the occurrence of sublethal and complex effects, such as alterations in the metabolism, developmental toxicity, endocrine disruption, neurotoxicity and immunotoxicity (Defarge et al. 2016; Schimpf et al. 2017; De Souza et al. 2017; Nardi et al. 2017). The accumulation or chronic nature of these effects may result in lower survival rates of non-target organisms, causing energetic imbalances and big losses in the wildlife populations sustaining the functioning of freshwater ecosystems.

The toxicity of glyphosate (Gly) depends on the concentration and on the kind of salt (isopropylamine salt, dimethylamine salt, monoammonium salt, and potassium salt) in the commercial formulations (Reno et al. 2015). In the safety information of the commercial products, acute toxicity (mortality) at 48 or 72 h is usually reported (Hua and Relyea 2014). However, this scarce information could lead to confusing conclusions about the long-term effects of pesticides. On the other hand, there is increasing awareness about the importance of gaining knowledge on the sublethal effects that pollutants could have on non-target species (Anton et al. 1993; Spurgeon et al. 2004; Venkateswara et al. 2003). This knowledge could help propose a value of maximum admissible environmental concentration for the protection of the aquatic biota.

In this line, in 2013 the National Research Council of the United States reported population models are necessary to integrate the effects of pesticides on survival and reproduction.

To detect sublethal effects, especially at the population level, the use of mathematical population models has been proposed. These models are very useful especially for understanding changes in populations with a structure of ages, states or stages, or with many changes in their life cycles, and have been used in toxicological tests (Chaparro and Canziani 2010; Santadino et al. 2014). However, little has been done to experimentally assess this goal. Forbes et al. (2016) reported that out of the 403 papers reviewed only 13 published papers used empirical laboratory data (i.e., modeling the concentrations and effects that were tested in the laboratory experiments), indicating the importance of gathering experimental data as trustable inputs to population models.

The matrix population models are powerful tools useful for the study of population dynamics, with applications in ecology, epidemiology and demography (Caswell 2001). Population models are very useful in risk assessments, since they can integrate potential effects of pesticide exposure on individual survival, reproduction, and growth, with relevant species-specific life history to project likely consequences for population persistence (Forbes et al. 2016).

Considering the huge rural extensions with genetically modified crops, the use of glyphosate-based herbicides all over the world, the need to know the sublethal effects on non-target species especially at a population level, the methodological limits of widely ecotoxicological studies, and that the potential recovery capacity of the exposed organisms remains poorly understood, the main goal of this work was to determine the effects of acute glyphosate pollution on the population dynamics of two freshwater cladoceran species and the potential for recovery of exposed organisms. We experimentally assessed the effects of glyphosate on two non-target freshwater cladoceran species; then we used this information to predict their population dynamics and the potential for recovery of exposed organisms.

The specific objectives were: (1) to determine the concentration causing 50% of mortality after 48 h of exposure (48 h-LC_{50}) by acute tests; (2) to establish the effects of 4-Gly formulations at the population level by a posteriori recovery tests; (3) to estimate the potential effects of 4-Gly on the population dynamics by a Monte Carlo simulation study; (4) to propose a methodology of intermediate complexity between acute and chronic tests that integrates key population parameters, such as survival, growth, fecundity, and *r*.

Materials and methods

The 4-Gly formulations selected were Eskoba® (Red Surcos), Panzer Gold® (Dow AgroSciences), Roundup Ultramax® (Monsanto) and Sulfosato Touchdown® (Syngenta Agro). The 4-Gly contained: 48% (w/v) isopropylamine salt; 60.8% (w/v) dimethylamine salt; 74.7% monoammonium salt; and 62% (w/v) potassium salt, respectively (CASAFE 2011).

The glyphosate-based formulations selected are among the most widely used in the pampean region and the Parana River floodplain (Argentina), the two ecoregions with the highest soybean production in the country (CONICET 2009; UNL 2010; Reno et al. 2015; Reno et al. 2016).

In these commercial formulations, the acid of glyphosate is turned into salt with the purpose of increasing its

solubility in water and for this reason the concentrations of glyphosate are reported as acid equivalent (a.e.) per liter (Monsanto 2009; Lanctôt et al. 2014).

Acute test

Before the tests, we prepared solutions of 1000 mg a.e. L^{-1} of the 4-Gly in sterile bidistilled water maintained under constant conditions of darkness at −4 °C until their analytic determination through high-performance liquid chromatography (HPLC) using a Dionex DX-100 ion chromatograph.

The concentrations of glyphosate in the solutions were: 1067; 1091; 914 and 993 mg a.e. L^{-1} in the formulations of Eskoba®, Sulfosato Touchdown®, Panzer Gold® and Roundup Ultramax® respectively. This stock solution was diluted in synthetic culture medium to prepare each one of the test concentrations to be used in the experiments with the two cladoceran species.

The stock cultures of *D. magna* were maintained in synthetic media containing 0.13 g K_2SO_4, 1.12 g $CaCl_2$, 1 g $NaHCO_3$ dissolved in 5 L of distilled water (ASTM 1980) and *C. dubia* in the synthetic media proposed by APHA (1998): 2.4 g SO_4Mg, 3.84 g $NaHCO_3$, 0.16 g KCl, and 2.4 g $CaSO_4.2H_2O$ dissolved in 20 L of distilled water. These media were used to do all the tests (acute and recovery ones). The cladocerans were fed with 40 µL (absorbance = 1.5 λ, 650 nm) of *Chlorella vulgaris* (strain CLV2, taken by the CISECE, Mexico) per organism and maintained in cultivation chambers under controlled and constant conditions: photoperiod 16 L: 8D and temperature 20 ± 1 °C.

The 48-h acute tests were performed with photoperiod 16 L: 8D and temperature 20 ± 1 °C. Twenty neonates (<24 h) of *C. dubia* and *D. magna* were divided into four groups of five specimens for each one of the treatments and controls (OECD 2004).

For the commercial formulation Panzer Gold® we tested 5 concentrations of glyphosate: 10, 5, 2.5, 1.25, 0.62 mg a.e. L^{-1} for *D. magna* and 6 concentrations for *C. dubia:* 2.5, 1.25, 0.62, 0.49, 0.31, and 0.15 mg a.e. L^{-1}.

For Roundup Ultramax® we tested 5 concentrations of glyphosate: 40, 20, 10, 5, 2.5 mg a.e. L^{-1} for *D. magna*, and 20, 10, 5, 2.5, 1.25 mg a.e. L^{-1} for *C. dubia*.

The concentrations tested and the 48 h-LC_{50} for Eskoba® and Sulfosato Touchdown® have already been reported by Reno et al. (2015).

The mortality was recorded as an indicator of acute toxicity and assessed by the immobilization of the organisms. As indicative of the toxic effect, in this work we considered the complete immobilization of the organisms and the absence of response after being gently stimulated or pushed by a metal rod.

The results were considered acceptable when the mortality in the control was ≤10%. For each species, we determined the lethal concentration 50 at 48 h (48 h-LC_{50}), where LC_{50} is the concentration causing 50% of mortality after 48 h of exposure (48 h-LC50) (Rand and Petrocelli 1985). The values of 48 h-LC_{50} with a 95% confidence level were determined by the probit analysis (Finney 1971). The pH and the dissolved oxygen (DO, mg L^{-1}) were measured at the beginning and at the end of all tests. The values were between 7.6 and 8.1 for the pH, and 6 and 8 mg L^{-1} for DO, within the normal limits established by APHA (1998).

Recovery test

To estimate the post-exposure population dynamics in the 4-Gly formulations and concentrations tested, recovery tests (RTs) were performed, following the procedure proposed by Reno et al. (2014): Each surviving individual of the acute tests was placed alone in a new 50 ml glass vessel with the respective glyphosate-free culture media for 15 days under the same conditions described for stock cultures (photoperiod 16 L: 8D and temperature 20 ± 1 °C).

The cladocerans were fed three times a week, with 40 µ (absorbance = 1.5 λ, 650 nm) of a suspension of *C. vulgaris*. At the same time, data were taken from each vessel, to determine the endpoints: survival (days), number of molts produced and released in the vessels (as indirect measure of growth, since the number of molts increases with body size), and fecundity (number of neonates released).

The values of the pH and the concentrations of DO were recorded at the beginning and at the end of each test, controlling that they were within the limits established by APHA (1998).

Construction of the matrix population models

With the results obtained from the RTs, we constructed matrix population models (MPM). These models have been used with good results to evaluate chronic effects of agricultural chemicals on non-target species (Forbes et al. 2016). They can be implemented when the population has discrete growth and a structure of ages, stages or states. Such populations are represented by a vector that depends on the time during which each specimen is in each one of the ages, stages or states that have been considered in the life cycle under study (Santadino et al. 2014).

The vector of age, stage or state over the time (t + 1) is obtained by multiplying the previous vector of age, state or stage by the time (t) in a transition matrix that represents the contributions of each age, stage or state over time. These elements, in a matrix where the states represent stages of the life cycle linked to the age (for example, successive larva stages), are measures of survival, or measures of fecundity in the case of the contributions of adults to the number of

eggs or neonates released. The information obtained in the RTs was summarized in a transition matrix to estimate the population parameters (Momo and Capurro 2006). The self-value of the transition matrix indicates whether the population is growing (module of dominant self-value higher than one), stable (module equal to one) or declining (module of self-value lower than one).

The states or stages of the life cycle of both cladocerans were: neonates—juveniles—adults. We also considered that in each case, the individuals have different potential fecundity that is null for neonates and juveniles and variable for adults, depending on the environment that they are in or have been exposed to. These stages are important in the development of cladocerans, and the possibility or not of giving up one stage to pass to another one can give relevant information about the expected long-term growing or declining effects on the population dynamics, after being exposed to different formulations and concentrations of glyphosate.

For each one of the concentrations at which a live specimen was recorded after 15-day RTs, we constructed two matrices: an optimist one (Fig. 1, A) and a pessimist one (Fig. 1, B) in which the values of incidence of a stage or state are the maximum and minimum possible for the studied population, respectively.

For the construction of the matrix, we first counted how many days the specimens took to molt in relation to the control, to have their first neonate (age of their first reproduction) and to turn from the juvenile stadium (J) to the adult one (A). For both species, we obtained the same average value of 5 molts (±1.03). That is to say that the cladocerans began to reproduce after 5 molts in the RTs. This parameter has been taken as the initial reference to construct the matrix of the treatments and the controls.

The values of the matrix were expressed as follows:

f (fecundity), estimated as the average of neonates (N) of the organisms that lived the 15 days of the test (the sum and subtraction of the standard deviation of the average give as a result optimistic and pessimist F, respectively).

S_{21} (N–J) = proportion of organisms that survived the acute test.

S_{22} (survival J–J) = proportion of juveniles that stayed in this state and did not complete the 5 molts needed to turn into adults.

This value can have a maximum (optimistic matrix) calculated from the equation:

$$\overline{X} = (X_1/t) + (X_2/t).....(X_n/t)$$

where X_n is the day when the last molt was recorded; t is the duration of the test (15 days). On the other hand, this value has a minimum = zero (0), i.e., all the organisms stayed in the juvenile state (pessimistic matrix).

S_{32} (survival J–A) = proportion of organisms that had 5 molts during the recovery test.

S_{33} (survival A–A) = the maximum value, when this parameter is = one (1), i.e., all the adults survived in this state until the end of the test (optimistic matrix).

The minimum value (pessimistic matrix) has been obtained from the equation:

$$\left(\overline{X}_{An}\right)/t_n$$

where $\overline{x}_{An}$ is the average of days that the organism survived after having the 5 molts, and t_n the duration of the test (15 days).

In the matrix population models, the self-values of higher module (called λ) are related to the asymptotic dynamics of the population. The logarithm of λ is the intrinsic population growth rate (r). That means that the population grows if the module of λ is higher than 1 (or $r > 0$) (Santadino et al. 2014).

Monte Carlo simulation

We performed a Monte Carlo simulation study of the matrix population models to estimate the probability distribution of the asymptotic population growth rate for each treatment and control. When matrix population models are based on parameters with an intrinsic variability, a unique estimation of the population growth rate does not allow measuring such variability. A Monte Carlo simulation study is a form of numerical analysis where a stochastic computer simulation of a mathematical model is performed to estimate

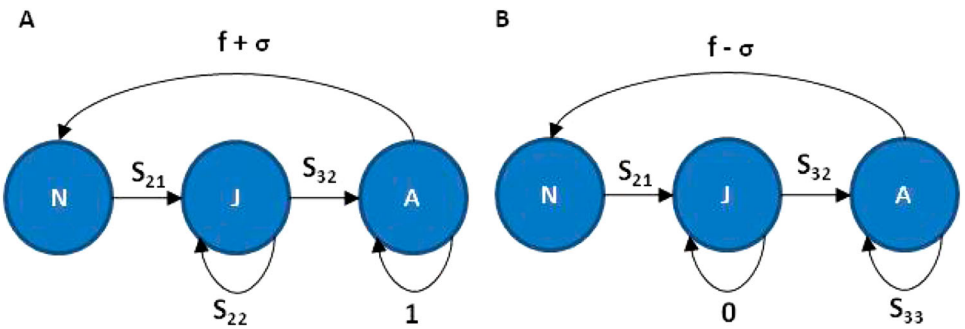

Fig. 1 Scheme of the model of population dynamics: Matrix A: optimistic matrix. B: pessimistic matrix. N neonate, J juvenile, A adult, f fertility, S_{21} survival N–J, S_{22} survival J–J, S_{32} survival J–A, S_{33} survival A–A, σ standard deviation

properties of the model that cannot be obtained analytically or by other approximation methods (Rubinstein and Kroese 2007). In order to make a population projection of the glyphosate-based formulation effects on survival and fecundity measured in the recovery tests, we used the two population transition matrices generated for each treatment as extreme possibilities for each population parameter of the matrix population model. In this way, by means of the Monte Carlo simulation, the variability in the parameters of the population matrix model is taken into account in the estimation of the population growth rate.

The simulation size was determined by assessing the effect of sample size on error estimates based on simulation results, which showed that for a sample size of 10^6 the variance of the estimates was negligible compared with the difference between treatments. Therefore, 10^6 stochastic population transition matrices were generated for each treatment by a uniform random sampling of each population parameter within the limits set by the two extreme transition matrices. On a matrix population model, once a stable population structure is attained, the growth rate λ is equal to the dominant eigenvalue of the transition matrix (Caswell 2001). For each random matrix, we computed the dominant eigenvalue, thus obtaining a 10^6 sample from the probability distribution of the projected population growth rate corresponding to each treatment.

The mean and 95% equal-tailed probability interval for the growth rate of each treatment were computed using the empirical distribution functions. The probability density function of the population growth rate was also estimated by a kernel diffusion method (Botev et al. 2010). All Monte Carlo computations were performed using the free software GNU Octave 3.8.2 (Octave community 2014). The probability density estimation was computed using the code provided by Botev et al. (2010).

Results

Acute test

Table 1 shows the values of 48 h-LC_{50} for the four glyphosate-based formulations studied. The values of 48 h-LC_{50} corresponding to Sulfosato Touchdown® were lower for both cladoceran species, *C. dubia* being the most sensitive one.

The specimens surviving the acute tests were used in the recovery tests.

Recovery tests

The organisms that survived until the end of the recovery test were previously exposed to the following concentration of glyphosate:

Eskoba®: 35, 30, 25, 20 mg a. e. L^{-1} for *D. magna*, and 18, 12, 8 mg a.e. L^{-1} for *C. dubia*.

Sulfosato Touchdown®: 0.5, 0.25 mg a.e. L^{-1} for *D. magna*, and 0.25, 0.125, 0.0625 mg a.e. L^{-1} for *C. dubia*.

Panzer Gold®: 2.5, 1.25, 0.625 mg a.e. L^{-1} for *D. magna*, and for *C. dubia*: 0.625, 0.49, 0.3125, 0.156 mg a.e. L^{-1}.

Roundup Ultramax®: 10, 5, 2.5 mg a.e. L^{-1} for *D. magna*, and 2.5, 1.25 mg a.e. L^{-1} for *C. dubia*.

In relation to the results of the Monte Carlo simulation, the average value of λ for *D. magna* exposed to all the formulations and concentrations evaluated was always lower than 1 (Fig. 2), so under these conditions, the population will not be able to survive over time after an acute exposure to the concentrations tested in this work.

In the case of *C. dubia*, the average value was also lower than one, except in the assays carried out with Roundup Ultramax®, where λ was 1.30 and 1.20. At both concentrations tested, the results were similar to 0.15 mg a.e. L^{-1} ($\lambda = 1.05$) of Panzer Gold® and to 0.06 mg a.e. L^{-1} ($\lambda = 1.01$) of Sulfosato Touchdown® (Fig. 3).

Furthermore, different neonatal mortality rates were recorded in the recovery tests for the two more toxic formulations: Panzer Gold® and Sulfosato Touchdown® (Table 2).

Discussion

Based on the results obtained in this work, the higher sensitivity of *C. dubia* compared to *D. magna* can be highlighted. Other authors also showed that C.dubia is less tolerant to xenobiotics: Regaldo (2013) found that *C. dubia* was more sensitive than *D. magna* and *Moinodaphnia macleayi* when they were exposed to chrome, lead and

Table 1 Effective concentrations for 50% of the population (LC_{50} mg e.a L^{-1}) obtained after 48 h exposure of *D. magna* and. *C. dubia* to the four glyphosate-based formulations tested. In brackets, limits of confidence ($\alpha = 0.05$)

Glyphosate-based formulations	48 h-LC_{50} (mg a.e. L^{-1})		References
	D. magna	*C. dubia*	
Eskoba®	29.48 (27.464–31.415)	14.49 (12.4–16.77)	Reno et al. (2015)
Roundup Ultramax®	11.68 (8.93–15.43)	4.84 (3.83–6.13)	In this work
Panzer Gold®	2.12 (1.68–2.67)	0.54 (0.44–0.65)	In this work
Sulfosato Touchdown®	1.62 (1.24–2.09)	0.31 (0.25–0.37)	Reno et al. (2015)

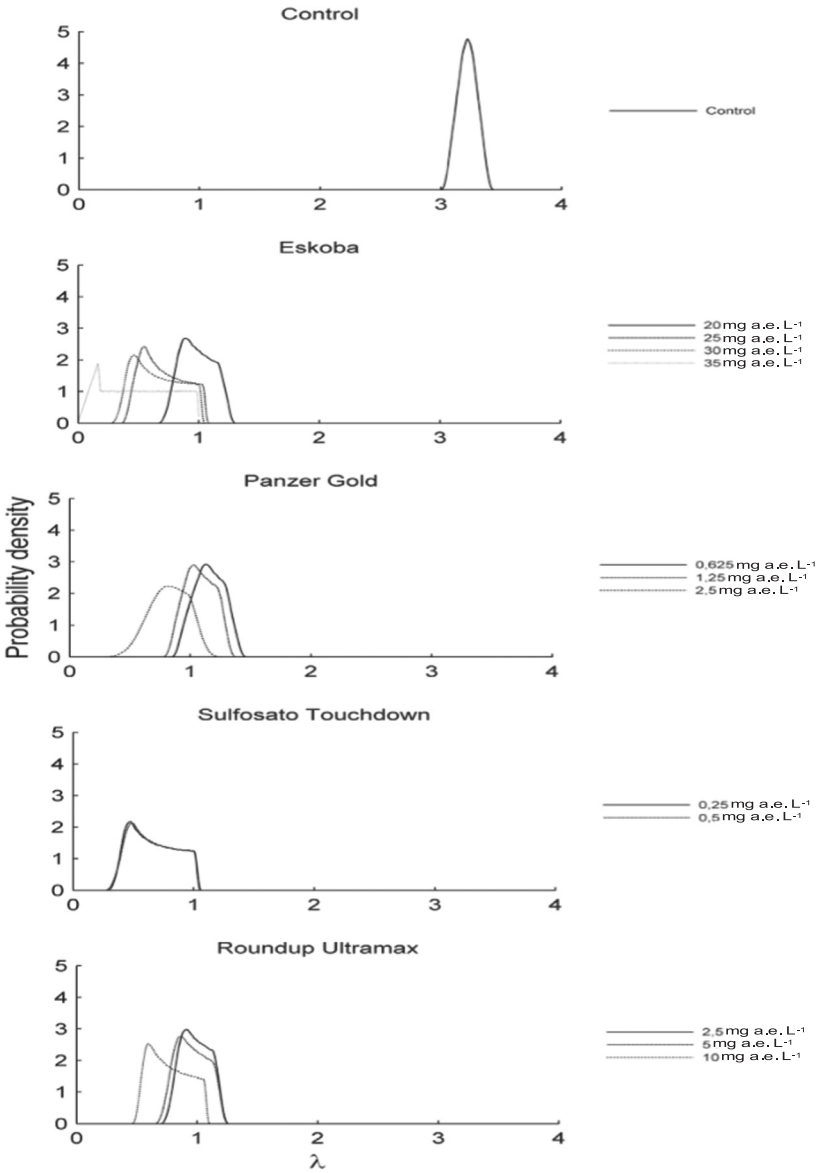

Fig. 2 Probability distribution of λ for *D. magna* obtained by Monte Carlo simulations for each of the 4-Gly formulations and previous exposure concentrations where the organisms survived until the end of the recovery tests

Table 2 Mortality of neonates (%) in the recovery tests with *D. magna* and *C. dubia*

Glyphosate-based formulations (mg a.e. L^{-1})	Neonates mortality (%)	
	D. magna	*C. dubia*
Panzer Gold®		
2.5	11.1	–
1.25	12.5	–
0.625	14.2	–
0.3125		18.8
0.156	–	28
Sulfosato Touchdown®		
0.25	–	19.7

copper. Wong and Dixon (1998), Rinderhagen et al. (2000), Olvera-Ramírez et al. (2010) and Reno et al. (2015) also reported that *C. dubia* was more sensitive than *D. magna* after exposing both species to various contaminants.

By comparing the 48 h-LC_{50} values found in this paper, with reports by other authors evaluating various commercial formulations of glyphosate, we can observe that 48 h-LC_{50} values differ between species and formulations, for example, *D. magna* showed values of 48 h-$LC_{50} = 7.9$ mg L^{-1} after being exposed to Faena® (356 % w/v potassium salt), 61.72 mg L^{-1} after exposure to Rondo® (36 % w/v monoammonium salt), and 190 mg L^{-1} after being exposed to Roundup® (48.0 % w/v isopropylamine salt). *C. dubia* showed toxicity values in the range 5.39 mg L^{-1} (Roundup®) to 415 mg L^{-1} (Rodeo®, 53.8% w/v

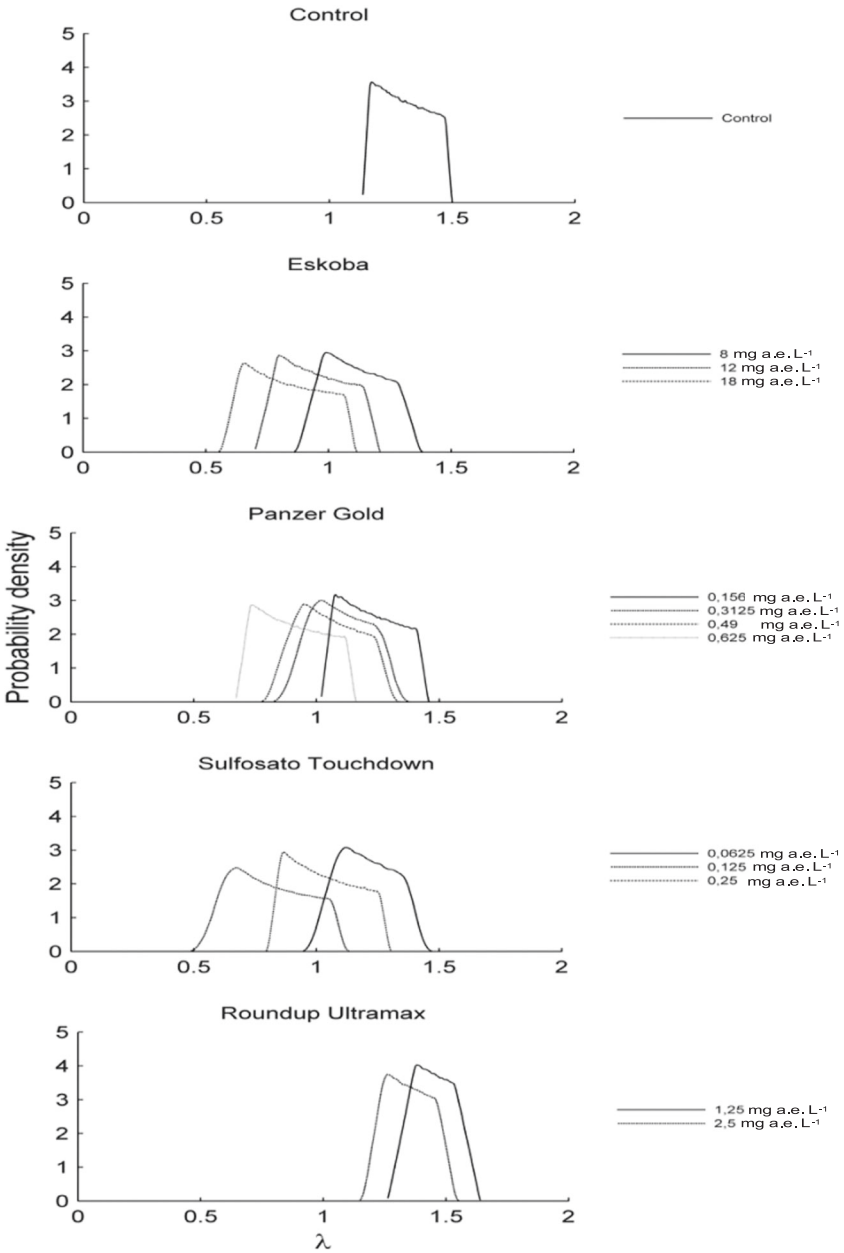

Fig. 3 Probability distribution of λ for *C. dubia* obtained by Monte Carlo simulations for each of the 4-Gly formulations and previous exposure concentrations where the organisms survived until the end of the recovery tests

isopropylamine salt) (Alberdi et al. 1996; Tsui and Chu 2003; Dominguez-Cortinas et al. 2008; Raipulis et al. 2009). On the other hand, Reno et al. (2014) recorded a 48 h-LC_{50} equal to 21.5 a.e. mg L^{-1} after exposing *Simocephalus vetulus* to the same commercial formulation (Eskoba®) evaluated in this work.

The differences found between the acute toxicities recorded in this paper and others considered here could be attributed to the composition of the commercial formulations (i.e., to the differences with respect to the chemical associations of the active ingredient and the proportion and nature of the surfactant or additives used in the commercial formulations), which could be more toxic than the active ingredient. In this sense Tsui and Chu (2003), reported that the surfactant (polyoxyethylene amine –POEA) of the commercial formulation Roundup®, was more toxic than the active principle and formulation, using *C. dubia* and *Acartia tonsa* as biological models.

The concentrations of glyphosate that have been evaluated in the recovery test are similar to or below the concentrations recorded in the environment by Aparicio et al. (2013): between 0.0005 and 0.56 mg Kg^{-1} in suspended

particulate matter, and also similar to or below the guiding levels reported by the Undersecretariat of Hydrological Resources, Argentina (2003), and the Canadian Water Quality Guidelines (2012) for the protection of the aquatic biota: between 0.2 and 2.7 mg a.e. L^{-1} for short-term exposure, corresponding to the modules of $\lambda < 1$. This condition indicates that populations that are exposed to acute glyphosate toxicity for 48 h do not have a tendency to recover from the stress events.

Hua and Relyea (2014) have demonstrated that most of the cladocerans did not recover at the end of the test (18 weeks) after being exposed to chlorpyrifos, despite the high degradation of this compound (2.8% in the third week). Other authors (Choung et al. 2013) have recently demonstrated that the populations exposed to concentrations of 10 µg L^{-1} of terbufos (an organophosphorus insecticide and nematicide) in mixture with atrazine were completely different to the control ones after the application (<48 h), attributing these differences to the elimination of the cladocerans and the reduction of the Hydracarina assemblages.

The mortality of neonates recorded in this study could be due to the genotoxic effects that could condition the viability of the progenies. Previous reports by Thai-Hoang et al. (2010) informed that after an acute exposure of 24 h, glyphosate affects the expression of genes that are fundamental to the metabolism of the fatty acids and steroids in *D. magna*. Cuhra et al. (2013) reported that at concentrations of Roundup of 0.45 mg L^{-1}, *D. magna* showed a reduction of fertility, an increase in the rate of abortion, and a reduction in the size of the neonates released.

The differences found between the acute toxicity results and the average of modules λ between similar or very close concentrations of glyphosate of different formulations could be attributed to the variety of compounds of these commercial formulations in addition to the active principle. Puglis and Boone (2011), Reno et al. (2014), Gagneten et al. (2014), among others, have reported that it is necessary to provide more information on all the components present in glyphosate-based commercial formulations.

The analysis of the effects of pesticides on the different demographic parameters that are usually performed does not show their real effects on the dynamics of the population. In this work, as in the ones performed by Emlen and Springman (2007), Harper et al. (2008), and Santadino et al. (2014), it was demonstrated that the analysis of matrix population models is a useful tool to know the effects on different ages, states or stages of the target populations. Forbes et al. (2016) reported that out of the 403 papers reviewed only 13 published papers used empirical laboratory data (i.e., modeling the concentrations and effects that were tested in the laboratory experiments), indicating the importance of gathering experimental data as trustable inputs to population models.

This survey provides empirical laboratory data obtained by laboratory experiments with post-modeling of population effects, which are very scarce, as was previously discussed. The importance of the data generated in this work, through population models coupled with ecotoxicological tests, is highlighted.

In addition, there is little knowledge about how species respond demographically to short-term and punctual pollution situations. In this line, Hanson and Stark (2012), Willson et al. (2012), Ibrahim et al. (2014), Macneale et al. (2014), reported that the inclusion of population models allows the integration of combined lethal and sublethal effects on populations and more detailed risk assessments. The performance of recovery trials and the analysis from MMP with Monte Carlo simulations allowed us to obtain a parameter (λ) that integrates relevant demographic variables, such as survival, growth and fecundity, making it possible to generate information of intermediate complexity between acute and chronic tests.

Conclusions

This study provides relevant information on the toxicity effects of different glyphosate-based formulations on the population dynamics of freshwater microcrustaceans. Since the effect of agricultural pollution at a population level and the potential recovery capacity of the exposed organisms remain poorly studied–partly because of methodological constraints–a methodology of intermediate complexity between the most commonly used acute and chronic tests was proposed. In brief, it consists of performing acute tests followed by recovery tests and a later analysis through MPM with Monte Carlo simulations. It showed to be very useful for integrating key demographic parameters such as survival, growth and fertility. Among the glyphosate-based formulations tested, Sulfosato Touchdown® was the most toxic, *C. dubia* being the most sensitive species.

This combined methodology—recovery tests followed by a Monte Carlo simulation study—is proposed to assess ecotoxicity at a population level. It predicts the possible fate of cladoceran populations after being exposed to an acute contamination event. This knowledge can be used in ecotoxicological evaluations and risk assessments and would contribute to environmental management through the establishment of guideline values for the protection of the aquatic biota. The significance of gaining knowledge on the sublethal effects that pollutants could have on non-target species is highlighted. Far from being of local importance, this study is of global interest in countries where agriculture is a significant diffuse source of contaminants, which might

end up in adjacent water bodies compromising water quality and generating toxicity to the aquatic biota.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This article does not contain any studies with human participants performed by any of the authors.

Informed consent Additional informed consent was obtained from all individual participants for whom identifying information is included in this article.

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