



Disruption of the hatching dynamics of zooplankton egg banks due to glyphosate application



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HIGHLIGHTS

- Invertebrate dormant egg banks are ecological and evolutionary reservoirs of species.
- Anthropogenic stressors can disrupt the process of emergence from egg banks.
- We analyzed how a glyphosate-based formulation affect the hatching dynamics of zooplankton.
- Glyphosate application caused imbalances in the hatching dynamics of egg banks.
- Changes in the active zooplankton might generate imbalances in natural food webs.

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ABSTRACT

Hatching rhythms of eggs banks are important processes because they favor species co-existence and promote resilience of ecosystems after natural disturbances. Anthropogenic stressors can disrupt such natural hatching dynamics. This work examines the effects of concentrations ranging from 1 to 8 mg l⁻¹ of a commercial glyphosate-based formulation (Sulfosato Touchdown®) on the hatching dynamics of zooplankton dormant stages, present in the sediment of a natural lake. Sediment samples were collected from the surface sediment (<10 cm deep) of an isolated shallow lake free from pesticide pollution. An *ex situ* emergence assessment method was carried out and four treatments plus one control (without pesticide) were performed with three replicate each. Zooplankton hatching from the resting stages was monitored during 30 days. In total, 30 zooplankton taxa were recognized. The species diversity decreased significantly at concentration above 2.7 mg l⁻¹ glyphosate. The proportion of cladocerans within hatching organisms decreased, while that of rotifers *Bdelloidea* increased in all treatments with glyphosate. Time of the first hatching (TFH), time of maximum hatching (TMH) and the frequency of hatchings (FH) of most zooplankton species were also altered. In conclusion, the application of a glyphosate-based pesticide selectively affected the hatching dynamic of zooplankton egg banks, which suggest that these resting structures are highly sensitive to the toxicity of the pesticide.

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1. Introduction

In aquatic ecosystems, the invertebrate dormant egg banks in the sediments constitute ecological and evolutionary reservoirs of

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species (Hairston, 1996). Among invertebrate communities, cladocerans, copepods and rotifers are important components of such egg banks in freshwater systems. They have the ability to form resting stages that allow them to survive under adverse environmental conditions (Alekseev and Lampert, 2001; Slusarczyk et al., 2005). The resting stages production is regulated by external natural mechanisms such as predation, competition, drought, low oxygen, nutrients and temperature conditions, among others (Brendonck and De Meester, 2003). The resting stages are usually deposited in bottom sediments and accumulated for long periods until conditions are favorable again (Hairston, 1996; Brendonck and

De Meester, 2003). The dormant stages in the sediments have been shown to contain a higher genetic and species diversity than that found in the active communities (Ortells et al., 2000; Vandekerckhove et al., 2005; Gerhard et al., 2016). Thus, egg banks as well as dormancy and hatching processes are essential to maintain the species co-existence and a high resilience rate of aquatic ecosystems after natural disturbances (Caceres, 1997; Brendonck and De Meester, 2003; Gyllström and Hansson, 2004).

Recent studies have suggested that anthropogenic stressors can disrupt the process of emergence from egg banks, causing not only negative impacts on the mechanisms of recolonization of the systems but also substantial changes in the active actual and potential communities (Nielsen et al., 2003; Angeler and García, 2005; Navis, 2015). However, few studies have reported so far the magnitude of such changes and their potential effects for the community and ecosystem dynamics (Brendonck and De Meester, 2003; Gyllström and Hansson, 2004). At an individual level, it has been demonstrated that, by increasing the application rate of the fire retardant Fire-Trol[®] 934, the emergence success of *Daphnia curvirostris* resting eggs decreases (Angeler et al., 2006). Glutaraldehyde was proven to be highly toxic to dormant eggs of *Artemia* sp. (Sano et al., 2003; Raikow et al., 2007); resting eggs of *D. mendotae* were also affected by glutaraldehyde and sodium hypochlorite (Raikow et al., 2007) and the insecticide Fenoxycarb affected the hatching success and changed the timing of hatching in dormant eggs of *D. magna* (Navis et al., 2013). While these studies demonstrate the susceptibility of the resting stages of some isolated species (mainly on *Daphnia* genus) to chemical stressors, information on the effects of aquatic pollutants on the dynamics of natural egg banks is currently needed.

One of the most important stressors affecting aquatic ecosystems is the discharge of chemicals associated with agricultural activities. In South America, several shallow lakes and streams, receive permanent punctual and diffuse discharges of pesticides (Marino and Ronco, 2005; Ronco et al., 2008, 2011). As a consequence, the presence of such compounds in surface waters near agricultural fields has increased noticeably (Peruzzo et al., 2008; Aparicio et al., 2013; Lupi et al., 2015; Ronco et al., 2008, 2016). Moreover, as in many other regions of the world, the increasing soybean production during the last decades has brought about an increasing use of glyphosate, which has become the most common pesticide in current agricultural practices (Giesy et al., 2000; CASAFE, 2011). Recent studies in the region have detected that the Río de la Plata basin and other sub basins associated with agriculture in Argentina are highly polluted with this pesticide and its metabolite AMPA, the main sinks of such compounds being bottom sediments (Peruzzo et al., 2008; Peluso et al., 2013; De Gerónimo et al., 2014; Ronco et al., 2016).

Ecotoxicity testings and biomonitoring studies in relation to the increasing inputs of glyphosate have found important negative effects in active populations of rotifers and microcrustaceans species. For example, a single application of a glyphosate-based formulation (Glifosato Atanor[®]) caused an important increase in the abundance of the rotifer *Lecane* spp. and the fraction of copepods within the zooplankton (Vera et al., 2012). Microcrustaceans reduced their life expectancy after 24 h of exposure to the glyphosate-based herbicide ESKOBA[®], of which *Simocephalus vetulus* also decreased its fertility and *Notodiptomus conifer* inhibited its sexual maturity (Reno et al., 2014).

Because of the key role of zooplankton in the aquatic ecosystems and the food webs, as the main food for small fish and top-down controller of phytoplankton (Lazzaro et al., 2003; Llope et al., 2012), the need to assess whether glyphosate-based formulations and their active components affect the hatching dynamics of zooplankton becomes highly relevant. This information would

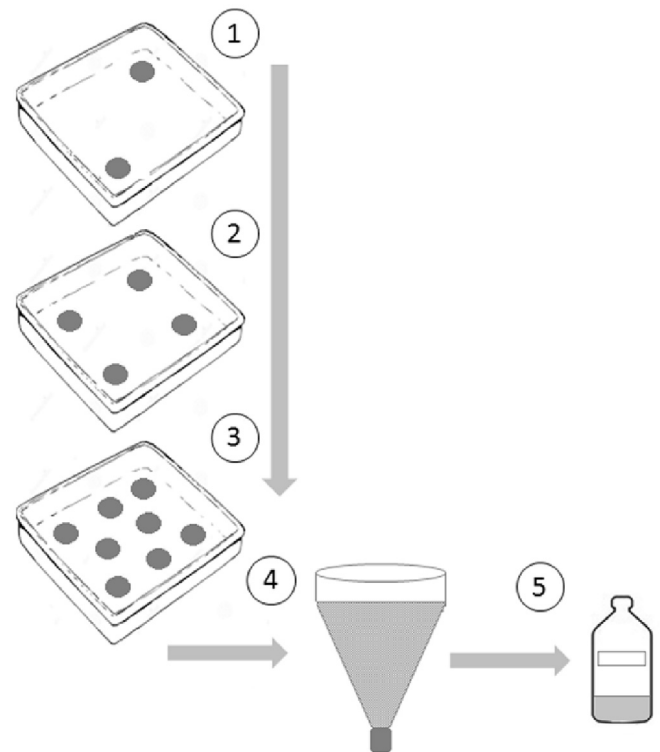


Fig. 1. Filtering procedure for the analysis of the zooplankton hatching. A succession of three perforated trays (1–3) was used followed by a filtering through a 20 μm mesh (4). The collected organisms were fixed and stored in glass bottles for counting.

allow understanding the magnitude of the effects of glyphosate on this community, making it possible to predict structural changes in the future. Besides, it would allow estimating the resilience of this community, i.e. its recovery rate after exposure, and the possible effects for the entire ecosystem.

While, to the best of our knowledge, there are no studies evaluating the effects of glyphosate on the hatching dynamics of zooplankton, previous research work analyzing other chemical compounds on hatching patterns have achieved opposite conclusions (Angeler et al., 2006; Henri et al., 2014; Navis, 2015). Therefore, despite the difficulty of making predictions about the possible responses of a given egg bank to glyphosate as a potential stressor; it is possible to expect a shift in the hatching dynamics. Taking into account the limited existing knowledge on this issue, the aim of this work was to analyze the effects of a commercial glyphosate-based formulation on the hatching dynamics of zooplankton dormant stages present in the sediment of a natural and permanent floodplain lake. It is hypothesized that the hatching dynamics of this community will be altered in the presence of the pesticide, which might cause changes in the structure of the active zooplankton assembly, generate imbalances in food webs and decrease water quality.

2. Methods

2.1. Sediment sampling

Zooplankton hatching from the resting stages was analyzed by an *ex situ* emergence assessment method proposed by Brendonck and De Meester (2003) and Garcia-Roger et al. (2008) and successfully used in previous studies in our laboratory (Battauz et al., 2014). Sediment samples were collected from the

Table 1
Glyphosate concentrations and its metabolite AMPA detected in the water of the different treatments after two days of application of the commercial formulation Sulfosato Touchdown®. nd: non detected glyphosate concentration in the sample.

N°	Nominal conc. ^a	GLYPHOSATE [mg/L]	AMPA [mg/L]	GLY + AMPA [mg/L]
C	0 mg/L	nd	nd	nd
T1	1 mg/L	0.6	0.3	0.9
T2	2 mg/L	1.4	0.5	1.9
T3	4 mg/L	2.7	0.7	3.4
T4	8 mg/L	5	1	6

^a Nominal concentrations were calculated from the % (w/v) of the isopropylamine (N-phosphonomethyl glycine salt) reported in the label of the commercial product.

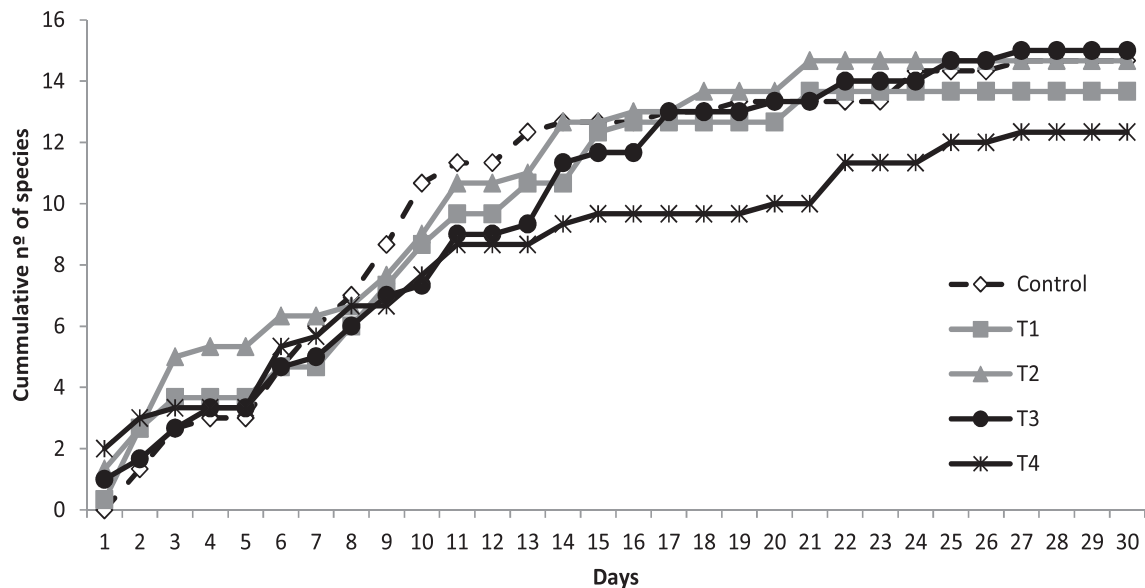


Fig. 2. Cumulative richness: number of species that hatched during the experimental period in each treatment.

Table 2
Results of the RM-ANOVA with Tukey *post hoc* test comparing the cumulative richness among treatments. Q values of the Tukey test are shown below the diagonal and p values are shown above the diagonal. Bold numbers indicate statistical significant differences between treatments.

	Sum of sqrs	df	MS	F	p
Between groups	90.062	4	22.515	33.42	<0.001
Tukey's pairwise comparisons					
	Control	T1	T2	T3	T4
Control		0.09409	0.2764	0.2285	0.0001
T1	3.559		0.00025	0.9935	0.0001
T2	2.817	6.376		0.00085	0.0001
T3	2.966	0.593	5.783		0.0001
T4	12.53	8.971	15.350	9.564	

Table 3
Exclusive species, i.e. those hatched in specific treatments and General species, i.e.: that hatched indistinctly in all treatments.

Exclusive species	
Control	<i>Brachionus angularis</i>
T1	<i>Brachionus havanaensis</i> ; <i>Keratella tropica</i> ; <i>Lecane cornuta</i>
T2	<i>Ceriodaphnia</i> sp.; <i>Lecane curvicornis</i>
T3	<i>Ephemeroporus</i> sp.; <i>Lecane lamellata</i> ; <i>L. rhenana</i>
T4	<i>Brachionus quadridentatus</i>
General species	
Bdelloidea; <i>Cephalodella</i> sp.; <i>Alona</i> sp.; <i>A. glabra</i> ; <i>Brachionus caudatus</i> ; <i>Lecane hamata</i> ; <i>L. inermis</i> ; <i>L. lunaris</i> ; <i>Lepadella patella</i> ; <i>Euchlanis</i> sp.; <i>Trichocerca</i> sp.	

surface sediment (<10 cm deep) of a small wetland located in the ecological reservoir of the University campus (Santa Fe, Argentina, 31°37' S, 60° 41' W). This wetland, consisting of 12 ha, belongs to the alluvial plain of the Paraná basin. During flood periods, it receives inputs from groundwater and other water bodies close to the Paraná River mainstream (Battaui et al., 2014). A total of nine sediment samples were taken from three equidistant points of the shoreline along transects of 50 m long.

2.2. Experimental design

In the laboratory, the sediment samples were pooled, thoroughly mixed, dried at 21 °C for 48 h and stored in the dark at 4 °C for 2 months (Vandekerckhove et al., 2005). The storage temperature was determined according the winter temperatures in the region (averages between 3.7 and 10.3 °C, CIM, Universidad Nacional del Litoral).

After the storage, the sediment was thawed at room temperature for one day. Subsequently, fifteen subsamples (20 g each) were placed in plastic trays (165 cm² of surface area) and covered with 0.5 L of sterile tap water and the respective pesticide concentration (see below). Four treatments plus one control (without pesticide) were performed with three replicate each.

During the experimental period, all the trays were maintained in an incubator with controlled temperature (25 °C) and photoperiod (16 h light: 8 h dark). The temperature for the emergence experiment was selected according the temperature of summers

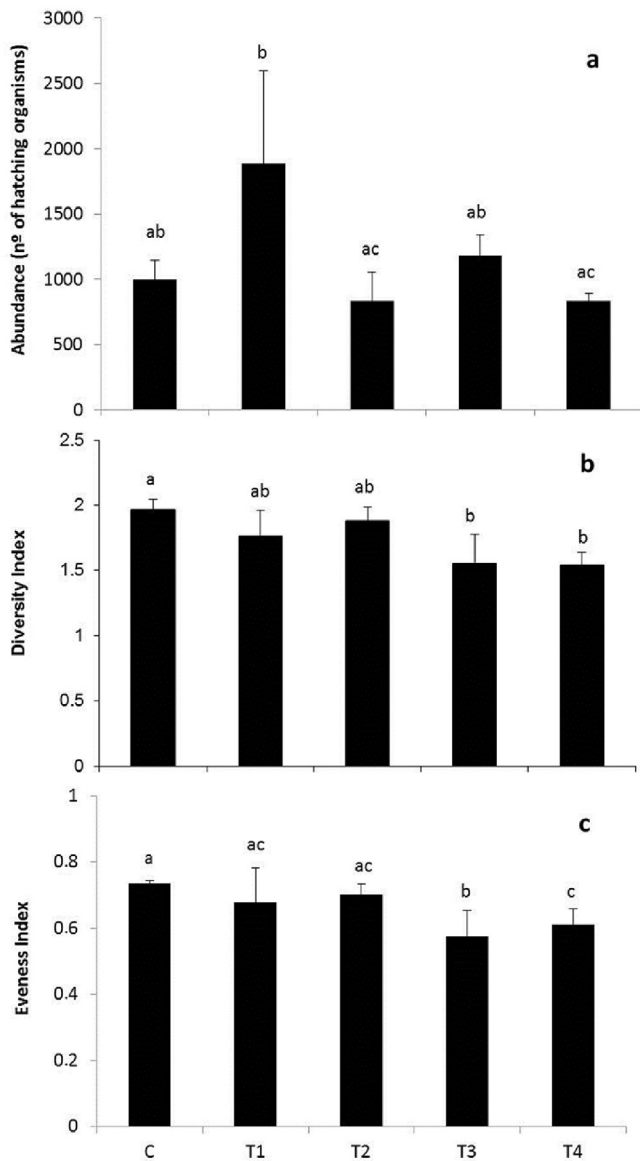


Fig. 3. a) Abundance of organisms that hatched in each treatment throughout the experiment (30 days). b) Diversity index (Shannon), considering the total number of species that hatched in each treatment at the end of the experiment. c) Evenness of species that hatched in each treatment throughout the experiment. Different letters indicate significant differences between treatments.

in the region (December–March: averages between 23.1 and 29 °C).

Hatching of plankton organisms from the resting stages in the sediment of each tray was monitored every day during the first 15 days. Afterward, the monitoring was performed every two days (resulting of 30 days of experimentation). The standardized procedure for the samplings consisted on filtering the supernatant water of each tray using a succession of three perforated trays, to prevent sediments suspension and finally a 20 µm mesh (Paggi et al., unpublished, Fig. 1). The collected organisms were fixed with 10% formalin. The samples were observed under an optical Nikon Eclipse (E100) microscope and taxonomic identification was made using different keys at the lowest level possible (Ruttner-Kolisko, 1974; Koste, 1978; Korovchinsky, 1992; Segers, 2002; Battistoni, 1995; Korínek, 2002; Alekseev, 2002; Korínek, 2002; among others). Hatched individuals were counted on a 1 mL Kolkwitz chamber.

2.3. Pesticide concentrations

A glyphosate commercial formulation was used (Sulfosato Touchdown®, Syngenta Agro) with 62% of the potassium salt and 38% of coadjuvants. The selection of a commercial formulation instead of the active principle isolated is based on that in this way is used in current agricultural practices. The applied concentrations for each treatment were 1, 2, 4 and 8 mg/L of the active principle (T1, T2, T3 and T4, respectively). The control consisted on a treatment without the application of the pesticide. The analytical determination was made from a water sample taken after one day of application using a chromatograph Dionex DX-100 ion equipped with a conductivity detector Waters 430, a suppressor column Dionex ASRS300, a column Dionex Ion Pack AS4A-SC and a pre-column Ion Pack AG4ASC. The eluent was NaOH 3.2 mM/Na₂CO₃ 7.2 mM. Actual concentrations of glyphosate and its metabolite AMPA are shown in Table 1.

2.4. Data analyses

To test the hypothesis, a total of eight general parameters were calculated: total abundance, total diversity (Shannon-Weaver index), total and cumulative richness (Stot and Scum), evenness (E) (Omori and Ikeda, 1984), the time of the first hatching (TFH), the time of maximum hatching (TMH) and the frequency of hatchings (FH). In addition, for the particular case of rotifers, the abundance *Bdelloidea* rotifers per day per treatment and their ratio in relation to the total zooplankton organisms (bdelloidea:totalzoo ratio) were analyzed.

The similarity analysis was used for the comparison of the taxonomic composition of hatchlings among treatments through the measurement of the Euclidian Distance (for TFH, FH and abundances). Results were expressed through a dendrogram following the Unweighted pair-group method with arithmetic mean (UPGMA), which showed the highest cophenetic correlation ($r = 0.8237$, $p < 0.05$). For testing the correlation between the three distance matrices, a Mantel test (Euclidean distance) was performed.

Differences among treatments in the cumulative richness were analyzed using a two-way ANOVA with repeated measures (RM). Differences in abundances, total diversity, evenness, TFH, TMH, FH, bdelloidea:totalzoo ratio and *Bdelloidea* per day among treatments were analyzed through one-way ANOVA with a significance level of $p < 0.05$. In the cases of significant differences, the Tukey post-test was used to assess which treatments were statistically different. In all cases the assumptions of normality and homoscedasticity were checked and the data were transformed to $\log x + 1$ when necessary.

3. Results

3.1. Hatching experiments

Of the total individuals that hatched within 30 days of experimentation, 30 taxa were recognized, 22 of which corresponded to Rotifera Monogononta, one to Rotifera *Bdelloidea* (unidentified), six to Cladocera and one to Cyclopoidea (Supplementary material, Table 1). In all treatments, the species richness increased fast until day 11 but later this increase slowed down. The number of species reached a constant value from the day 25 in all treatments (Fig. 2).

From day 15 onwards, the species richness of the treatment with the highest concentration of glyphosate (T4) remained below the values registered in the other treatments (Fig. 2), this difference being statistically significant (RM ANOVA, $p < 0.001$). Among the

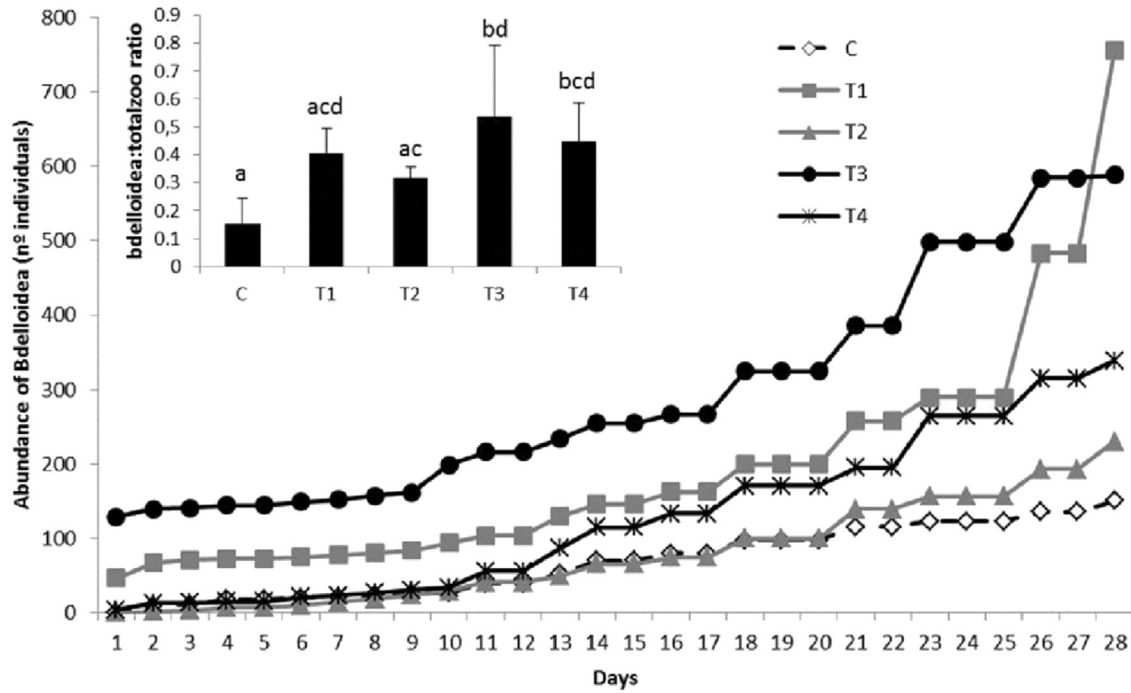


Fig. 4. Cumulative number of *Bdelloidea* rotifers throughout the experiment and bdelloidea: totalzoo ratio per each treatment. Different letters indicate significant differences between treatments.

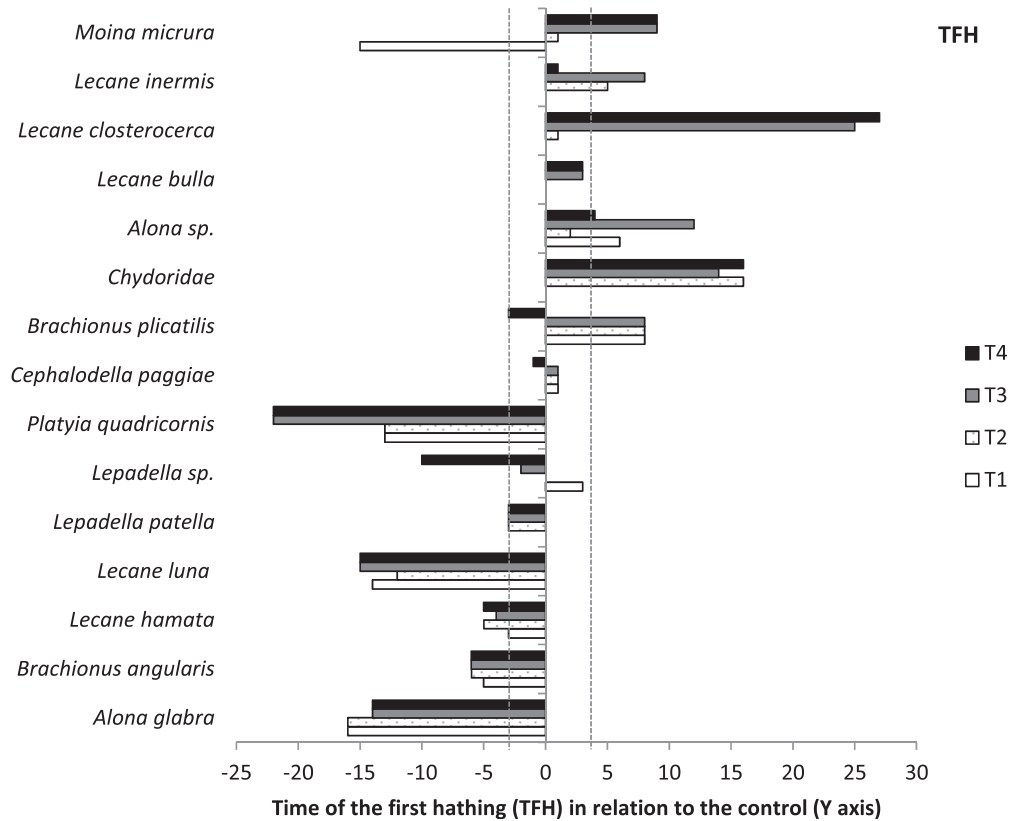


Fig. 5. Time of the first hatching (TFH) of each species per treatment. The TFH values for each species were plotted in relation to the control values (i.e.: to the value obtained from each treatment, the respective control value was suppressed). Thus, the negative numbers represent less TFH respect to control and the positive numbers represent a greater TFH than the one of control. The bars that exceed the dotted lines differ statistically from the respective control ($p < 0.05$).

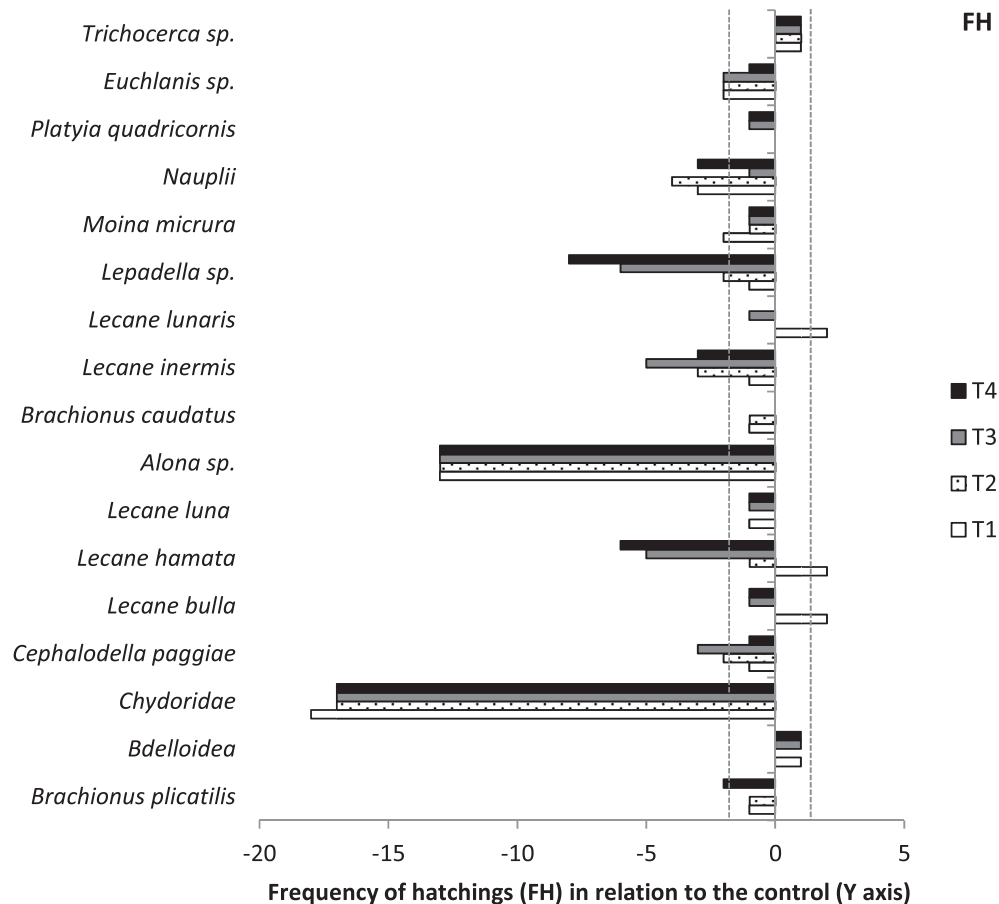


Fig. 6. Frequency of hatchings (FH) of each species per treatment. The FH values for each species were plotted in relation to the control values (i.e.: to the value obtained from each treatment, the respective control value was suppressed). Thus, the negative numbers represent less FH respect to control and the positive numbers represent a greater FH than the one of control. The bars that exceed the dotted lines differ statistically from the respective control ($p < 0.05$).

other treatments, T1 and T2 also differed significantly as well as T2 and T3 (RM ANOVA, $p < 0.001$; Table 2).

Half of the most abundant species (11 out of 22) were present in all treatments, while the other half (10 of 22) were exclusive of one of them (Table 3). The only brachionid rotifer present in all treatments was *B. caudatus*, half lecanid rotifer species were exclusive to some treatments, particularly in the intermediate pesticide concentrations, suggesting a specie-specific sensitivity within these groups. The cladoceran *Ceriodaphnia* sp. was exclusive of T2, however, only one individual of this species was observed during the whole experiment.

3.2. Abundance, diversity and evenness

The total abundance varied between 833 and 1179 individuals. As an exception, the treatment T1 recorded a total of 1884 individuals on average, of which more than 60% consisted of *Bdelloidea* rotifers. Significant differences were registered between treatments regarding this parameter (Fig. 3a). The species diversity (Shannon index) remained similar in the Control, T1 and T2, but decreased significantly in T3 and T4 (Fig. 3b). The evenness decreased in T3 and T4 with respect to the other treatments with lower glyphosate concentrations (Fig. 3c).

3.3. *Bdelloidea* rotifers

Of the total of registered taxa, the *Bdelloidea* rotifers showed a high hatching rate in all treatments with glyphosate, especially in

T1 at the end of the experimental period. On the contrary, the hatching rate of *Bdelloidea* remained low and practically constant in the Control (Fig. 4). Similarly, the *bdelloidea*:totalzoo ratio in T1, T2, T3 and T4 significantly surpassed the *bdelloidea*:totalzoo ratio in the Control (Fig. 4). In average, the amount of *bdelloidea* per day was also very high in treatments with glyphosate, especially in T1 (79.5 ind. l^{-1}), T3 (57.7 ind. l^{-1}) and T4 (35.6 ind. l^{-1}) in comparison with the control (15.7 ind. l^{-1}), being this difference statistically significant for T1 and control ($F = 2.99$, $p = 0.04$) and T3 and control ($F = 3.79$, $p = 0.01$).

3.4. Time of the first hatching (TFH), time of maximum hatching (TMH) and the frequency of hatchings (FH)

Out of the total of more abundant species, seven taxa (33%) showed a similar pattern of occurrence, 7 species (33%) delayed their TFH while other 7 species (33%) hatched earlier than the respective controls (Supplementary material Table II; Fig. 5).

Most of the analyzed species maintained or moderately increased the FH compared to the control. The other species decreased their FH throughout the study. This decrease was very marked in most cladocerans and rotifers of the genera *Lecane* and *Lepadella*, especially in treatments with the highest concentration of glyphosate (Supplementary material, Table II; Fig. 6).

The TMH was variable, depending on the treatment. However, half of the species delayed the TMH (generally coincident with the TFH) while the other half reached their maximum hatching early (Supplementary material Table II; Fig. 7).

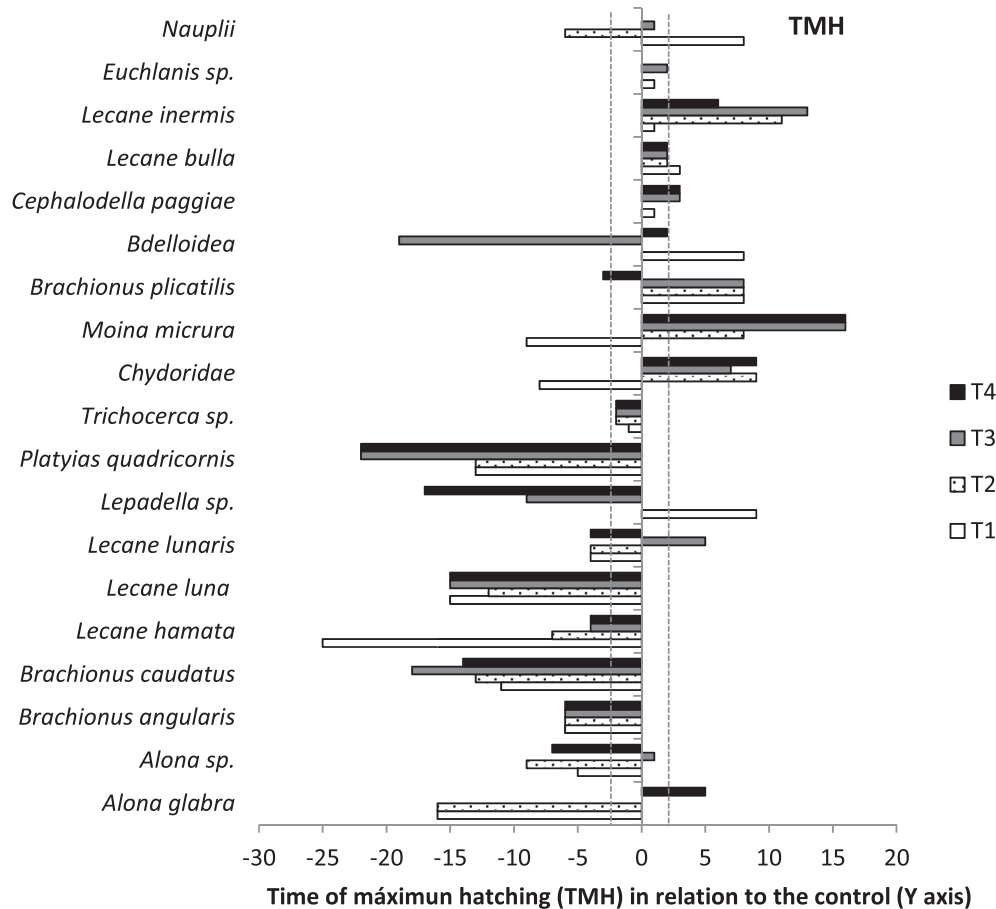


Fig. 7. Time of the maximum hatching (TMH) of each species per treatment. The FH values for each species was plotted in relation to the control values (i.e.: to the value obtained from each treatment, the respective control value was suppressed). Thus, the negative numbers represent less FH respect to control and the positive numbers represent a greater FH than the one of control. The bars that exceeded the dotted lines differ statistically from the respective control ($p < 0.05$).

In particular, in the presence of glyphosate, *Bdelloidea* rotifers maintained the TFH and FH in comparison with the Control treatment. This result associated with the increase in their abundance suggests that the herbicide has benefited the development of *Bdelloidea* rotifers. Among cladocerans, *Alona glabra* shortened its TFH and increased its FH suggesting that it may also be benefited by the presence of glyphosate. Conversely, among Monogononta rotifers hatching patterns were variable. For example, *Lecane bulla* delayed the first hatching even though it maintained the FH, *Lepadella* species shortened the TFH but decreased the FH, and *L. inermis* delayed its TFH and decreased the FH.

The similarity analyses comparing the taxonomic composition of each treatment showed that T1 and T2 were different from T3 and T4, indicating the existence of a potential herbicide tolerance limit for zooplankton species. Finally, the control is distinguished from the four groups treated with glyphosate (Fig. 8). The Mantel test showed a significant relation between the three matrices (corr. coef.: 0.5542; $p = 0.0078$).

4. Discussion

Zooplankton egg banks and their hatching dynamics are important for the seasonal succession and maintenance of the diversity of functional groups, favoring the establishment of complex food webs in natural systems (Parker et al., 1996; Caceres, 1997). Besides providing greater quantity and quality of resources, complex trophic structures also facilitate the resilience to environmental stress (Vandermeer et al., 1998). Here we demonstrated that

the application of a glyphosate-based pesticide selectively altered the hatching dynamic of the zooplankton egg banks present in the sediment, suggesting that these resting structures, which are resistant to desiccation, severe changes in temperature, and digestion among other negative factors for life (Carvalho and Wolf, 1989), are highly sensitive to the toxicity of the pesticide.

Together with taxon richness, the abundance and diversity of hatchlings decreased in the treatments exposed to the glyphosate-based herbicide, especially in the higher concentrations. This result is consistent with previous research reports that found negative effects on the dynamics of the dormant communities when exposed to other toxic substances (Marcial et al., 2005; Angeler et al., 2006; Jiang et al., 2007; Henri et al., 2014). Conversely, a recent outdoor mesocosm experiment showed no significant effects of a carbamate pesticide (Carbaryl) on the dormant zooplankton communities relative to the active one (Navis, 2015). However, since only one exposure concentration was analyzed in this work, different results might be obtained with the application of other concentrations or commercial formulations differing in their mode of action.

Unbalances in the active zooplankton structure could have important consequences for the food web dynamics as they are an important food source for many fish and invertebrates and exert control over the phytoplankton (Sommer et al., 2003; Lazzaro et al., 2003; Llope et al., 2012). In the present study we also found that the glyphosate-based herbicide reduced the evenness of the hatching assemblages at the end of the experiment, with a remarkable increase of bdelloids relative to other species. From an

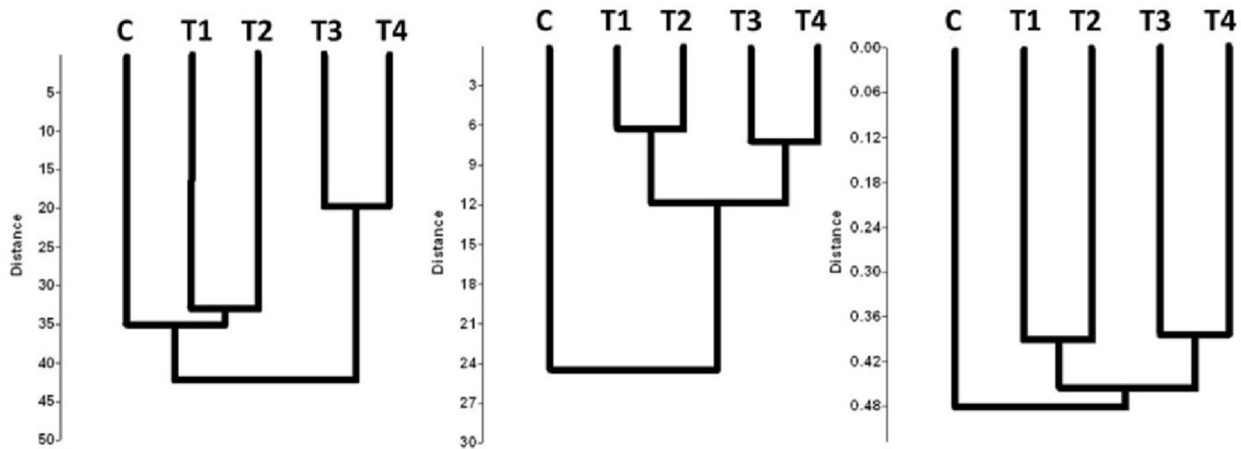


Fig. 8. Comparison of the taxonomic composition of hatchlings among treatments (C: control; T1–T4: treatments with the herbicide) through the measurement of the Euclidian Distance for TFH (a) and FH (b), and abundances (c) following the Unweighted pair-group method with arithmetic mean (UPGMA).

ecotoxicological point of view, there are previous studies evaluating the effects of glyphosate formulations on the active community of zooplankton (Chen et al., 2004; Vera et al., 2012; Reno et al., 2014; Baker et al., 2016); however, the noticeable increase in bdelloids at the expense of other species has not been previously documented.

Another cause of unevenness may be related to the decrease of cladocerans, particularly Chydoridae, in treatments with higher glyphosate concentrations. This effect is consistent with laboratory and field experiments showing that active cladocerans are more sensitive to the toxic effects of pesticides, compared with active rotifers and copepods (José de Paggi, 1997; Reno et al., 2014). In addition, the fact that the Cyclopoid nauplii were not affected by the pesticide suggests that the effects of glyphosate on dormant stages during the hatching process differ among and within species and provides the hypothesis that dormant stages mirror the same order of sensitivity possessed by active organisms.

An important trait of biological cycles is the ability to synchronize lifecycles with environmental and biological factors (Grist and Gurney, 1997; Lara-Lopez and Neira, 2008; Battauz et al., 2014). The establishment of such seasonal rhythms gives organisms the advantage of optimizing resources while promoting species coexistence (Hairston and Kearns, 2002). Here, we found that the HF, TMH and TFH were modified in most species due to the glyphosate application. While these results have limitations to be directly inferred to the field, it is plausible to assume that if such effects on the specific hatching rhythms (furtherance or delays) occur under natural conditions, imbalances in biological cycles may be caused. Accordingly, their interactions with other species (e.g.: preys, predators or competitors) and their particular sensitivity to natural environmental changes (droughts, oxygen depletion, changes in temperature or hydroperiod) may be strongly disrupted as well. Finally, treatments with 0.6 and 1.4 mg/L (T1 and T2) produced a similar moderate effect in the assemblies, while the higher concentrations (2.7 and 5; i.e.: T3 and T4) produced more severe alterations (e.j.: less richness, abundance and diversity).

The effects caused by the herbicide on the hatching dynamics of zooplankton suggest that this substance has the property to pass or associate with the membranes covering the resistance stages. Although the life cycle of the active ingredient (glyphosate) is short (c.a. within 14 days; Giesy et al., 2000; Perez-Jones et al., 2007; Degenhardt et al., 2012), one application was enough to cause negative effects in the analyzed community. Accordingly, other authors showed that certain pollutants are able to accumulate in resting stages, affecting the hatching and survival of the animals

(Wyn et al., 2007; Rogalski, 2015; Navis et al., 2015). For example, Aránguiz-Acuña and Serra (2016) have recently suggested that arsenic accumulates transgenerationally not only in females, but also in diapausing eggs, reinforcing our hypothesis. Together, these studies show that although diapausing stages can provide protection against extreme natural conditions, their susceptibility to certain anthropogenic stressors could seriously affect natural communities.

Several studies have demonstrated the relatively rapid recovery of zooplankton communities following the removal of perturbations due to their high dispersal rates (Louette and De Meester, 2005; Cáceres and Soluk, 2002) and the presence of dormant stages in sediments (De Stasio, 1989; Hairston, 1996) supporting the assumption of high resilience. However, recovery failure has been documented in some zooplankton species, suggesting that high resilience may not be universal (McNaught et al., 1999; Sarnelle and Knapp, 2004; Gutierrez et al., 2016). Probably, the sensitivity of resting stages to some specific environmental stressors (such as pesticides) may be playing a role in such recovery hampering. Taking into account that agricultural expansion is one of the major threats to biodiversity conservation in continental waters all over the world, further analysis along this line is of crucial importance. Since a special focus should also be placed on the analysis of the resistance and resilience of aquatic systems contaminated with glyphosate, the hatching process is suggested as a useful monitoring tool for such kind of studies.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.chemosphere.2016.12.110>.

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