JEB®

Journal of Environmental Biology

p-ISSN: 0254-8704 • e-ISSN: 2394-0379 • CODEN: JEBIDP
Journal website : www.jeb.co.in ★ **E-mail :** editor@jeb.co.in

Triveni Enterprises (Educational Services Set-up)

Original Research

DOI : http://doi.org/10.22438/jeb/43/1/MRN-1471

Effect of a glyphosate formulation on freshwater plankton: A community combined metric approach

W.M. Polla[1], L. Regaldo[1,2], U. Reno[1,2], A. Popielarz[3], S. Gervasio[3], V. Fernández[1] and A.M. Gagneten[1]*

[1]Laboratorio de Ecotoxicología. Departamento de Ciencias Naturales. Facultad de Humanidades y Ciencias. Universidad Nacional del Litoral (UNL). CP 3000. Santa Fe. Argentina
[2]Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Godoy Cruz 2290, Buenos Aires, Argentina
[3]Instituto Nacional de Tecnología (INTEC – CONICET - UNL). Parque Tecnológico Litoral Centro. CP 3000, Santa Fe. Argentina

***Corresponding Author Email :** amgagneten@gmail.com

Paper received: 08.04.2020 *Revised received*: 17.07.2020 *Accepted*: 25.08.2020

Abstract

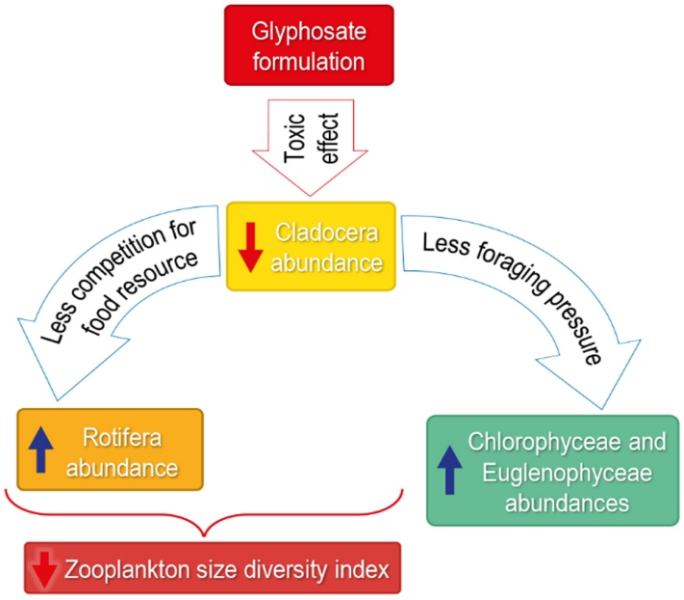
Aim: The aim of this study was to experimentally assess the effects of glyphosate formulation on plankton composition by using different community parameters through a mesocosm experiment.

Methodology: A 600 l mesocosm experiment was performed for 7 days, including a control (without glyphosate) and two concentrations of glyphosate.

Results: Glyphosate caused a significant decrease in cladoceran density and a significant increase in rotifer, Chlorophyceae, and Euglenophyceae densities. In addition, zooplankton size diversity as well as microalgal evenness diminished.

Interpretation: The decrease in cladoceran density may have benefited rotifers since they are less competitive for food resources. Moreover, the decrease in cladoceran foraging pressure over Chlorophyceae and Euglenophyceae may have benefited them. The different tolerances and competitiveness within the plankton components make the structure of this community a good indicator of environmental disturbance.

Key words: Community parameters, Glyphosate, Herbicide, Mesocosm, Zooplankton



How to cite : W.M. Polla, L. Regaldo, U. Reno, A. Popielarz, S. Gervasio, V. Fernández and A.M. Gagneten: Effect of a glyphosate formulation on freshwater plankton: A community combined metric approach. *J. Environ. Biol.*, **43**, 35-42 (2022).

Introduction

Agricultural practices have increased in the last years to meet the population's food demands. To this end, two approaches have been used: (i) expansion, by the use of new land, usually in environmentally fragile areas, (ii) intensification, by increasing the productivity of cultivated land. Intensification of agricultural practices involves the use of high amounts of fertilizers and pesticides. The agrochemicals applied can reach freshwater bodies by drift, leaching or runoff, affecting non target organisms (Amorós *et al.,* 2007; Sasal *et al.,* 2015). There is a growing concern about the pollution of continental water bodies by agrochemicals, because their species and habitat diversity as well as their ecosystem services are declining (Erwin, 2009). Therefore there is an urgent need to study the effects of agrochemicals on the aquatic biota and the processes that support environmental biodiversity and its functionality.

Glyphosate [N-(phosphonomethyl)glycine] (Gly), a broad-spectrum postemergence non-selective herbicide, is the most globally used for weed control in urban and rural areas (Annett *et al.,* 2014). Wide use and ubiquity of glyphosate implies a continuous exposure for the biota, demanding an extensive analysis of its effects on the environment (Primost *et al.,* 2017). Glyphosate toxicity to non-target freshwater organisms has been largely documented (Rico-Martinez *et al.,* 2012; Annett, *et al.,* 2014; Kharat *et al.,* 2020), but most of these studies focus on single-species effects or lethal toxicity, and the information is incipient and fragmented. Ecologically relevant effects cannot be predicted based on single-species approaches because they depend on both the interaction between organisms (intra- and interspecific) and their relation with the environment. In this way, experimental ecosystem approaches allow us to understand how pesticides may affect ecosystems as a whole (Lozano *et al.,* 2018).

Plankton is a potential bio-indicator of aquatic pollution since they have short generation time, respond rapidly to the environmental changes and anthropogenic disturbances (DeLorenzo *et al.,* 2001; Resh, 2008). Regarding the variety of responses between the components of zooplankton, it has been demonstrated that microcrustaceans, especially cladocerans, are more sensitive to pesticides than rotifers, which seem to tolerate them better (Vera *et al.,* 2012). Moreover, it has been suggested that there could be a direct relation between the size and sensitivity to pollutants in different components of zooplankton community, the largest being the most sensitive (Hanazato, 2001). Regarding microalgae, it has been suggested that different classes can respond to pesticides in different ways. Pesticides can affect microalgae through direct toxicity, or indirectly by modifying the nutrient cycle and then, the nutrient sources for microalgae (Pérez *et al.,* 2007).

The use of combined metrics (e.g., species diversity, richness, density and body size diversity) may allow a better comprehension of the effects of stress factors (such as a herbicide application) on the plankton community as well as their influence on the structure and function of the community, because these metrics provide different information. The species richness provides qualitative information on the community composition; this metric is usually complemented with quantitative information, the relative densities. Moreover, both metrics can be integrated into the diversity index. Several authors have focused on the importance of joining these traditional taxonomic metrics with others that provide different information (Mouillot *et al.,* 2006; Gallardo *et al.,* 2011). In this sense, the size diversity index, which integrates body size and densities, may provide information about food web energy fluxes, feeding ecology, trophic structure, and niche segregation between species or their developmental stages (Basset *et al.,* 2004; Gallardo *et al.,* 2011). In light of the above, this study was carried out to assess the effects of glyphosate formulation on the plankton composition and variability by using different community parameters through a mesocosm experiment.

Materials and Methods

The experiment was performed outdoors in 600l rectangular pools (0.5 m deep) for 7 days with permanent aeration. A combination of lake water and tap water was used in a proportion 1/3 v/v to ensure a gradual acclimation of the assemblages. Tanks were inoculated with microalgae, zooplankton and a floating plant, *Nymphoides* sp., collected from shallow lakes of middle Parana River floodplain. *Nymphoides* sp. was added to the tanks, covering about one third of the surface in order to encourage natural conditions providing refuge for the fauna and contributing to nutrient cycling. After adding assemblages, the mesocosms were left undisturbed for three days for stabilization. At day four, glyphosate was homogeneously dripped into the corresponding pools, and water was carefully stirred to ensure homogeneity of glyphosate in pool water.

The pools were left to stabilize for 2 h. The experiment consisted of three treatments: one control (C0) and two concentrations (C1 and C2) of the glyphosate commercial formulation (CREDIT®, Nufarm S.A., Buenos Aires, Argentina) containing 48% isopropylamine salt of N-phosphonomethyl glycine and unspecified inert ingredients and adjuvants. Each treatment was replicated, thus a total of six mesocosms were established and monitored for 7 days. Samplings were made at days: 1 (2 h after adding the herbicide) (T1), 3 (T2), 5 (T3) and 7 (T4). At each sampling time, pool water samples were taken for estimation of glyphosate (500 ml amber glass bottles). Water samples of zooplankton and microalgae were also taken, and physicochemical parameters were monitored: pH, temperature (°C), conductivity (µS cm^{-1}), and dissolved oxygen (mg l^{-1} O_2) using Hanna portable probes. Also, at each sampling time water samples were taken for soluble reactive phosphorus, ammonium, nitrite, and nitrate determinations, according to APHA (2017). Water samples were also taken for chlorophyll analysis, according to APHA (2017). A total of 30 samples were taken at each sampling time. The selected concentrations of glyphosate were close to those reported by Relyea (2005), Pérez *et al.* (2007) and Vera *et al.* (2010). The glyphosate concentrations were determined using a SHIMADZU Prominence 20A Series chromatograph equipped with a fluorescence detector (SHIMADZU RF-10AxL; SHIMADZU Corporation, Kyoto, Japan) and a column (Phenomenex Luna NH2 Part No. 00G-4378-Y0). The effective concentrations used in the experiments were 6.2

mg l^{-1} (±0.49) for C1 and 12.7 mg l^{-1} (±1.82) for C2, Quantitative zooplankton samples were collected with a trap-tube sampler of 45 µm pore mesh (total filtered volume 8 l: three integrated samples from different points of the tanks), fixed and colored with formaldehyde 4% and erythrosine.

The individuals were identified and quantified using specific taxonomic keys (Koste, 1978; Reid, 1985; Smirnov, 1992; Paggi, 1995) in 1ml Sedgewick Rafter chamber in an optical microscope (Olympus Cx31). Up to 3 aliquots were examined until at least 100 individuals of the most frequent species were counted: otherwise, the whole sample was quantified. The total body length of zooplanktons was measured with a microscale attached to the microscope eyepiece. Water for microalgae quantification was sampled with a 100 ml bottle (taken in the subsurface layer, integrating different points of the tanks) and fixed with 1% of Lugol acidified solution. Qualitative samples were collected with a 10 µm pore mesh and fixed with formaldehyde 4%. Microalgae were quantified using an inverted microscope and sedimentation chambers until at least 100 individuals of the most frequent species (Utermöhl, 1958). Individuals were considered as the unit present in the samples (unicell, colony, coenobium or filament). Species were identified with an optical microscope (Olympus CX31). Bacillariophyta frustules were treated with hydrogen peroxide (100 volumes) and hydrochloric acid at 80°C for 2 h, and washed with distilled water before permanent mounting with Naphrax® (refractive index = 1.74) (Battarbee, 1986). Taxonomic identification was carried out as perTell and Conforti (1986);Komárek and Anagnostidis (1999; 2005); Metzeltin *et al.* (2005); Komárek (2013).

Data analysis: To assess the possible difference in the environmental parameters between treatments, Kruskal-Wallis tests were performed. Also, Freedman tests were conducted to assess the possible variations of these variables through time.

The community attributes estimated for both zooplankton and microalgae were: Shannon diversity index (Shannon and Weaver, 1964), species richness (number of species) and individual density (ind. l^{-1}). Also, for zooplankton, the size diversity index was estimated using "diversity 08" (Quintana *et al.,* 2008). Kruskal-Wallis tests were applied to assess the difference in zooplankton and microalgae community attributes between treatments. To analyze the effect of time on the structure of each assemblage, Freedman tests were performed for each treatment. Linear correlations (Spearman test) were performed using glyphosate concentrations detected in both treatments at each sampling time and some individuals' taxa densities in order to identify possible effects of the pesticide. Zooplankton total body lengths were grouped into 10 size ranges (100 µm), the total density per rank was calculated for each pool and the replicate averages per treatment were compared using Chi^2 test.

Results and Discussion

All environmental parameters measured, i.e., dissolved oxygen (76% – 88.7%), temperature (16.7 – 22.5 °C), pH (6.3 – 6.7) and conductivity (175 – 192.7µs s^{-1}) did not vary significantly either in time or between treatments (p>0.05).Nitrites decreased significantly over time in C2 (p=0.008) (T0: 0.05, T4: 0.002 mgl^{-1}). Nitrates (2.3 – 2.7 mg l^{-1}), phosphates (0.3 – 3.5 mg l^{-1}) and ammonia (0.008 – 0.5 mg l^{-1}) did not vary significantly either through time or between treatments (p>0.05). Chlorophyll a (2.4 – 9 µg l^{-1}) did not vary significantly either over time or between treatments (p>0.05). Nevertheless, it tended to increase in C1 (T0: 4, T4: 12.9 µg l^{-1}) and C2 (T0: 6.4, T4: 9 µg l^{-1}) over time. The degradation rate of glyphosate was 0.2 mg l^{-1} day^{-1} in C1 and 0.4 mg l day^{-1} in C2 (Table 1). Zooplankton species richness and

Table 1: Concentration of glyphosate (mg l^{-1}) in experimental pool water inoculated with microalgae, zooplankton and the floating plant *Nymphoides* sp.

	T0	T1	T2	T3	T4
C0	0.2 ± 0.0	0.4 ± 0.4	0.3 ± 0.0	0.1 ± 0.0	0.7 ± 0.0
C1	6.2 ± 0.5	6.4 ± 1.1	5.5 ± 0.4	5.6 ± 0.3	4.9 ± 0.5
C2	12.7 ± 1.8	12.5 ± 1.1	10.3 ± 0.9	11.5 ± 0.4	15.5 ± 0.5

Values are mean ± standard deviation

Table 2: Species richness and Shannon diversity index for zooplankton and microalgae exposed to glyphosate

		Zooplankton		Microalgae	
		T0	T4	T0	T4
Richness	C0	32.0 ± 2.8	21.5 ± 0.7	25.0 ± 7.1	13.0 ± 1.4
	C1	31.0 ± 1.4	26.5 ± 2.1	25.5 ± 10.6	12.5 ± 0.7
	C2	34.0 ± 4.2	30.5 ± 3.5	22.0 ± 2.8	11.0 ± 2.8
Shannon diversity	C0	2.4 ± 0.1	2.2 ± 0.0	2.3 ± 0.4	1.7 ± 0.1
	C1	2.3 ± 0.6	2.0 ± 0.0	2.0 ± 0.2	1.7 ± 0.2
	C2	2.5 ± 0.1	2.0 ± 0.1	2.0 ± 0.3	1.2 ± 0.1

Values are mean ± standard deviation

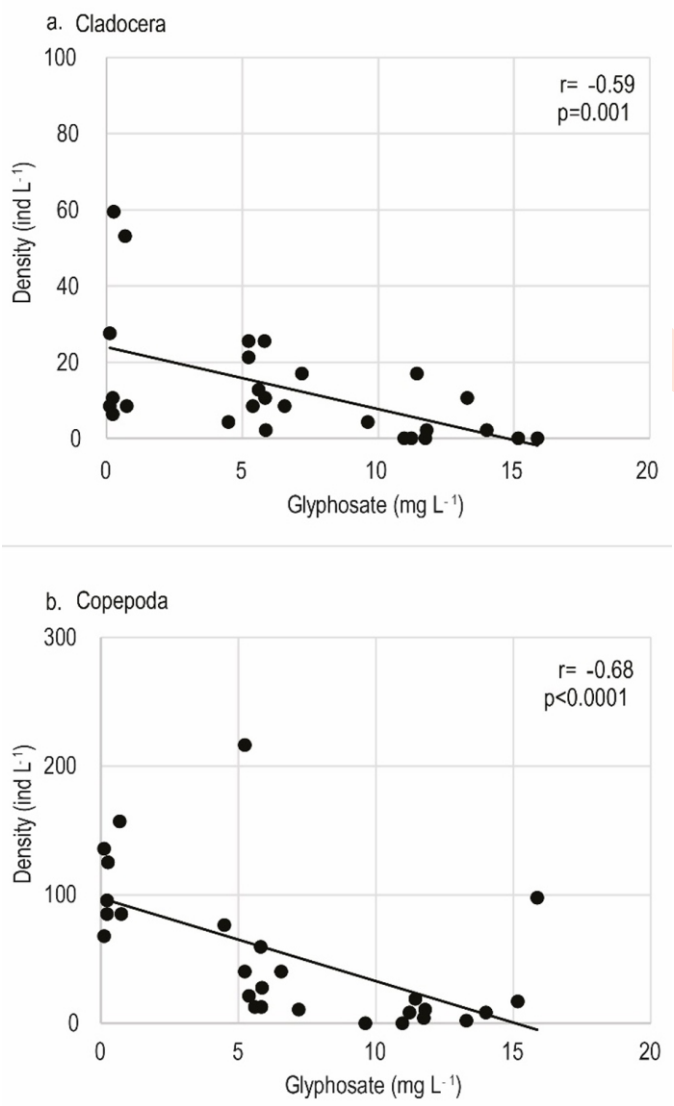


Fig. 1: Inverse correlation between glyphosate concentration and (a) Cladocera and (b) Copepoda densities.

Zooplankton Shannon diversity index did not vary significantly either over time or between treatments ($p>0.05$) (Table 2). Conversely, cladoceran density decreased significantly in C1 and C2 (T4: C1: 14.9, C2: 0 ind. l^{-1}) with respect to C0 (T4: C0: 54.1 ind.l^{-1}) ($p=0.0005$). Also, a significant inverse correlation was observed between glyphosate concentration (C1 and C2) and cladoceran and copepod densities ($r= -0.59$ $p=0.001$; $r= -0.68$ $p<0.0001$) (Fig. 1). Total rotifer density increased significantly in both C1 and C2 (T4: C1: 2418, C2: 2391 ind. l^{-1}) with respect to C0 (T4: C0: 170 ±0.001 ind. l^{-1}) ($p=0.003$). The most abundant rotifer taxa were *Asplachna* sp., *Brachionus calyciflorus* and *B. angularis*; their densities also increased significantly in C1 and C2 ($p=0.006$) (Fig. 2). At the end of the experiment, the size diversity index was lower in C1 (2.26) and C2 (2.23) with respect to C0 (3.17).Similarly, the size spectra were significantly lower in C1 (70-800 µm) and C2 (70-700 µm) than in C0 (70-1500 µm) (Chi2 test, C0 vs. C1: $x^2=102$ $p<0.001$; C0 vs. C2: $x^2= 107.9$ $p<0.001$) (Fig. 3 a).

Microalgae species richness and Shannon diversity index did not vary significantly either over time or between treatments ($p>0.05$) (Table 2). There were no significant differences between treatments in the microalgae class densities ($p>0.05$). Also, no significant correlation between different microalgae class densities and the glyphosate concentration was observed ($p> 0.05$). Nevertheless, in T4 the individuals' density distribution per class was significantly more even in the control than in treatments (Chi2 test, C0 vs. C1: $x^2= 54$ $p<0.001$; C0 vs. C2: $x^2=54.2$ $p <0.001$) (Fig. 3 b.). Chlorophyceae and Euglenophyceae densities increased significantly in C1 over time

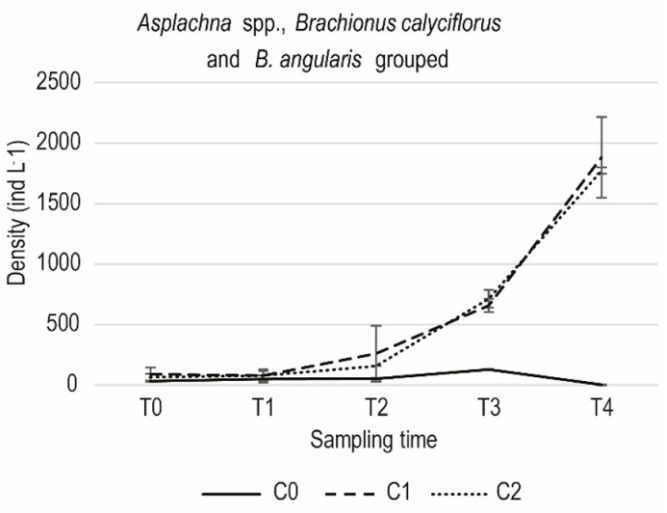


Fig. 2: Grouped densities of *Asplachna* sp., *Brachionus calyciflorus* and *B. angularis* in C0: control, C1: 6.2 mg l^{-1} and C2: 12.7 mg l^{-1} at different sampling times.

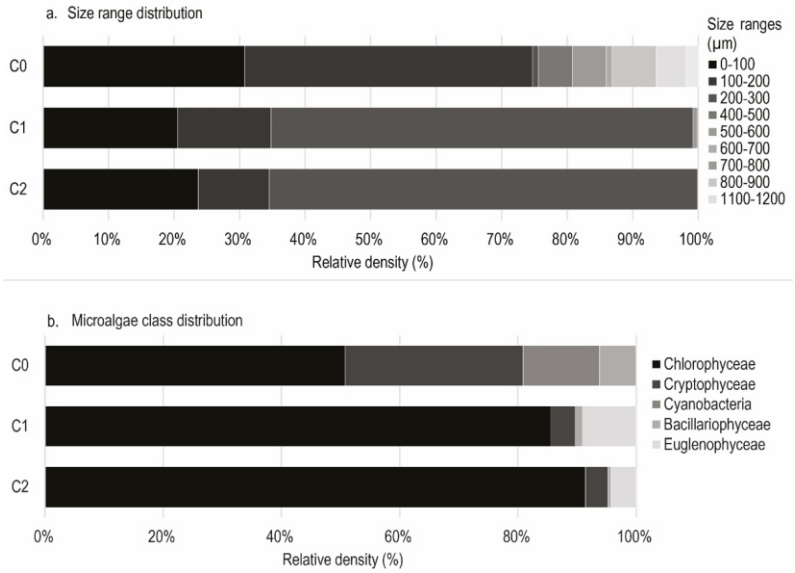


Fig. 3: (a) Zooplankton relative density distribution in the different size ranges at the end of the experiment (T4); (b) Microalgae relative density distribution in the different classes at the end of the experiment (T4). C0: control, C1: 6.2 mg l^{-1} and C2: 12.7 mg l^{-1}.

($p<0.05$ in both cases) and tended to increase in C2 over time (Fig. 4). The density of Bacillariophyceae tended to decrease in C1 (T0: 3803, T4: 536 ind. l^{-1}) and C2 (T0: 2735, T4: 357 ind. l^{-1}) over time ($p=0.07$ and 0.08). For both assemblages studied in this work, zooplankton and microalgae, the Shannon diversity index and the species richness did not reflect the herbicide effects. Although they are widely employed, several authors have questioned the use of these two taxonomic metrics alone to assess anthropic effects on the environment (Reizopoulou *et al.,* 1996; Gallardo *et al.,* 2011). They have argued that these metrics cannot necessarily discriminate between natural stress and anthropogenic impact. Moreover, some authors have pointed out that these metrics have limitations to detect changes at the community level (Mouillot *et al.,* 2006). Instead, Morais da Rosa *et al.* (2020) stated that through organism density, it is possible to perceive the factors that affect reproductive events, changing the number of individuals and promoting variations in the dominance patterns. Copepod and Cladoceran densities were negatively affected by the glyphosate formulation. Microcrustaceans, especially cladocerans, have been proposed to be particularly

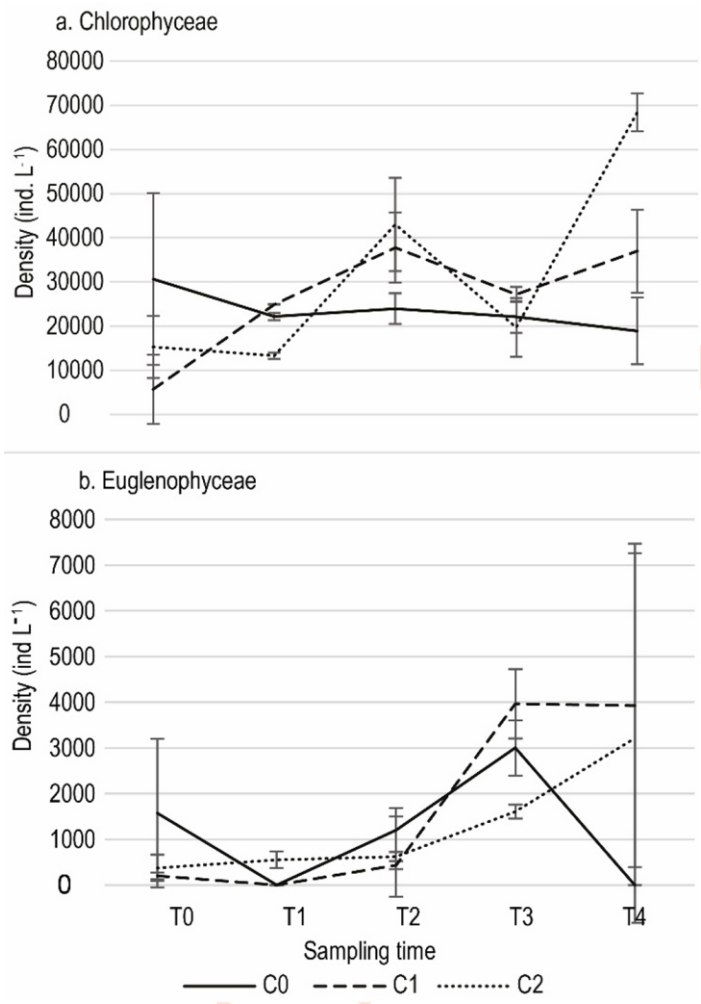


Fig. 4: Density of (a) Chlorophyceae and (b) Euglenophyceae in C0: control, C1: 6. 2 mg l^{-1} and C2: 12.7 mg l^{-1} at different sampling times.

sensitive to environmental disturbances (Sakamoto *et al.,* 2006; Rico-Martinez *et al.,* 2012; Vera *et al.,* 2012).

Some authors explained this sensitivity by the fact that microcrustaceans are especially exposed to chemicals after molting and that also large-sized animals molt more times than smaller ones prior to reproduction (Gliwicz and Sieniawska, 1986). On the contrary, rotifer density increased significantly in the treatments with glyphosate formulation. On the one hand, it may have been due to their tolerance to pesticides, which has been shown in several studies (Wendt-Rasch *et al.,* 2003; Chang *et al.,* 2005). On the other hand, their low competitiveness for food resources with respect to microcrustaceans could have benefited rotifers by the decrease in microcrustacean densities (Hanazato 1998, Vera *et al.,* 2010). In this study *Brachionus calyciflorus*, *B. angularis* and *Asplachna* sp. showed a particular density increase. This suggests that these species are particularly tolerant to the glyphosate formulation, and their densities could be used as indicative of glyphosate contamination. In congruence with the above-discussed, the size diversity index was lower in the treatments with glyphosate formulation with respect to the control.

This relation between zooplankton size and its sensitivity has been proposed by several authors (Gallardo *et al.,* 2011). Gliwicz and Sieniawska (1986) found a positive interaction between body size and sensitivity to lindane in cladocerans. Havens and Hanazato (1993) proposed that rotifers have more diverse life histories than microcrustaceans, which is why they are likely to adapt better to the environmental changes as this group contains both tolerant and sensitive taxa. The observed co-occurrence of different tolerances and competitiveness within the zooplankton community determines the community structure. This attribute is a good indicator of environmental disturbance, since zooplankton community plays a key role in freshwater environments as intermediate links in trophic networks (Mbandzi *et al.,* 2018).

Consequently, the changes in this community structure can affect other trophic levels through top-down and bottom-up effects (Carpenter and Kitchells, 1995). Also as the zooplankton community quickly responds to environmental factors (Perbiche-Neves *et al.,* 2013), its response to contaminants could be informative of the impacts on the ecosystem as a whole (Hanazato, 2001). Conversely to zooplankton, microalgae did not seem to have been directly affected by the herbicide but by zooplankton foraging pressure: Chlorophyceae and Euglenophyceae increased through time in glyphosate formulation treatments, which was also observed in the increase of chlorophyll a. These responses could have been triggered by changes in zooplankton foraging pressure because of the decrease in microcrustacean density. This top-down effect has been proposed in several studies (Sinistro *et al.,* 2007). On the other hand, a positive effect of glyphosate formulations may have occurred for such groups since this herbicide may have provided phosphate as a source for algae (Austin *et al.,* 1991; Pérez *et al.,* 2007).

Bacillariophyceae density decreased with time in glyphosate treatments. For instance, Pérez *et al.* (2007) and Vera *et al.* (2010) reported a decrease in Bacillariophyceae density by exposing a periphyton assemblage to Roundup® in mesocosms. Also, Vera *et al.* (2012) reported that diatoms appeared as the periphytic algae most sensitive to Glyphosate Atanor® in mesocosms; in addition, Wood *et al.* (2016) stated that freshwater diatoms can be promising bioindicators of toxicity of herbicide with different modes of action. It is important to note that according to the safety data sheet of the glyphosate formulation used in this work, the product is slightly toxic to aquatic organisms (LC50> 10 to 100 mg l^{-1}) (US EPA, 2012). The results obtained in this work show that concentrations <10 mg l^{-1} of glyphosate can produce changes in the plankton community, potentially affecting the integrity of freshwater environments. In this sense, the use of different analysis scales (microcosm, mesocosm, and field) and indicators (survival, growth, fertility, species density, diversity) allows for more accurate risk assessments of herbicide formulations. In conclusion, the use of combined metrics of plankton community would be an appropriate approach to identify different effects of glyphosate formulations. Since alterations in zooplankton size structure can modify the microalgae community, the change in the whole plankton community could be a good approach to assess herbicide effects.

Acknowledgment

This research was supported by the National University of the Littoral (Research Project CAI+D: 50120110100215LI).

Add-on Information

Authors' contribution: W.M. Polla, L. Regaldo, U. Reno: Formal analysis, Investigation, Methodology, Review and Editing; **A. Popielarz:** Methodology; **S. Gervasio, V. Fernández:** Methodology, Review and Editing; **A.M. Gagneten:** Writing – Review and Editing, Supervision, Project administration. Funding acquisition.

Research content: The research content of manuscript is original and has not been published elsewhere.

Ethical approval: Not applicable.

Conflict of interest: The authors declare that there is no conflict of interest.

Data from other sources: Not applicable.

Consent to publish: All authors agree to publish the paper in *Journal of Environmental Biology*

References

Amorós, I., J.L. Alonso, S. Romaguera and J.M. Carrasco: Assessment of toxicity of a glyphosate-based formulation using bacterial systems in lake water. *Chemosphere,* **67**, 2221-2228 (2007).

Annett, R., H.R. Habibi and A. Hontela: Impact of glyphosate and glyphosate-based herbicides on the freshwater environment. *J. Appl. Toxicol.,* **34**, 458-479 (2014).

APHA (American Public Health Association): Standard Methods for the Examination of Water and Wastewater, 23th Edn., APHA, AWWA, WPCF, Washington DC, USA (2017).

Austin, A.P., G.E. Harris and W.P. Lucey: Impact of an organophosphate herbicide (Glyphosate R) on periphyton communities developed in experimental streams. *B. Environ. Contam. Tox.,* **47**, 29-35 (1991).

Basset, A., F. Sangiorgio and M. Pinna: Monitoring with benthic macroinvertebrates: Advantages and disadvantages of body size descriptors. *Aquat. Conserv.,* **14**, 43-58 (2004).

Battarbee, R.: Diatom Analysis. In: Handbook of Holocene Palaeoecology and Palaeohydrology (Eds.: B.E. Berglund) John Wiley and Sons, Chichester, UK, pp. 527-570 (1986).

Carpenter, S.R. and J.F. Kitchell: The Trophic Cascade in Lakes. Cambridge Studies in Ecology. Cambridge University Press, Cambridge, NY, USA, p. 385 (1995).

Chang, K.H., M. Sakamoto and T. Hanazato: Impact of pesticide application on zooplankton communities with different densities of invertebrate predators: An experimental analysis using small-scale mesocosms. *Aquat. Toxicol.,* **72**, 373-382 (2005).

DeLorenzo, M.E., G.I. Scott and P.E. Ross: Toxicity of pesticides to aquatic microorganisms: A review. *Environ. Toxicol. Chem.,* **20**, 84-98 (2001).

Erwin, K.L.: Wetlands and global climate change: The role of wetland restoration in a changing world. *Wetl. Ecol. Manag.,* **17**, 71 (2009).

Gallardo, B., S. Gascón, X. Quintana and F.A. Comín: How to choose a biodiversity indicator–redundancy and complementarity of biodiversity metrics in a freshwater ecosystem. *Ecol. Indic.,* **11**, 1177-1184 (2011).

Gliwicz, M.Z. and A. Sieniawska: Filtering activity of Daphnia in low concentrations of a pesticide. *Limnol. Oceanogr.,* **31**, 1132-1138 (1986).

Hanazato, T.: Response of a zooplankton community to insecticide application in experimental ponds: A review and the implications of the effects of chemicals on the structure and functioning of freshwater communities. *Environ. Pollut.,* **101**, 361-373 (1998).

Hanazato, T.: Pesticide effects on freshwater zooplankton: An ecological perspective. *Environ. Pollut.,* **112**, 1-10 (2001).

Havens, K.E. and T. Hanazato: Zooplankton community responses to chemical stressors: a comparison of results from acidification and pesticide contamination research. *Environ. Pollut.,* **82**, 277-288 (1993).

Kharat, T.L., K.B. Rokade and K.B. Shejule: Effect of Roundup 41% (glyphosate) on blood serum biochemical parameters of freshwater fish, *Rasbora daniconius*. *J. Environ. Biol.,* **41**, 222-227 (2020).

Komárek, J.: Cyanoprokaryota. 3. *Heterocytous* genera. In: Süswasserflora von mitteleuropa [Freshwater Flora of Central Europe] (Eds.: B. Büdel, G. Gärtner, L. Krienitz and M. Schagerl). Springer Spektrum, Berlin, Heidelberg, p. 8 (2013).

Komárek, J. and K. Anagnostidis: Cyanoprokariota. 1. Chroococcales. In: Subwasserflora von mitteleuropa [Freshwater Flora of Central Europe] (Ed.: G. Fisher). Stutgart, Jena, p. 548 (1999).

Komárek, J. and K. Anagnostidis: Cyanoprokaryota 2. Oscillatoriales. In: Süsswasserflora von mitteleuropa [Freshwater Flora of Central Europe] (Eds.: B. Büdel, L. Krienitz, G. Gärtner and M. Scnagerl). ElsevierSpektrum, Heidelberg, p. 759 (2005).

Koste, W.: Rotatoria. The rotifers of Central Europe. 2nd Edn., Gebruder Borntraeger, Berlin and Stuttgart, p. 673 (1978).

Lozano, V.L., A. Vinocur, C.S. García, L. Allende, D.S. Cristos, D. Rojas, M. Wolansky and H. Pizarro: Effects of glyphosate and 2,4-D mixture on freshwater phytoplankton and periphyton communities: A microcosms approach. *Ecotox. Environ. Safe.,* **148**, 1010-1019 (2018).

Mbandzi, N., R.J. Wasserman, S.H.P. Deyzel, N.G. Vine and A.K. Whitfield: River flow, zooplankton and dominant zooplanktivorous fish dynamics in a warm-temperate South African estuary. *J. Fish Biol.,* **92**, 1747-1767(2018).

Metzeltin, D., L. Bertalot and F. Garcia-Rodriguez: Diatoms of Uruguay: Compared with other taxa from South America and elsewhere. Ruggell, A.R.G. GantnerVerlag KG, p. 736 (2005).

Morais da Rosa, L., L.D.S. Cardoso, L.R. Rodrigues and D. da Motta-Marques: Density versus biomass responses of zooplankton to environmental variability in a subtropical shallow lake. *Inland Waters*, **11**, 44-56 (2021).

Mouillot, D., S. Spatharis, S. Reizopoulou, T. Laugier, L. Sabetta, A. Basset and T. Do Chi: Alternatives to taxonomic-based approaches to assess changes in transitional water communities. *Aquat. Conserv.,* **16**, 469-482 (2006).

Paggi, J.C.: Cladocera. In: Ecosistemas de aguas continentales. Metodología para su estudio [Ecosystems of Continental Waters. Methodology for Their Study] (Eds.: E. Lopretto and G. Tell). Ed. Sur, La Plata, Argentina, pp. 909-951 (1995).

Perbiche-Neves, G., C. Fileto, J. Laço-Portinho, A. Troguer and M. Serafim-Júnior: Relations among planktonic rotifers, cyclopoid copepods and water quality in two Brazilian reservoirs. *Lat. Am. J. Aquat. Res.,* **41**, 138-149 (2013).

Pérez, G.L., A. Torremorell, H. Mugni, P. Rodriguez, M.S. Vera, M. Do Nascimento, L. Allende, J. Bustingorry, R. Escaray and I. Izaguirre: Effects of the herbicide roundup on freshwater microbial communities: A mesocosm study. *Ecol. Appl.,* **17**, 2310-2322 (2007).

Primost, J.E., D.J. Marino, V.C. Aparicio, J.L. Costa and P. Carriquiriborde: Glyphosate and AMPA, "pseudo-persistent" pollutants under real-world agricultural management practices in the mesopotamic Pampas agroecosystem, Argentina. *Environ. Pollut.,* **229**, 771-779 (2017).

Quintana, X.D., S. Brucet, D. Boix, R. López-Flores, S. Gascón, A. Badosa, J. Sala, R. Moreno-Amich and J.J. Egozcue: A Nonparametric method for the measurement of size diversity with emphasis on data standardization. *Limnol. Oceanogr.,* **6**, 75-86 (2008).

Reid, J.W.: Chave de identificação e lista de referências bibliográficas para as espécies continentais sulamericanas de vida livre da orden Cyclopoida (Crustacea, Copepoda) [Identification key and list of bibliographic references for free-living South American continental species of the order Cyclopoida (Crustacea, Copepoda)]. Instituto de Biociências, Universidade de São Paulo, Brazil (1985).

Reizopoulou, S., M. Thessalou-Legaki and A. Nicolaidou:Assessment of distubance in mediterranean lagoons: an evaluation of methods. *Mar. Biol.,* **125**, 189-197 (1996).

Relyea, R.A.: The impact of insecticides and herbicides on the biodiversity and productivity of aquatic communities. *Ecol. Appl.,* **15**, 618-627 (2005).

Resh, V.H.: Which group is best? Attributes of different biological assemblages used in freshwater biomonitoring programs. *Environ. Monit. Assess.,* **138**, 131-138 (2008).

Rico-Martínez, R., J.C. Arias-Almeida, I.A. Pérez-Legaspi, J. Alvarado-Flores and J.L. Retes-Pruneda: Adverse effects of herbicides on freshwater zooplankton. In: Herbicides-Properties, Synthesis and Control of Weeds (Ed.: M.N. Hasaneen). Intech Open, Rijeka, Croatia, pp. 405-434 (2012).

Sakamoto, M., K.H. Chang and T. Hanazato: Inhibition of development of anti-predator morphology in the small cladoceran *Bosmina* by an Insecticide: Impact of an anthropogenic chemical on prey–predator interactions. *Freshwater Biol.,* **51**, 1974-1983 (2006).

Sasal, M.C., L. Demonte, A. Cislaghi, E.A. Gabioud, J.D. Oszust, M.G. Wilson, N. Michlig, H.R. Beldoménico and M.R. Repetti: Glyphosate loss by runoff and its relationship with phosphorus fertilization. *J. Agr. Food. Chem.,* **63**, 4444-4448 (2015).

Shannon, C. and W. Weaver: The Mathematical Theory of Communication. Urbana: University of Illinois Press, Illinois, USA (1964).

Sinistro, R., M.L. Sánchez, M.C. Marinone and I. Izaguirre: Experimental study of the zooplankton impact on the trophic structure of phytoplankton and the microbial assemblages in a temperate wetland (Argentina). *Limnologica,* **37**, 88-99 (2007).

Smirnov, N.N.: Mesozoic Anomopoda (Crustacea) from Mongolia. *Zool. J. Linn. Soc.,* **104**, 97–116 (1992).

Tell, G. and V. Conforti: Euglenophyta pigmentadas de la Argentina [Pigmented euglenophyta from Argentina]. (Ed.: J. Cramer) Bibliotheca Phycologica, Berlin, p. 301 (1986).

US EPA (United States Environmental Protection Agency): Technical Overview of Ecological Risk Assessment Analysis Phase: Ecological Effects Characterization. Last updated on July 14, 2020. Available from: https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/technical-overview-ecological-risk-assessment-0 (2012).

Utemohl, H.: Zur Vervollkommung der quantitativen phytoplankton-methodik [To Perfection the Quantitative Phytoplankton Method]. Internationale Vereinigung für theoretische und angewandte Limnologie. *Mitteilungen,* **9**, 1-38 (1958).

Vera, M.S., E. Di Fiori, L. Lagomarsino, R. Sinistro, R. Escaray, M.M. Iummato, A. Juárez, M.C. Ríos de Molina, G. Tell and H. Pizarro: Direct and indirect effects of the glyphosate formulation Glifosato Atanor® on freshwater microbial communities. *Ecotoxicology,* **21**, 1805-1816 (2012).

Vera, M.S., L. Lagomarsino, M. Sylvester, G.L. Pérez, P. Rodríguez, H. Mugni, R. Sinistro, M. Ferraro, C. Bonetto and H. Pizarro: New evidences of Roundup® (glyphosate formulation) impact on the periphyton community and the water quality of freshwater ecosystems. *Ecotoxicology,* **19**, 710-721(2010).

Wendt-Rasch, L., U. Friberg-Jensen, P. Woin and K. Christoffersen: Effects of the pyrethroid insecticide cypermethrin on a freshwater community studied under field conditions. II. Direct and indirect effects on the species composition. *Aquat. Toxicol.,* **63**, 373-389 (2003).

Wood, R.J., S.M. Mitrovic, R.P. Lim and B.J. Kefford: How benthic diatoms within natural communities respond to eight common herbicides with different modes of action. *Sci. Total Environ.,* **557**, 636-643 (2016).