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Comparative impact of two glyphosate-based formulations in interaction with *Limnoperna fortunei* on freshwater phytoplankton

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ABSTRACT

Although contamination and invasive species are two of the most relevant anthropogenic drivers affecting ecosystems, their joint impact on the environment has been poorly investigated. Glyphosate, directly or indirectly, contaminates freshwater systems which in turn may be invaded by the golden mussel *Limnoperna fortunei*. Under laboratory conditions, we studied the combined effect of technical-grade glyphosate, Roundup Max® and Glifosato Atanor®, in scenarios with and without *L. fortunei,* on phytoplankton from Salto Grande Reservoir (Uruguay River, Argentina). We expected that the effects of the interaction on phytoplankton and water quality would vary with the form of herbicide applied. The assay was conducted for 14 days (Tf) using 3-L bottles as experimental units. Eight treatments were performed in triplicate: C: Control; M: mussel; G: technical-grade glyphosate acid; R: Roundup Max®; A: Glifosato Atanor®; MG: mussel + technical-grade glyphosate acid, MA: mussel + Glifosato Atanor® and MR: mussel + Roundup Max®. The active ingredient was applied at 6 ppm. The dissipation of glyphosate in water was 1.5–2.6 times higher in presence of mussels. Treatments G and A showed an increase in phytoplankton abundance, mainly the cyanobacteria *Microcystis* spp. wich rised to 289% and 639% at Tf, respectively, relative to their values at Ti. Roundup Max® limited the growth of *Microcystis* spp., as its abundance decreased 59% relative to Ti. *L. fortunei* reduced phytoplankton abundances at Tf. Evenness increased significantly in M, MG, MR and MA, while it decreased in G, R and A relative to C. The interaction of factors produced a significant synergistic increase in periphyton; periphytic chlorophyll *a* concentration was $0.81 \pm 0.02\ \mu g\ cm^{-2}$ for MR; $0.09 \pm 0.02\ \mu g\ cm^{-2}$ for MA and $0.02 \pm 0.01\ \mu g\ cm^{-2}$ for MG. *Limnoperna fortunei* appeared as the driving force in the interaction. The assay described here allows for the rapid assessment of the impact of these types of agents on freshwater.

1. Introduction

Some human activities are direct drivers of ecosystem change, such as habitat fragmentation, climate change, species overexploitation, pollution and the introduction of invasive species (Millennium Ecosystem Assessment, 2005). In turn, direct drivers are influenced by indirect ones (e.g. technological development and the phenomenon of economic globalization), which also affect the structure and functioning of ecosystems. Most of these drivers are studied separately, but in fact they act simultaneously and the results of the interactions may be synergistic, antagonistic or additive (Townsend et al., 2008).

Over the past 50 years, the world population has doubled (World Population 2017) leading to an increase in human demands (Ojima et al., 1993). This resulted in greater land use and other practices that degrade soil fertility and water quality (Clark et al., 1986; Turner et al., 1993). Further intensification of human activities has caused the removal of native species, introduction of invasive species, changes in hydrological flows, and pollution of land, air and water (IGBP, 1990; WCED, 1987). The expansion of agricultural land has intensified the application of fertilizers and herbicides (Foley et al., 2005). An excessive input of nutrients to water bodies by the use of fertilizers is one of the main sources of pollution, reducing the capacity of these environments to assimilate and process wastes in the water. In consequence, numerous inland water bodies are deteriorated due to eutrophication. On the other hand, cyanobacterial blooms have emerged as an increasingly common event in lakes and coastal waters, and related eutrophication has become an issue of global concern (Bell and Codd, 1996; Carmichael, 1994; Conroy et al., 2005; Kalff, 2002).

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The herbicide glyphosate is the active ingredient of different commercial formulations applied worldwide; over 826 million kg of glyphosate were globally used for agricultural and non-agricultural practices in 2014 (Benbrook, 2016). In Argentina, where glyphosate is the main agrochemical used, over 320 million kg were sold in 2013 (CASAFE, 2013). Although glyphosate is applied to control undesirable weeds, it can reach water bodies by direct application or by indirect transport through wind and runoff after intense rainfall. Commercial formulations contain the active ingredient (glyphosate), inert elements and water. The active ingredient corresponds to a glyphosate salt, usually the isopropylamine salt (IPA), but there are also formulations with potassium or ammonium salt. The inert elements are solvents, surfactants and humectants of unknown composition which increase the permeability of the plant cuticle acting as a barrier to herbicide uptake (Lanctôt et al., 2014). The formulations vary depending on the type and concentration of the active component and on the type and concentration of surfactant or adjuvant aggregate (Giesy et al., 2000). Studies using algae and other organisms such as amphibians agree that the components of glyphosate-based formulations (i.e. surfactants and additives) contribute to the majority of the toxicity of the commercial formulations (Relyea and Jones, 2009; Fuentes et al., 2011; Lipok et al., 2010).

The golden mussel *Limnoperna fortunei* is an invasive freshwater species native to Southeast Asia, which was introduced to the coasts of La Plata River in the 1990s (Pastorino et al., 1993) through the ballast water of transoceanic ships. Adults are sessile and feed by filtering plankton and organic matter from water; they are involved in transferring part of the organic matter from the water column to the benthos, which may lead to the alteration of nutrient dynamics (Karatayev et al., 1997). The mussel-induced impacts are manifold and include increased water transparency due to its high filtration rates of up to 350 mL h^{-1} $individual^{-1}$ (Sylvester et al., 2005), as well as decreased phytoplankton abundance (Cataldo et al., 2012b) by inducing changes in the structure and function of freshwater ecosystems (Boltovskoy et al., 2009). In addition, the golden mussel exerts a grazing control on seston, modifying the proportion and availability of nutrients which may generate serious imbalances such as those favoring blooms of *Microcystis* spp. (Cataldo et al., 2012a).

Previous studies have shown that *L. fortunei* can reduce the half-life of glyphosate by more than 4-fold at a rate of 50.2 ± 3.4 mg per gram of mussel dry weight per day (Di Fiori et al., 2012).

Pizarro et al. (2015a) evaluated the impact of the combined effect of technical-grade glyphosate and *L. fortunei* on freshwater microbial communities using three concentrations of acid glyphosate (1, 3 and 6 ppm) in outdoor mesocosms. These authors found that the joint effect depended on the concentration of herbicide used and that the higher the herbicide dose, the higher the total phosphorus concentration and the larger the availability of phosphates. In addition, they reported that metaphyton was abundant in the treatments with glyphosate and mussels due to increased availability of nutrients and that both stressors acted synergistically on phosphate concentration, bacterioplankton abundance and water turbidity. Gattás et al. (2016), who investigated the combined effect of *L. fortunei* plus acid glyphosate with that of *L. fortunei* plus Roundup Max® on microbial communities in outdoor mesocosms, obtained different results according to the type of herbicide.

Taking into account that herbicides are currently applied as commercial formulations, the present work is focused on the effect of *Limnoperna fortunei* in combination with glyphosate and two widely used commercial formulations (Roundup Max® and Glifosato Atanor®) on water quality and phytoplankton using microcosms under controlled laboratory conditions. We analyzed physical and chemical variables of the water and the phytoplankton specific composition and diversity in an experiment of 14-days long. We used natural water from the Salto Grande Reservoir, which is a sink for the agrochemicals used in the region and harbors the mussel since the 2000s. We hypothesized that the effect of the herbicides and the golden mussel on phytoplankton and water quality would vary depending on whether they are used alone or in combination. We expected that the type of herbicide used in combination with mussels would influence the results.

2. Materials and methods

2.1. Experimental design

We conducted a manipulative laboratory experiment during April 2015 using water from the Salto Grande Reservoir (31°15′38, 16″S; 57°57′11, 84″W). It is a large (780 km^2), subtropical reservoir built in 1979 by damming the Uruguay River, where cyanobacterial blooms are increasingly common in mid-summer (Berón, 1990; Quiros and Luchini, 1983; De León and Chalar, 2003; Chalar, 2006). Boltovskoy et al. (2013) have reported a significant increase in the abundance of *L. fortunei* larvae in Salto Grande Reservoir since 2000. This, together with an extensive agrochemical use due to the dramatic expansion of the industrial agriculture in the region, makes the Salto Grande Reservoir a suitable model for studying the interaction between both anthropogenic stressors.

The assay was performed in an incubation chamber for 14 days under controlled light (1250 ± 180 Lux, LD 12:12 photoperiod), aeration and temperature (24 ± 1 °C) conditions. We used 24 microcosms (experimental units) consisting of 3-L plastic (PET, polyethylene terephthalate) bottles (Fig. 1) filled with water from the Salto Grande Reservoir, which was collected on the day of the beginning of the experiment. Air was supplied to each experimental unit via a centrally located aeration hose (10 mm in diameter) to allow recirculation of water and avoid sedimentation. Eight treatments were performed in triplicate: C: Control; M: mussel; G: technical-grade glyphosate acid (95% purity, CAS: 1071-83-6); R: Roundup Max® (74.7% of *N*-(phosphonomethyl) glycine monoammonium salt (CAS: 40465-66-5) and 25.3% of inert ingredients and adjuvants); A: Glifosato Atanor® (43.8% of *N*-(phosphonomethyl) glycine monopotassium salt (CAS: 39600-42-5) and 56.2% of inert ingredients and adjuvants); and treatments with mussels and herbicide, namely MG: mussel + technical-grade glyphosate acid, MA: mussel + Glifosato Atanor® and MR: mussel + Roundup Max®. Treatment C only included water from Salto Grande. Both Roundup Max® and Glifosato Atanor® are among the most used herbicides in Argentina. The active ingredient was applied at a concentration of 6 ppm in treatments G, R, A, MG, MR and MA. We chose this

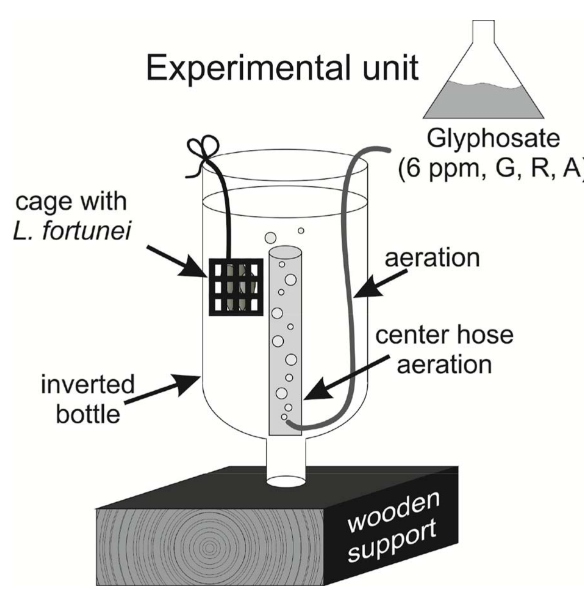

Fig. 1. Scheme of an experimental unit (microcosms) showing the cage with individuals of *L. fortunei* for treatments with mussels. G: technical-grade glyphosate acid; R: Roundup Max®; A: Glifosato Atanor®.