Harmful Algae 80 (2018) 130–139

Contents lists available at ScienceDirect

Harmful Algae

journal homepage: www.elsevier.com/locate/hal

Low-dose stimulation of growth of the harmful alga, *Prymnesium parvum*, by glyphosate and glyphosate-based herbicides

Brittanie L. Dabney[a], Reynaldo Patiño[b,*]

[a] Department of Environmental Toxicology and Texas Cooperative Fish and Wildlife Research Unit, Texas Tech University, Lubbock, TX, 79409-1163, USA
[b] U.S. Geological Survey, Texas Cooperative Fish and Wildlife Research Unit and Departments of Natural Resources Management and Biological Sciences, Texas Tech University, Lubbock, TX, 79409-2120, USA

ARTICLE INFO

Keywords:
N-(phosphonomethyl) glycine
Phosphonate
Golden alga
HAB
Hormesis

ABSTRACT

Glyphosate-based herbicides (GBH) are widely used around the globe. While generally toxic to phototrophs, organic phosphorus in glyphosate can become available to glyphosate-resistant phytoplankton and contribute to algal bloom development. Few studies have examined the effects of GBH on growth of eukaryotic microalgae and information for the toxic bloom-forming haptophyte, *Prymnesium parvum*, is limited. Using a batch-culture system, this study examined the effects on *P. parvum* growth of a single application of Roundup Weed and Grass Killer Super Concentrate Plus® (Roundup SC), Roundup Weed and Grass Killer Ready-to-Use III® (Roundup RtU), and technical-grade glyphosate at low concentrations [0–1000 µg glyphosate acid equivalent (ae) l^{-1}]. Roundup formulations differ in the percent of glyphosate as active ingredient (Roundup SC, ~50%; Roundup RtU, 2%), allowing indirect evaluation of the influence of inactive ingredients. Roundup SC enhanced exponential growth rate at 10–1000 µg glyphosate ae l^{-1}, and a positive monotonic association was noted between Roundup SC concentration and early (pre-exponential growth) but not maximum cell density. Glyphosate and both Roundup formulations enhanced growth rate at 100 µg glyphosate l^{-1}, but only Roundup SC and glyphosate significantly stimulated early and maximum density. This observation suggests the higher concentration of inactive ingredients and other compounds in Roundup RtU partially counteracts glyphosate-dependent growth stimulation. When phosphate concentration was varied while maintaining other conditions constant, addition of Roundup SC and glyphosate at 100 µg l^{-1} influenced growth more strongly than equivalent changes in phosphate-associated phosphorus. It appears, therefore, that low doses of glyphosate stimulate growth by mechanisms unrelated to the associated small increases in total phosphorus. In conclusion, glyphosate and GBH stimulate *P. parvum* growth at low, environmentally relevant concentrations. This finding raises concerns about the potential contribution to *P. parvum* blooms by glyphosate-contaminated runoff or by direct application of GBH to aquatic environments.

1. Introduction

Harmful algal blooms (HAB) are a major threat to aquatic ecosystems. They can impair water quality, cause massive fish kills, and harm human health (Anderson et al., 2015; O'Neil et al., 2012; Roelke et al., 2016). The brackish water haptophyte *Prymnesium parvum* (golden alga) is an HAB species that has caused considerable damage to inland aquatic ecosystems worldwide. In the United States, it was first reported in Texas in 1985 (Southard et al., 2010) after a large fish kill on the Pecos River, and since then its presence has been recorded in at least 22 other states (Israël et al., 2014; Roelke et al., 2016). Toxins released by golden alga irritate the gills of fishes and other aquatic organisms and disrupt respiration and ionoregulation (Roelke et al., 2016; Svendsen et al., 2018). Allelopathic or toxic activities of *P. parvum* also have been associated with reduced abundances or impaired growth of phytoplankton (Fistarol et al., 2003; Granéli and Johansson, 2003a; Michaloudi et al., 2009) and zooplankton species (Brooks et al., 2010; Michaloudi et al., 2009).

Golden alga is a mixotrophic species, a trait that may give it a competitive edge for organic and inorganic resources (Burkholder et al., 2008; Dölger et al., 2017). Previous field studies have shown that golden alga presence or abundance generally associates positively with levels of inorganic and organic phosphorus and organic nitrogen, but negatively with inorganic nitrogen (Israël et al., 2014; Vanlandeghem et al., 2015). In nutrient depleted environments, golden alga may enhance its toxin production to suppress growth of other plankton (Granéli and Johansson, 2003b; Uronen et al., 2005). Because phosphorus is a limiting nutrient in most freshwater ecosystems, its

* Corresponding author.
E-mail address: Reynaldo.patino@ttu.edu (R. Patiño).

https://doi.org/10.1016/j.hal.2018.11.004
Received 24 July 2018; Received in revised form 5 November 2018; Accepted 6 November 2018
Available online 17 November 2018
1568-9883/ Published by Elsevier B.V.

accumulation from anthropogenic sources is considered a major factor generally contributing to HAB formation (Anderson et al., 2002; Ho and Michalak, 2017; Schindler, 1987, 2006). One potential anthropogenic source of phosphorus may be organophosphate pesticides.

Glyphosate [*N*-(phosphonomethyl) glycine] is a synthetic phosphonate that inhibits 5-enolpyruvylshikimate-3-phosphate synthase within the shikimate metabolic pathway, which is necessary for the production of aromatic amino acids and plant growth (Brito et al., 2018; Pollegioni et al., 2011). Glyphosate-based herbicides (GBH) are the most widely used herbicides worldwide (Benbrook, 2016) and in the U.S. they rank first for agricultural use and second for household use (Grube et al., 2011). Surveys at urban and agricultural areas have found that concentrations in the water column vary depending on application time of the herbicide and time of sampling (Battaglin et al., 2014; Hanke et al., 2010; Mahler et al., 2017; Peruzzo et al., 2008). Maximum concentrations of glyphosate in streams and lentic ecosystems of the U.S. have been reported at ~70 and 300 μg l^{-1}, respectively (Battaglin et al., 2014), and even higher concentrations were recorded in surface waters of agricultural regions in Argentina – up to 700 μg l^{-1} (Peruzzo et al., 2008). Earlier studies suggested that GBH are of low risk to human and environmental health (Giesy et al., 2000; Solomon and Thompson, 2003). At present, however, GBH and other organophosphate pesticides are receiving increased attention as potential human carcinogens and as toxicants to terrestrial and aquatic organisms and ecosystems (Guyton et al., 2015; Myers et al., 2016; Relyea, 2005; Thompson et al., 2006; Van Bruggen et al., 2018). Impacts of GBH to aquatic environments may also include eutrophication caused by the increased levels of total phosphorus (Gattás et al., 2016; Pizarro et al., 2016; Vera et al., 2010, 2012; Wang et al., 2016).

Phytoplankton responses to glyphosate appear to be species-dependent. Some species have relatively high tolerance to glyphosate and, aided by the increase in total phosphorus available for growth, can become dominant in phytoplankton communities of impacted water bodies (Gattás et al., 2016; Pizarro et al., 2016; Vera et al., 2012, 2010; Wang et al., 2016). In addition, in the presence of inorganic phosphorus, glyphosate and GBH seem capable of directly stimulating growth of cyanobacteria and certain algae at exposure concentrations within the mg l^{-1} range (Daouk et al., 2013; Wang et al., 2016; Drzyzga and Lipok, 2018) – well above concentrations normally observed in aquatic environments. Growth stimulation at much lower concentrations of glyphosate (μg l^{-1} range) in the presence of inorganic phosphorus also was reported, however, for at least one species of green microalga (Wong, 2000). In regards to golden alga, a recent microcosm study found that the relative abundance of this species did not change during a 28-day exposure to glyphosate [Roundup Original MAX®, up to 100 μg of glyphosate acid equivalent (ae) l^{-1}] (Yates and Rogers, 2011). This study did not report absolute abundances before or during the exposures (Yates and Rogers, 2011), however, and assessments of the potential effects of the herbicide on growth of individual species (negative, positive, or no effect) cannot be made.

The objectives of the present study are to determine and compare the effects on golden alga growth of environmentally relevant concentrations of glyphosate using two Roundup formulations and technical-grade glyphosate. Technical-grade glyphosate was used in order to disentangle the potential effects of inactive ingredient present in commercial formulations from those of the neat compound. Additionally, experiments were conducted in phosphate-sufficient and -deficient conditions to characterize potential interactions between inorganic (phosphate) and organic (phosphonate) phosphorus on golden alga growth.

2. Materials and methods

2.1. Stock cultures

Golden alga (*P. parvum*, UTEX-2797) was obtained from UTEX Culture Collection of Algae (The University of Texas at Austin, Texas, USA). The strain used is from the Colorado River (Texas) and is the most widespread strain in US inland waters (Lutz-Carrillo et al., 2010). Stock cultures for this study were maintained in culture medium at salinity of 5, which is within the salinity range associated with golden alga habitat in the USA (Israël et al., 2014; Vanlandeghem et al., 2015).

The standard base medium for this study was previously described (Rashel and Patiño, 2017). Briefly, it consists of a modified UTEX artificial sea water medium (https://utex.org/products/artificial-seawater-medium) containing NaCl (Fisher Scientific S271), $MgSO_4{\cdot}7H_2O$ (Sigma Aldrich 230391), KCl (Fisher Scientific P217) and $CaCl_2{\cdot}2H_2O$ (Sigma Aldrich C3881) at final concentrations of 84 mM, 2.84 mM, 2.16 mM, 0.54 mM, respectively. Salinity and pH were 5 and ~8.1, respectively. Culture medium was enriched with f/2 levels of nitrogen ($NaNO_3$; Fisher Scientific BP360) and phosphorous ($NaH_2PO_4.H_2O$; Fisher Scientific S25562 A), trace metals and vitamins (Guillard, 1975). In the trace metals solution, ferrous ammonium sulfate in the original recipe was replaced with an equimolar amount of ferric chloride (Sigma Aldrich 236489). All ingredients were added before autoclaving except vitamins. After autoclaving, filter-sterilized (Nalgene, 0.45 μm, sterile analytical filter unit, Thermo Fisher Scientific Inc., Waltham, MA, USA) vitamin B12 (Fisher Scientific BP862), biotin (Sigma B4639), thiamine (Sigma Aldrich T1270), and sodium bicarbonate (93 μM; Sigma Aldrich S5761) were added.

Stock cultures were grown non-axenically in 250-ml Erlenmeyer flasks filled with 100 ml of standard base medium. These cultures were maintained in an incubator (I36LLVL; Percival Scientific Inc. Perry, IA, USA) at 22 °C and 12:12 h light:dark photoperiod with a light intensity of ~6500 lx and gently swirled once daily. Stock cultures in late exponential growth were used to inoculate experimental cultures.

2.2. Test chemicals

Technical-grade glyphosate (CAS 1071-8 3-6; Restek 32426, 99% purity) was purchased from Thomas Scientific (Swedesbord, NJ, USA). Two formulations of the glyphosate-based herbicide, Roundup (Monsanto, USA) were used that differ in the percent of active ingredient, glyphosate isopropylamine salt. They are Roundup Weed and Grass Killer Super Concentrate Plus® (Roundup SC), which contains 50.2% of active ingredient [431 g l^{-1} of glyphosate acid equivalent (ae)], and Roundup Weed and Grass Killer Ready-to-Use III® (Roundup RtU), which contains 2% of active ingredient (12 g of glyphosate ae l^{-1}). Inactive ingredients in Roundup include surfactants, and the only known difference between the two Roundup formulations is the presence of 2% pelargonic and related fatty acids in Roundup RtU. For all experiments, working stock solutions of test chemicals were prepared in deionized water at a concentration of 10,000 μg glyphosate ae l^{-1} for use in experimental cultures.

2.3. Experimental cultures

Experiments were performed in a temperature-controlled room with an average target ambient temperature of 24 °C. The light sources were wide spectrum fluorescent tube bulbs with an average measured intensity of 6000 lx (at surface of culture rack) that were set on a 12:12 h photoperiod cycle. Culture flasks were swirled once daily. Cell abundance was determined every 3 days by hemocytometry (Rashel and Patiño, 2017) and cultures ran for 21 days, the time when *P. parvum* growth reached stationary phase in all treatments. The limit of detection for the cell counting procedure used in this study is 333 cells ml^{-1} (Rashel and Patiño, 2017). Ambient temperature and light intensity were monitored continuously and weekly, respectively, with a Fisherbrand™ Temperature Data Logger (TEMP101 A) and a Fisher Scientific Light Meter (06-662-64). All treatments within an experiment were conducted in triplicate flasks.

2.4. Roundup SC dose-response

The effect of Roundup SC on *P. parvum* growth was examined at nominal concentrations of 0, 0.1, 1, 10, 100, and 1000 µg glyphosate ae l^{-1}. These concentrations of glyphosate bracket the range found in most aquatic environments (Battaglin et al., 2014; Peruzzo et al., 2008). Cultures containing standard base medium were inoculated with 10,000 cells ml^{-1} of *P. parvum* (from day 16 stock cultures) in a final volume of 100 ml prior to the addition of Roundup SC.

2.5. Technical-grade glyphosate and roundup formulations

The response of *P. parvum* to technical-grade glyphosate (glyphosate), Roundup SC, and Roundup RtU and a control were compared. Standard base medium was inoculated with 10,000 cells ml^{-1} (from day 16 stock cultures) in a final volume of 100 ml. Test chemicals at final nominal concentrations of 100 µg glyphosate ae l^{-1} were then added to the culture flasks. The concentration of 100 µg l^{-1} was based on the results of the preceding experiment and is within values reported in aquatic habitats (Battaglin et al., 2014).

2.6. Roundup SC in phosphorus-limited medium

The amount of phosphate added to the culture medium was varied to achieve nominal concentrations of 0, 20, 40, 60, 80 and 100% of the f/2 concentration in standard base medium; namely, 100% phosphate represents the regular concentration and 0% phosphate is standard base medium without the addition of phosphate. Flasks were inoculated with 10,000 cells ml^{-1} (from day 17 stock cultures) and incubated with or without Roundup SC at 100 µg glyphosate ae l^{-1}. The estimated concentration of total phosphorus derived from phosphate at 100% is 1123 µg l^{-1}, and each 20% increment represents an increase of 225 µg l^{-1}. The estimated concentration of total phosphorus derived from glyphosate at 100 µg l^{-1} is 18.3 µg l^{-1}.

2.7. Technical-grade glyphosate and roundup formulations in phosphorus-limited medium

The influence of phosphate concentration on *P. parvum* growth was assessed with and without glyphosate, Roundup SC, or Roundup RtU. The phosphate concentrations used were 100 and 20% of the concentration in standard base medium, and the concentration of glyphosate was 100 µg glyphosate ae l^{-1}. A day 18 stock culture was used to inoculate experimental flasks to achieve 10,000 cells ml^{-1} in a final volume of 100 ml.

2.8. Growth indices and statistical analysis

Growth indices estimated were exponential growth rate (r, day^{-1}) and early and maximum cell density (cells ml^{-1}) (Rashel and Patiño, 2017). Growth rate was calculated using the following equation (Guillard, 1973; Wood et al., 2005), where N_1 and N_2 are cell densities at times t_1 and t_2 ($t_2 > t_1$):

$$r = \frac{\ln N_2 - \ln N_1}{t_2 - t_1}$$

Times were chosen so that they bracket the linear portion of the ln-transformed growth curve in each individual replicate. The highest cell count was considered as the maximum cell density achieved by each replicate, and abundance on day 3 was used as index of early (pre-exponential) growth (Rashel and Patiño, 2017). Mean values (± SEM) are reported for all growth indices.

Growth responses at a constant phosphate concentration were analyzed using 1-way ANOVAs to determine if significant differences occurred between treatment concentrations (Roundup SC, section *2.3.1*) or glyphosate formulations (all 3 test chemicals at single concentration, section *2.3.2*). Two-way ANOVAs were used when phosphate was also varied; independent factors in these experimental designs where phosphate and glyphosate (Roundup SC) concentrations (section *2.3.3*), or phosphate and glyphosate formulation (section *2.3.4*). If significant factor or interaction effects were found, post-hoc Sidak's multiple comparison tests were performed to assess pairwise differences within and across factors. Additionally, when experimental variables had multiple numeric (quantitative) levels [i.e., varying glyphosate (section *2.3.1*) or phosphate (section *2.3.2*) concentration], correlation analyses were conducted between each growth index and the varying independent variable to assess the existence of trends. These correlation analyses were done to complement ANOVA results. The non-parametric Spearman's test was selected because it does not assume linear relationships between covariates; namely, it determines the strength of linear or nonlinear monotonic associations. Statistical analyses were conducted with GraphPad Prism 7 (GraphPad Software, Inc., La Jolla, CA, USA).

3. Results

3.1. Roundup SC dose-response

Average ambient temperature and light intensity during this experiment were 23.7 ± 0.41 °C and 6000 lx, respectively. In semi-log plots (not shown), exponential growth phases were observed on days 3–12 for concentrations of 0–10 µg ae l^{-1} and days 3–9 for 100–1000 µg ae l^{-1}. Visual inspection of growth curves indicated that cultures exposed to concentrations of 10–1000 µg l^{-1} had higher abundance during the exponential growth phase compared to the lower concentrations including the control (Fig. 1). Control cultures reached maximum density at ≥ day 21 but in the presence of Roundup SC maximum density was observed earlier, at days 15–18 (Fig. 1).

Results of one-way ANOVA (Table 1) and pairwise contrasts showed no significant differences in r among the control group and 0.1–1 µg glyphosate ae l^{-1}; however, significant increases in r relative to control values were observed at 10–1000 µg l^{-1} (Sidak's post-hoc, $p < 0.05$; Fig. 1). Growth rates at 1000 (0.58 day^{-1}), 100 (0.52 day^{-1}) and 10 (0.44 day^{-1}) µg glyphosate ae l^{-1} were 45, 30 and 10% higher than the control group (0.40 day^{-1}). Correlation analysis indicated the existence of a positive (monotonic) association between glyphosate concentration and r ($\rho = 0.81$, $df = 16$, $p < 0.0001$).

One-way ANOVA showed significant differences in early growth (day 3 abundance) between treatments (Table 1). Although Sidak's multiple comparisons test failed to show significant pairwise differences between individual treatments ($p > 0.05$), results of correlation analysis indicated the existence of a strong positive association between glyphosate concentration and early density ($\rho = 0.79$, $df = 16$, $p = 0.0009$).

One-way ANOVA showed significant differences in maximum density between treatments (Table 1). Compared to the control values, maximum densities in the presence Roundup SC were only higher in the 0.1 µg l^{-1} treatment group (Sidak's post-hoc, $p < 0.05$; Fig. 1), and results of correlation analysis did not show a monotonic association between glyphosate concentration and maximum density ($\rho = -0.26$, $df = 16$, $p = 0.30$).

3.2. Technical-grade glyphosate and roundup formulations

Average ambient temperature and light intensity were 24.2 ± 0.20 °C and 6000 lx, respectively. In semi-log plots (not shown), exponential growth phases were observed between days 3 and 9 for all treatments receiving glyphosate (100 µg ae l^{-1}), and between days 3 and 12 for the control group. Visual inspection of growth curves indicated that cultures exposed to glyphosate and Roundup SC showed increased abundance during the exponential phase of growth compared to the control or Roundup RtU treatments (Fig. 2). In addition, while

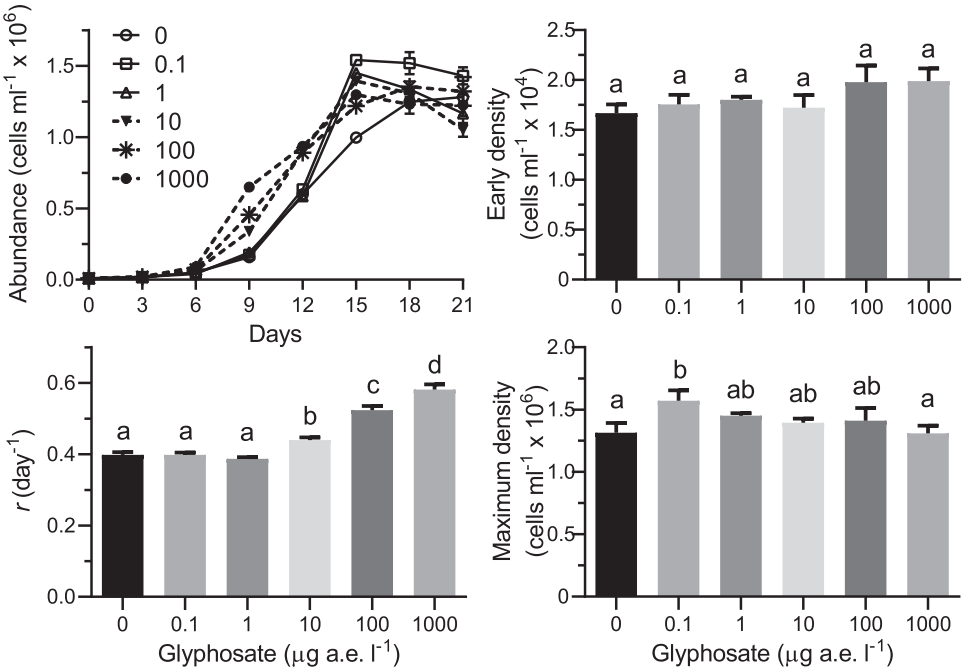

Fig. 1. Effects of glyphosate in Roundup Weed and Grass Killer Super Concentrate Plus® at various concentrations (acid equivalents, ae) on golden alga abundance (growth curves), exponential growth rate (r), early density, and maximum density. Bars associated with common letters are not significantly different ($\alpha = 0.05$). Markers and bars represent means ($n = 3$), and error bars represent standard error of the means.

control and Roundup RtU cultures achieved maximum density at ≥ 21 days, the glyphosate and Roundup SC treatments reached maximum yield earlier, at days 15–18 (Fig. 2).

One-way ANOVA showed significant effects of glyphosate formulation on r, early density, and maximum density (Table 1). Growth rates in all cultures with glyphosate and GBH were significantly higher from controls, especially for the technical-grade glyphosate and Roundup SC treatments (Sidak's post-hoc, $p < 0.05$; Fig. 2). Growth rates with glyphosate and Roundup SC were also significantly higher than with Roundup RtU (Fig. 2). The highest and lowest r were observed in the Roundup SC (0.52 day^{-1}) and control (0.38 day^{-1}) cultures, representing an increase of ~37% in the presence of Roundup SC at 100 µg glyphosate ae l^{-1} (Fig. 2). Early and maximum density were generally higher in cultures with glyphosate and Roundup SC (Sidak's post-hoc, $p < 0.05$; Fig. 2) and treatment-related differences for both endpoints were similar, except that early growth did not differ significantly between the Roundup SC and RtU treatments.

3.3. Roundup SC in phosphorus-limited medium

Average ambient temperature and light intensity were 20.1 ± 2.2 °C and 6000 lx, respectively. In semi-log plots, the linear portion of the growth curves bracketed days 3–9 for 100-80% phosphate concentration (f/2 level = 100%) with or without Roundup SC

Table 1
Output of one- and two-way ANOVA on data collected in this study. Growth endpoints (Response) examined included exponential growth rate (r), maximum density, and early density. Effects tested included glyphosate concentration or formulation [technical-grade glyphosate, Roundup Weed and Grass Killer Super Concentrate Plus® (Roundup SC), and Roundup Weed and Grass Killer Ready-to-Use III®], and phosphate concentration. DFn = degrees of freedom numerator. DFd = degrees of freedom denominator.

Treatments	Response	Effects	DFn, DFd	F-value	p-value
Roundup SC dose-response	r	Roundup SC concentration	5, 12	223.2	< 0.0001
	Maximum density		5, 12	5.849	0.0058
	Early density		5, 12	4.192	0.0195
Technical-grade glyphosate and roundup formulations	r	Glyphosate formulation	3, 8	39.68	< 0.0001
	Maximum density		3, 8	27.71	0.0001
	Early density		3, 8	9.942	0.0045
Roundup SC in phosphorus-limited medium	r	Roundup SC concentration	1, 24	144	< 0.0001
		Phosphate concentration	5, 24	388.5	< 0.0001
		Interaction	5, 24	12.4	< 0.0001
	Maximum density	Roundup SC concentration	1, 24	44.78	< 0.0001
		Phosphate concentration	5, 24	178.2	< 0.0001
		Interaction	5, 24	6.442	0.0006
	Early density	Roundup SC concentration	1, 24	123.4	< 0.0001
		Phosphate concentration	5, 24	6.495	0.0006
		Interaction	5, 24	22.55	< 0.0001
Technical-grade glyphosate and roundup formulations in phosphorus-limited medium	r	Glyphosate formulation	3, 16	114.5	< 0.0001
		Phosphate concentration	1, 16	97.65	< 0.0001
		Interaction	3, 16	30.46	< 0.0001
	Maximum density	Glyphosate formulation	3, 16	485.4	< 0.0001
		Phosphate concentration	1, 16	708	< 0.0001
		Interaction	3, 16	23.46	< 0.0001
	Early density	Glyphosate formulation	3, 16	4.338	0.0204
		Phosphate concentration	1, 16	0.9205	0.3516
		Interaction	3, 16	4.013	0.0263

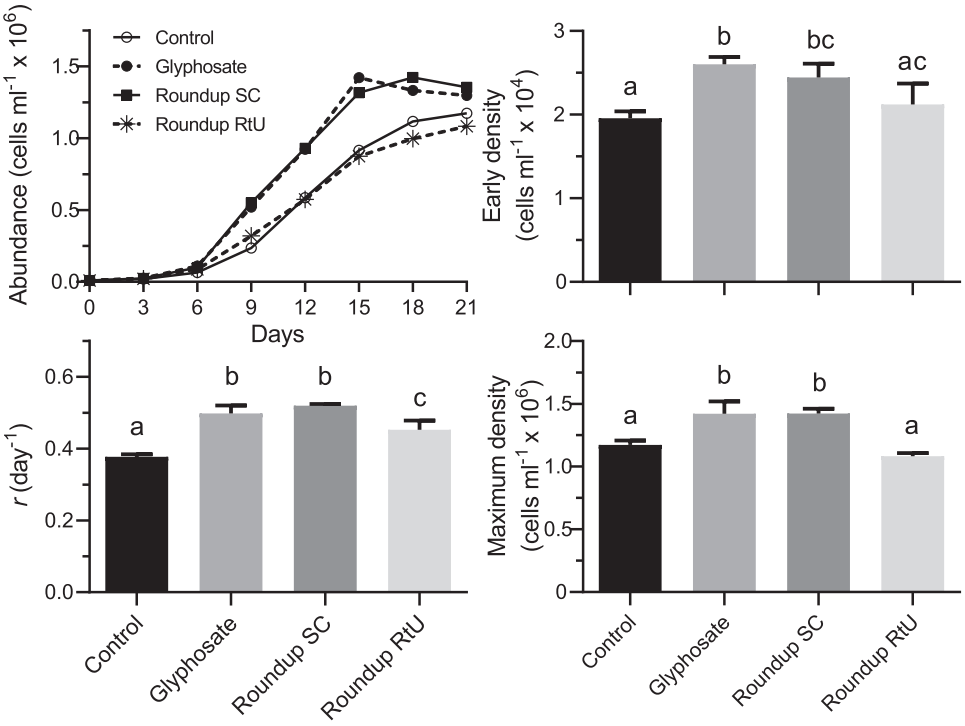

Fig. 2. Effects of technical-grade glyphosate (Glyphosate), Roundup Weed and Grass Killer Super Concentrate Plus® (Roundup SC), and Roundup Weed and Grass Killer Ready-to-Use III® (Roundup RtU) on golden alga abundance (growth curves), exponential growth rate (r), early density, and maximum density relative to a treatment control. The concentration of glyphosate used was 100 µg acid equivalent l^{-1}. Bars associated with common letters are not significantly different ($\alpha = 0.05$). Markers and bars represent means ($n = 3$), and error bars represent standard error of the means.

(100 µg glyphosate ae l^{-1}), days 3–12 for 60-20% phosphate with or without Roundup SC, days 3–12 for 0% phosphate without Roundup SC, and days 3–6 for 0% phosphate with Roundup SC (not shown). Visual inspection of growth curves indicated that maximum abundance was generally reached on days 18–21 at phosphate concentrations ≥ 60% and on day 15 or earlier (day 6 for 0% phosphate with Roundup SC) at lower concentrations (Fig. 3). It was also evident that cell abundance was associated positively with phosphate concentration and generally also with presence of Roundup SC (Fig. 3).

Results of 2-way ANOVA indicated that phosphate concentration, Roundup SC and their interaction had significant effects on r, early density and maximum density (Table 1). Growth rate did not differ between 80 and 100% phosphate in either the presence or absence of Roundup SC, but seemed to gradually decrease with decreasing phosphate concentration in both cases (Sidak's post-hoc, $p < 0.05$; Fig. 3). Correlation analysis showed a strong positive association between phosphate concentration and r. Spearman's rho was 0.92 and 0.80 in the absence and presence of Roundup SC, respectively ($df = 16$, $p < 0.0001$). At each phosphate concentration, r was generally higher in the presence of Roundup SC except in treatments with 60% and 40% phosphate, where differences were not significant (Fig. 3).

In the absence of Roundup SC, early cell density was affected by phosphate concentration but the association did not follow a directional pattern; namely, early growth was lower than the control (100% phosphate) at 60, 40 and 0% phosphate concentrations but not at 80 or 20% (Sidak's post-hoc, $p < 0.05$; Fig. 3). In addition, correlation analysis did not reveal a significant monotonic association between these variables ($\rho = 0.36$, $df = 16$, $p = 0.14$). In the presence of Roundup SC, however, early cell density was significantly stimulated at each phosphate concentrations except in the 80% phosphate treatment (Sidak's post-hoc, $p < 0.05$; Fig. 3). Curiously, as phosphate concentration decreased, early cell density generally increased in cultures containing Roundup SC (Sidak's post-hoc, $p < 0.05$; Fig. 3), and this negative relationship was confirmed by results of correlation analysis ($\rho = -0.73$, $df = 16$, $p = 0.0005$).

Maximum density in the absence of Roundup SC was similar among the 100, 80 and 60% phosphate concentrations but decreased at lower concentrations while in the presence of Roundup SC, maximum density steadily decreased across the entire range of phosphate concentrations (Sidak's post-hoc, $p < 0.05$; Fig. 3). Correlation analysis confirmed this positive association. Spearman's rho was 0.97 and 0.95 in the absence or presence of Roundup SC ($df = 16$, $p < 0.0001$). In addition, maximum density was generally higher in treatments receiving Roundup SC except in cultures with 0% phosphate, where cell densities were similar with or without Roundup SC; these pairwise differences, however, were statistically significant only at 100 and 20% phosphate (Fig. 3).

3.4. Technical-grade glyphosate and roundup formulations in phosphorus-limited medium

Average ambient temperature and light intensity were 23.3 ± 1.8 °C and 6000 lx, respectively. In semi-log plots, the linear portion of the growth curves in all treatments occurred between days 3 and 9 (not shown). Maximum yields were achieved at variable culture times depending on treatment (range was 15–21 days; Fig. 4). Visual inspection of growth curves indicated a positive association between cell abundance and phosphate concentration and, generally, also between abundance and presence of glyphosate (100 µg l^{-1}), especially technical-grade glyphosate and Roundup SC (Fig. 4).

Results of 2-way ANOVA showed significant effects of glyphosate formulation, phosphate concentration and their interaction on r and maximum density, and of glyphosate formulation and the interaction between glyphosate formulation and phosphate concentration on early density (Table 1). In cultures with standard phosphate concentration (100%), those that received glyphosate had significantly higher r compared to the controls, and r values in the technical-grade glyphosate or Roundup SC treatments were significantly higher than in the Roundup RtU treatment (Sidak's post-hoc, $p < 0.05$; Fig. 4). The highest r recorded was for the Roundup SC treatment (0.48 day^{-1}), a value 30% higher than the control (0.37 day^{-1}). In cultures receiving 20% phosphate, the addition of glyphosate and Roundup SC yielded significantly higher r than either Roundup RtU or the control (Sidak's post-hoc, $p < 0.05$). The highest and lowest r were observed in cultures receiving glyphosate (0.48 day^{-1}) and Roundup RtU (0.33 day^{-1}). The two Roundup formulations showed significantly lower r at

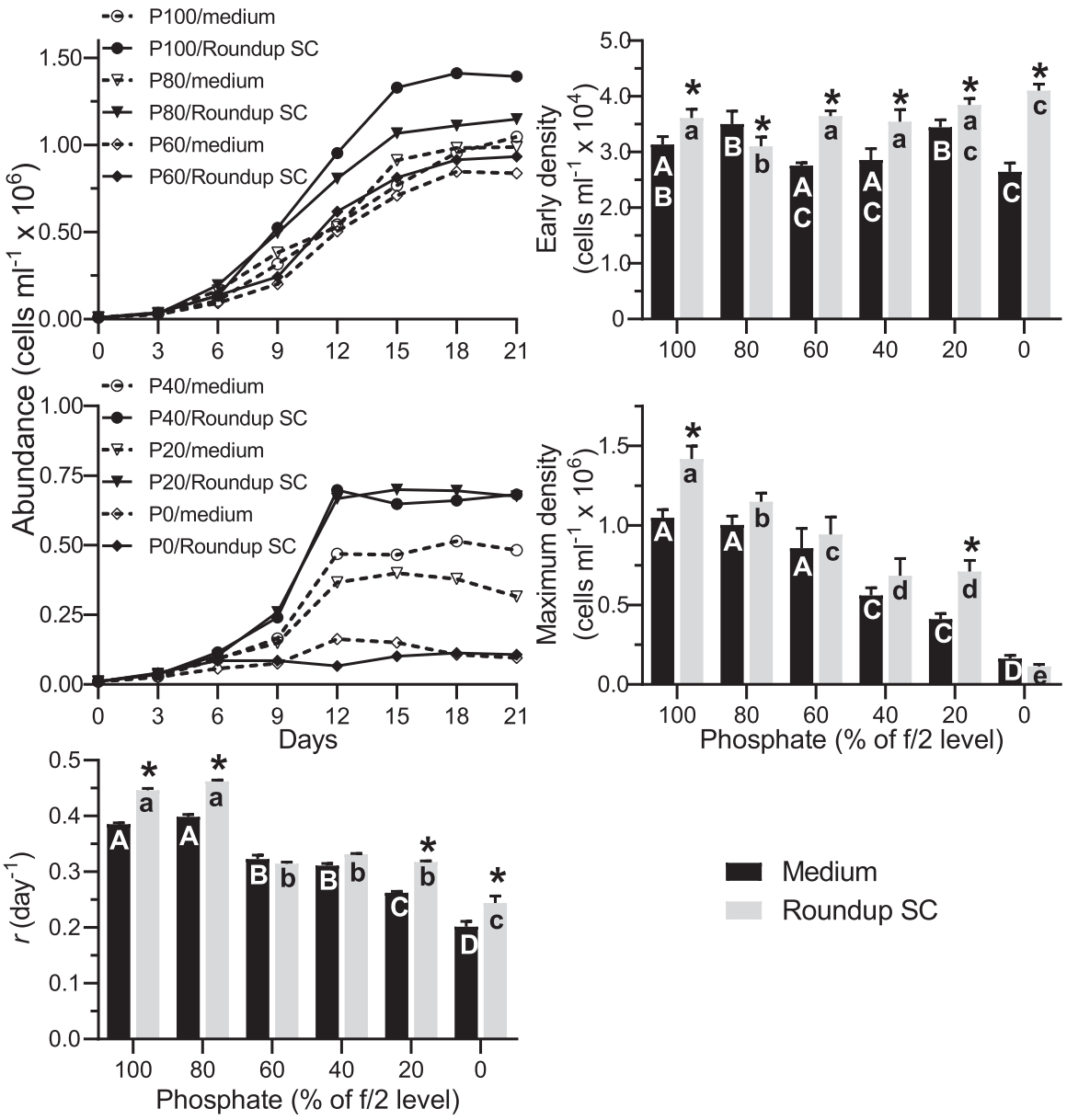

Fig. 3. Effects of glyphosate in Roundup Weed and Grass Killer Super Concentrate Plus® (Roundup SC) under varying concentrations of phosphate on golden alga abundance (growth curves), exponential growth rate (*r*), early density, and maximum density. The concentration of glyphosate was 100 µg acid equivalent l^{-1}, and the concentration of phosphate is expressed as percent of the f/2 nutrient level in standard medium (100% = P100). Bars associated with common letters are not significantly different (α = 0.05) in the absence (capitalized letters) or presence (small letters) of Roundup SC. Asterisks indicate significant difference in the absence (medium) of presence of Roundup SC within each phosphate concentration. Markers and bars represent means (n = 3), and error bars represent standard error of the means.

20% phosphate compared to 100% (Sidak's post-hoc, $p < 0.05$; Fig. 4).

Early density was stimulated by technical-grade glyphosate at 100% phosphate concentration and by Roundup SC at 20% (Sidak's post-hoc, $p < 0.05$; Fig. 4) and, within each glyphosate treatment, a reduction in early cell density at the lower phosphate concentration was observed only in the presence of glyphosate (Fig. 4). In contrast, the effects of glyphosate and Roundup SC on maximum density were of greater magnitude and statistically significant at either phosphate concentration (Sidak's post-hoc, $p < 0.05$; Fig. 4); however, Roundup RtU had no effect on maximum density relative to the respective controls at either phosphate concentration (Fig. 4). In addition, a reduction in maximum density was consistently observed at the lower phosphate concentration within all treatments (Sidak's post-hoc, $p < 0.05$; Fig. 4).

4. Discussion

The most significant finding of this study is that growth of golden alga was stimulated by glyphosate and GBH at low, environmentally relevant concentrations. It is also notable that this stimulation occurred under phosphorus-sufficient conditions. Using Roundup SC, the highest concentration at which no effect on *r* occurred was 1 µg glyphosate ae l^{-1}, and growth stimulation was observed at 10–1000 µg l^{-1}. Increases in growth rate relative to control values were 10, 30, and 45 percent higher at 10, 100, and 1000 µg glyphosate ae l^{-1}. At 100 µg glyphosate l^{-1}, Roundup RtU and glyphosate also stimulated *r*. Among the other growth indices examined, early cell density was also positively associated with glyphosate concentration (in Roundup SC) but the association between maximum density (yield) and glyphosate concentration was not linear or monotonic. The reason for the latter observation is

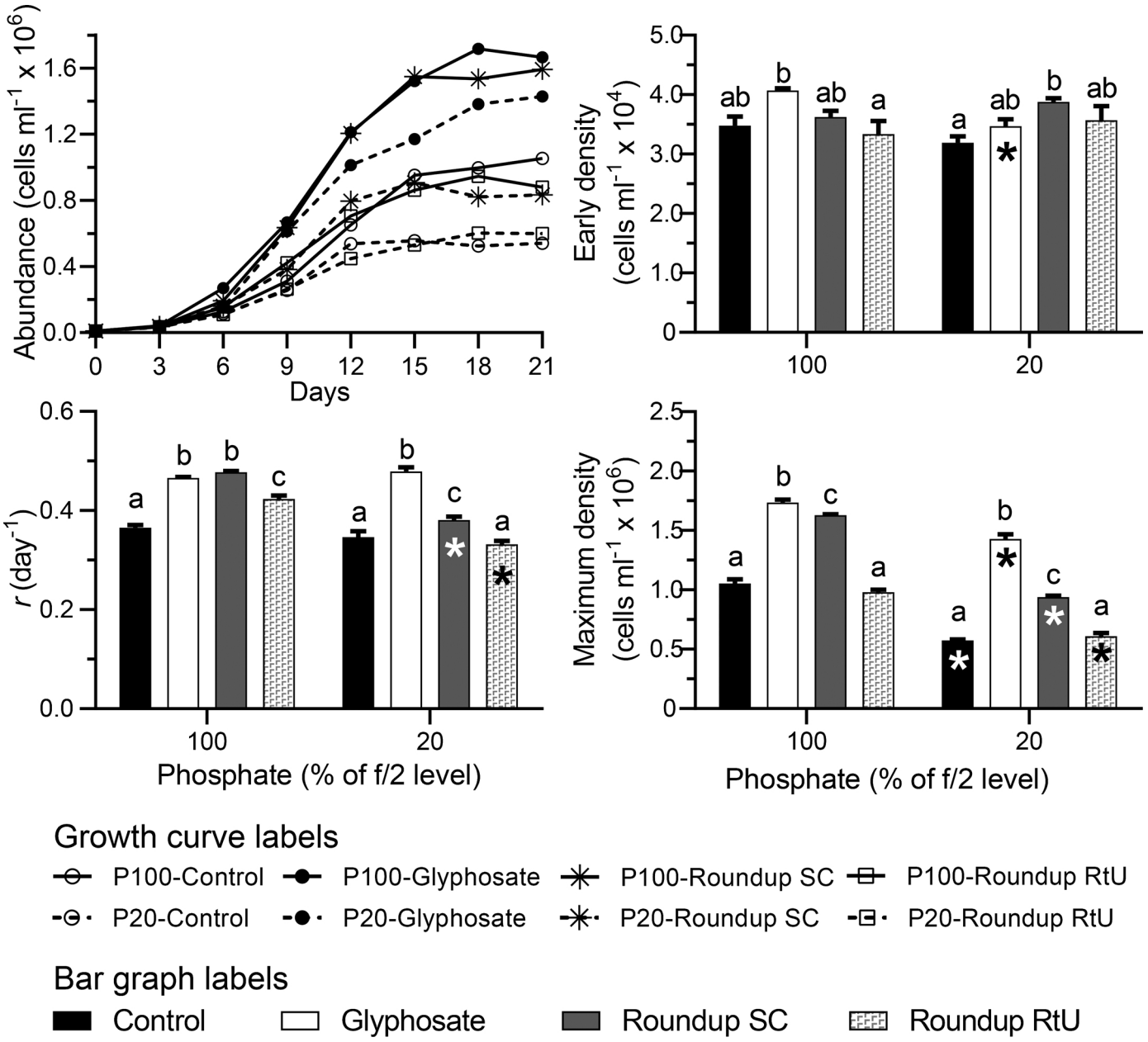

Fig. 4. Effects of technical-grade glyphosate (Glyphosate), Roundup Weed and Grass Killer Super Concentrate Plus® (Roundup SC), and Roundup Weed and Grass Killer Ready-to-Use III® (Roundup RtU) on golden alga abundance (growth curves), exponential growth rate (*r*), early density, and maximum density relative to a treatment control and at two different phosphate concentrations. The concentration of glyphosate (acid equivalent) was 100 µg l^{-1} and the concentration of phosphate is expressed as percent of the f/2 nutrient level in standard medium (100% = P100). Bars associated with common letters are not significantly different ($\alpha = 0.05$). Asterisks indicate significant difference between corresponding bars across phosphate concentrations. Markers and bars represent means ($n = 3$), and error bars represent standard error of the means.

uncertain but could be at least partly due to the shorter duration of the exponential growth phase at the higher Roundup SC concentrations. This is the first clear documentation of a stimulatory effect of an herbicide at environmentally relevant concentrations on growth of a haptophyte under phosphorus-sufficient conditions, and this is also among few studies available that have reported stimulation of microalgal growth at glyphosate exposure concentrations within the µg l^{-1} range.

An earlier microcosm study found that the relative abundance of golden alga is unaffected by exposure to 100 µg glyphosate ae l^{-1} in Roundup Original MAX®, which contains 48.7% of glyphosate as the active ingredient (Yates and Rogers, 2011). This earlier study did not report absolute abundances and inferences about glyphosate effects on growth of individual species in the microcosm, and therefore a comparison to the present results cannot be reliably made. It is also possible that differences in experimental conditions – e.g., defined culture media in present study versus lake water in the earlier one (Yates and Rogers, 2011) – can influence the sensitivity of golden alga to glyphosate.

The range of glyphosate concentrations used in this study did not allow for the determination of the tolerance limit for golden alga (concentration over which growth is suppressed) but it is clearly higher than 1 mg l^{-1}, the highest concentration tested. In the green microalga, *Scenedesmus quadricauda*, stimulatory and inhibitory effects of glyphosate on growth were reported within the µg l^{-1} range and ≥ 2 mg l^{-1}, respectively (Wong, 2000). In the related species, *S. vacuolatus*, the range of stimulatory and inhibitory glyphosate concentrations are both

much higher: 2–20 mg l^{-1} and > 20 mg l^{-1}, respectively (Daouk et al., 2013). It appears, therefore, that the range of glyphosate concentrations that produces a hormetic growth response (low-dose stimulation followed by higher-dose inhibition; Calabrese and Baldwin, 2003) varies among inland freshwater microalgae even among closely related species, and similar observations have been made with marine microalgae (Wang et al., 2016). High tolerance to glyphosate seems to be particularly widespread among cyanobacteria, including the HAB species *Microcystis aeruginosa* (Forlani et al., 2008). In the latter species, stimulatory effects on growth also were described in the µg l^{-1} range under phosphorus-starved conditions (Qiu et al., 2013).

In the present study, the percentage (or the composition) of inactive ingredient in the GBH formulations appears to have influenced growth responses to glyphosate. Stimulation of algal growth (*r*, early and maximum density) at a concentration of 100 µg glyphosate ae l^{-1} in Roundup SC (~50 percent inactive ingredient) was generally similar in magnitude to the response to technical-grade glyphosate at the equivalent exposure concentration. When using the same exposure concentration of glyphosate in Roundup RtU (98 percent inactive ingredient), however, growth stimulation was still evident but was clearly reduced relative to the other experimental treatments. While the exact composition of inactive ingredients is not listed on the product labels, polyethoxylated tallow amines are commonly used in GBH formulations and these compounds can be toxic to algae (Lipok et al., 2010; Tsui and Chu, 2003). Also, ready-to-use formulations typically contain 2% pelargonic acid, which in some plant species synergizes with glyphosate to enhance herbicidal activity (Wehtje et al., 2009). Thus, the higher concentration of potentially toxic compounds derived from inactive ingredient or the presence of pelargonic acid in Roundup RtU may have diminished the stimulatory effect of glyphosate on golden alga growth.

Contaminated runoff discharge or direct application of high concentrations of GBH or glyphosate (mg l^{-1} range) to surface waters can increase the concentration of total phosphorus that is available for primary productivity (Gattás et al., 2016; Pérez et al., 2007; Pizarro et al., 2016; Vera et al., 2012, 2010). Moreover, several species of glyphosate-tolerant cyanobacteria (Forlani et al., 2008; Lipok et al., 2007; Qiu et al., 2013; Saxton et al., 2011) and microalgae (Wang et al., 2016) can reportedly utilize glyphosate as sole source of phosphorus. Tolerance of these taxa to glyphosate coupled with increases in total phosphorus may explain the observed increases in their abundance following application of high concentrations of glyphosate (or GBH) in micro- and mesocosm [or even monoculture (Daouk et al., 2013)] systems (Daouk et al., 2013; Drzyzga and Lipok, 2018; Pizarro et al., 2016; Saxton et al., 2011; Vera et al., 2012; Wang et al., 2016). In cases where growth stimulation occurs at very low concentrations of glyphosate (µg l^{-1} range), however, explanations based on increased nutrient availability may not be generally applicable. When the concentration of phosphate was increased from 80 to 100% of f/2 levels – equivalent to an increase of 225 µg l^{-1} – in the absence of glyphosate, golden alga growth did not change indicating that total phosphorus in 80%-phosphate medium (~898 µg l^{-1}) is sufficient for maximum growth under the present culture conditions. On the other hand, when 10–1000 µg glyphosate ae l^{-1} – equivalent to 1.83–183 µg l^{-1} – were added to medium containing 100% phosphate, growth was significantly stimulated. Therefore, under phosphorus-sufficient conditions, the stimulatory effect of glyphosate at low concentrations cannot be explained by the associated minor increases in total phosphorus. Stimulatory effects of glyphosate and GBH on golden alga growth rate were also observed at low phosphate concentrations (20%) and even in its absence (0%). Growth stimulation under phosphorus-limited conditions, however, could have been at least partly due the increase in total phosphorus associated with glyphosate (e.g., Wang et al., 2016). Constituent nitrogen in glyphosate is also unlikely to be responsible for growth stimulation even if all of it became bioavailable. Relative to the concentration of sodium nitrate-associated nitrogen in experimental media (~12.3 mg l^{-1}), the concentration of glyphosate-associated nitrogen is negligible (0.8–80 µg l^{-1} at 10–1000 µg glyphosate l^{-1}).

Low-dose growth effects of glyphosate and GBH have been described in an increasing number of plant species and particularly in those where toxicity is observed at the label-recommended (higher) application rates (Brito et al., 2018; Cedergreen, 2008a, 2008b; Duke, 2018). While toxicity at high glyphosate concentrations seems to involve inhibition of the shikimate metabolic pathway, stimulating mechanisms at low concentrations have received little research attention and are not well understood (Brito et al., 2018; Cedergreen and Olesen, 2010; Duke, 2018; Pollegioni et al., 2011). It has been reported, however, that growth stimulation by low doses of glyphosate is accompanied by increases in carbon assimilation and photosynthesis (Brito et al., 2018; Cedergreen and Olesen, 2010; Nascentes et al., 2018). In addition, application of glyphosate at low rates seems to cause an increase in shikimic acid production in some cases, the opposite of what normally occurs at the higher toxic rates (Brito et al., 2018). Further research is needed to understand the mechanisms of low-dose growth stimulation by glyphosate in golden alga, other algae, and plants.

Sterile media were used in the present study but stock and experimental cultures were not strictly axenic and a confounding effect of coexisting bacteria on the response of golden alga to glyphosate cannot be ruled out. Visual inspection of culture flasks and light-microscopical inspections during cell counting, however, did not reveal overt signs of bacterial growth in stock or experimental cultures. In addition, growth stimulation by glyphosate under axenic conditions has been previously reported for some algal species (Wang et al., 2016).

5. Conclusions

This study showed that glyphosate and GBH formulations stimulate growth of golden alga at low, environmentally relevant concentrations. Growth stimulation was observed at glyphosate concentrations as low as 10 µg l^{-1} (containing an equivalent phosphorus concentration of 1.83 µg l^{-1}) under phosphorus-sufficient conditions, suggesting that growth stimulation is unlikely to be the result of minor increases in glyphosate-derived total phosphorus. The concentration of glyphosate at which growth inhibition occurs in golden alga could not be determined in the present study but based on present observations and data for other microalgae, it is likely in the mg l^{-1} range (Daouk et al., 2013; Wong, 2000). Thus, the growth response of golden alga to glyphosate is likely to follow a hormetic pattern. Growth responses to Roundup SC and technical-grade glyphosate were similar to each other but higher than to Roundup RtU, suggesting that the high level of inactive ingredient or presence of additional constituents in Roundup RtU interferes with the ability of glyphosate to stimulate growth. Results of this study raise concerns about the potential contribution to the formation of golden alga blooms by glyphosate-contaminated runoff discharge or the direct application of GBH to aquatic environments (e.g., to control nuisance macrophytes). Similar concerns have been raised previously in regards to cyanobacterial HAB species (Qui et al., 2013),

An understanding of low-dose stimulation of growth by glyphosate can be complicated in plants due inconsistent responses often observed in association with variable conditions such as differences in growth stage and physiological status (Belz and Duke, 2014). Uneven exposures to herbicide by the different plant parts also can be a source of variability. The use of aquatic microalgae as models for the study of low-dose herbicide effects may help overcome some of these difficulties. Namely, exposures are uniform around the surface of single-cell organisms in liquid media, exposure levels can be estimated with reasonable precision (as exposure concentrations), and culture systems are based on well-established and highly replicable growth cycles.

Conflict of interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

Acknowledgements

Dr. Rick Relyea provided a detailed critique of an early draft of this manuscript. The Texas Cooperative Fish and Wildlife Research Unit is jointly supported by U.S. Geological Survey, Texas Tech University, Texas Parks and Wildlife Department, The Wildlife Management Institute, and U.S. Fish and Wildlife Service. This study was supported by Texas Tech University intramural funds. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.[SS]

References

Anderson, C.R., Moore, S.K., Tomlinson, M.C., Silke, J., Cusack, C.K., 2015. Living with harmful algal blooms in a changing world: strategies for modeling and mitigating their effects in coastal marine ecosystems. In: Ellis, J., Sherman, D.J. (Eds.), Coastal and Marine Hazards, Risks, and Disasters. Elsevier, Amsterdam, pp. 495–561. https://doi.org/10.1016/B978-0-12-396483-0.00017-0.

Anderson, D.M., Glibert, P.M., Burkholder, J.M., 2002. Harmful algal blooms and eutrophication: nutrient sources, composition, and consequences. Estuaries 25, 704–726.

Battaglin, W.A., Meyer, M.T., Kuivila, K.M., Dietze, J.E., 2014. Glyphosate and its degradation product AMPA occur frequently and widely in U.S. soils, surface water, groundwater, and precipitation. J. Am. Water Resour. Assoc. 50, 275–290. https://doi.org/10.1111/jawr.12159.

Belz, R.G., Duke, S.O., 2014. Herbicides and plant hormesis. Pest Manag. Sci. 70, 698–707.

Benbrook, C.M., 2016. Trends in glyphosate herbicide use in the United States and globally. Environ. Sci. Eur. 28, 3. https://doi.org/10.1186/s12302-016-0070-0.

Brito, I.P.F.S., Tropaldi, L., Carbonari, C.A., Velini, E.D., 2018. Hormetic effects of glyphosate on plants. Pest Manag. Sci. 74, 1064–1070. https://doi.org/10.1002/ps.4523.

Brooks, B.W., James, S.V., Valenti, T.W., Urena-Boeck, F., Serrano, C., Berninger, J.P., Schwierzke, L., Mydlarz, L.D., Grover, J.P., Roelke, D.L., 2010. Comparative toxicity of *Prymnesium parvum* in inland waters. J. Am. Water Resour. Assoc. 46, 45–62. https://doi.org/10.1111/j.1752-1688.2009.00390.x.

Burkholder, J.A.M., Glibert, P.M., Skelton, H.M., 2008. Mixotrophy, a major mode of nutrition for harmful algal species in eutrophic waters. Harmful Algae 8, 77–93. https://doi.org/10.1016/j.hal.2008.08.010.

Calabrese, E.J., Baldwin, L.A., 2003. Hormesis: the dose-response revolution. Annu. Rev. Pharmacol. Toxicol. 43, 175–197. https://doi.org/10.1146/annurev.pharmtox.43.100901.140223.

Cedergreen, N., 2008a. Is the growth stimulation by low doses of glyphosate sustained over time? Environ. Pollut. 156, 1099–1104. https://doi.org/10.1016/j.envpol.2008.04.016.

Cedergreen, N., 2008b. Herbicides can stimulate plant growth. Weed Res. 48, 429–438. https://doi.org/10.1111/j.1365-3180.2008.00646.x.

Cedergreen, N., Olesen, C.F., 2010. Can glyphosate stimulate photosynthesis? Pestic. Biochem. Physiol. 96, 140–148. https://doi.org/10.1016/j.pestbp.2009.11.002.

Daouk, S., Copin, P.J., Rossi, L., Chèvre, N., Pfeifer, H.R., 2013. Dynamics and environmental risk assessment of the herbicide glyphosate and its metabolite AMPA in a small vineyard river of the Lake Geneva catchment. Environ. Toxicol. Chem. 32, 2035–2044. https://doi.org/10.1002/etc.2276.

Dölger, J., Nielsen, L.T., Kiørboe, T., Andersen, A., 2017. Swimming and feeding of mixotrophic biflagellates. Sci. Rep. 7, 1–10. https://doi.org/10.1038/srep39892.

Drzyzga, D., Lipok, J., 2018. Glyphosate dose modulates the uptake of inorganic phosphate by freshwater cyanobacteria. J. Appl. Phycol. 30, 299–309. https://doi.org/10.1007/s10811-017-1231-2.

Duke, S.O., 2018. The history and current status of glyphosate. Pest Manag. Sci. 74, 1027–1034. https://doi.org/10.1002/ps.4652.

Fistarol, G.O., Legrand, C., Granéli, E., 2003. Allelopathic effect of *Prymnesium parvum* on a natural plankton community. Mar. Ecol. Prog. Ser. 255, 115–125. https://doi.org/10.3354/meps255115.

Forlani, G., Pavan, M., Gramek, M., Kafarski, P., Lipok, J., 2008. Biochemical bases for a widespread tolerance of cyanobacteria to the phosphonate herbicide glyphosate. Plant Cell Physiol. 49, 443–456. https://doi.org/10.1093/pcp/pcn021.

Gattás, F., Vinocur, A., Graziano, M., dos Santos Afonso, M., Pizarro, H., Cataldo, D., 2016. Differential impact of *Limnoperna fortunei*-herbicide interaction between Roundup Max® and glyphosate on freshwater microscopic communities. Environ. Sci. Pollut. Res. 23, 18869–18882. https://doi.org/10.1007/s11356-016-7005-6.

Giesy, J.P., Dobson, T., Solomon, K.R., 2000. Ecotoxicological risk assessment for Roundup® herbicide. Reviews of Environmental Contamination and Toxicology. Springer, New York, New York, NY, pp. 35–120.

Granéli, E., Johansson, N., 2003a. Effects of the toxic haptophyte *Prymnesium parvum* on the survival and feeding of a ciliate: the influence of different nutrient conditions. Mar. Ecol. Prog. Ser. 254, 49–56. https://doi.org/10.3354/meps254049.

Granéli, E., Johansson, N., 2003b. Increase in the production of allelopathic substances by *Prymnesium parvum* cells grown under N- or P-deficient conditions. Harmful Algae 2, 135–145. https://doi.org/10.1016/S1568-9883(03)00006-4.

Grube, A., Donaldson, D., Kiely, T., Wu, L., 2011. Pesticides Industry Sales and Usage: 2006 and 2007 Market Estimates, U.S. Environmental Protection Agency. Washington, DC. .

Guillard, R.R.L., 1975. Culture of phytoplankton for feeding marine invertebrates. Culture of Marine Invertebrate Animals. Springer, Springer, Boston, MA, pp. 29–60.

Guillard, R.R.L., 1973. Division rates. In: Jr, S. (Ed.), Handbook of Phycological Methods: Culture Methods and Growth Measurements. Cambridge University Press, Cambridge, pp. 289–311.

Guyton, K.Z., Loomis, D., Grosse, Y., Ghissassi, F.El, Benbrahim-Tallaa, L., Guha, N., Scoccianti, C., Mattock, H., Straif, K., 2015. Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate. Lancet Oncol. 16, 490–491. https://doi.org/10.1016/S1470-2045(15)70134-8.

Hanke, I., Wittmer, I., Bischofberger, S., Stamm, C., Singer, H., 2010. Relevance of urban glyphosate use for surface water quality. Chemosphere 81, 422–429. https://doi.org/10.1016/j.chemosphere.2010.06.067.

Ho, J.C., Michalak, A.M., 2017. Phytoplankton blooms in Lake Erie impacted by both long-term and springtime phosphorus loading. J. Great Lakes Res. 43, 221–228. https://doi.org/10.1016/j.jglr.2017.04.001.

Israël, N.M.D., VanLandeghem, M.M., Denny, S., Ingle, J., Patiño, R., 2014. Golden alga presence and abundance are inversely related to salinity in a high-salinity river ecosystem, Pecos River, USA. Harmful Algae 39, 81–91. https://doi.org/10.1016/j.hal.2014.06.012.

Lipok, J., Owsiak, T., Młynarz, P., Forlani, G., Kafarski, P., 2007. Phosphorus NMR as a tool to study mineralization of organophosphonates—the ability of *Spirulina* spp. to degrade glyphosate. Enzyme Microb. Technol. 41, 286–291. https://doi.org/10.1016/j.enzmictec.2007.02.004.

Lipok, J., Studnik, H., Gruyaert, S., 2010. The toxicity of Roundup® 360 SL formulation and its main constituents: glyphosate and isopropylamine towards non-target water photoautotrophs. Ecotoxicol. Environ. Saf. 73, 1681–1688. https://doi.org/10.1016/j.ecoenv.2010.08.017.

Lutz-Carrillo, D.J., Southard, G.M., Fries, L.T., 2010. Global genetic relationships among isolates of golden alga (*Prymnesium parvum*). J. Am. Water Resour. Assoc. 46, 24–32. https://doi.org/10.1111/j.1752-1688.2009.00388.x.

Mahler, B.J., Van Metre, P.C., Burley, T.E., Loftin, K.A., Meyer, M.T., Nowell, L.H., 2017. Similarities and differences in occurrence and temporal fluctuations in glyphosate and atrazine in small Midwestern streams (USA) during the 2013 growing season. Sci. Total Environ. 579, 149–158. https://doi.org/10.1016/j.scitotenv.2016.10.236.

Michaloudi, E., Moustaka-Gouni, M., Gkelis, S., Pantelidakis, K., 2009. Plankton community structure during an ecosystem disruptive algal bloom of Prymnesium parvum. J. Plankton Res. 31, 301–309. https://doi.org/10.1093/plankt/fbn114.

Myers, J.P., Antoniou, M.N., Blumberg, B., Carroll, L., Colborn, T., Everett, L.G., Hansen, M., Landrigan, P.J., Lanphear, B.P., Mesnage, R., Vandenberg, L.N., vom Saal, F.S., Welshons, W.V., Benbrook, C.M., 2016. Concerns over use of glyphosate-based herbicides and risks associated with exposures: a consensus statement. Environ. Heal. 15, 19. https://doi.org/10.1186/s12940-016-0117-0.

Nascentes, R.F., Carbonari, C.A., Simões, P.S., Brunelli, M.C., Velini, E.D., Duke, S.O., 2018. Low doses of glyphosate enhance growth, CO_2 assimilation, stomatal conductance and transpiration in sugarcane and eucalyptus. Pest. Manag. Sci. 74, 1197–1205. https://doi.org/10.1002/ps.4606.

O'Neil, J.M., Davis, T.W., Burford, M.A., Gobler, C.J., 2012. The rise of harmful cyanobacteria blooms: the potential roles of eutrophication and climate change. Harmful Algae 14, 313–334.

Pérez, A.G.L., Torremorell, A., Mugni, H., Rodríguez, P., Vera, M.S., Allende, L., Bustingorry, J., Escaray, R., Ferraro, M., Izaguirre, I., Pizarro, H., Morris, D.P., Zagarese, H., 2007. Effects of the herbicide Roundup on freshwater microbial communities: a mesocosm study. Ecol. Appl. 17, 2310–2322.

Peruzzo, P.J., Porta, A.A., Ronco, A.E., 2008. Levels of glyphosate in surface waters, sediments and soils associated with direct sowing soybean cultivation in north pampasic region of Argentina. Environ. Pollut. 156, 61–66. https://doi.org/10.1016/j.envpol.2008.01.015.

Pizarro, H., Vera, M.S., Vinocur, A., Pérez, G., Ferraro, M., Menéndez Helman, R.J., dos Santos Afonso, M., 2016. Glyphosate input modifies microbial community structure in clear and turbid freshwater systems. Environ. Sci. Pollut. Res. 23, 5143–5153. https://doi.org/10.1007/s11356-015-5748-0.

Pollegioni, L., Schonbrunn, E., Siehl, D., 2011. Molecular basis of glyphosate resistance-different approaches through protein engineering. FEBS J. 278, 2753–2766. https://doi.org/10.1111/j.1742-4658.2011.08214.x.

Qiu, H., Geng, J., Ren, H., Xia, X., Wang, X., Yu, Y., 2013. Physiological and biochemical responses of *Microcystis aeruginosa* to glyphosate and its Roundup® formulation. J. Hazard. Mater. 248, 172–176. https://doi.org/10.1016/j.jhazmat.2012.12.033.

Rashel, R.H., Patiño, R., 2017. Influence of genetic background, salinity, and inoculum size on growth of the ichthyotoxic golden alga (*Prymnesium parvum*). Harmful Algae 66, 97–104. https://doi.org/10.1016/j.hal.2017.05.010.

Relyea, R.A., 2005. The impact of insecticides and herbicides on the biodiversity and productivity of aquatic communities. Ecol. Appl. 15, 618–627. https://doi.org/10.1890/03-5342.

Roelke, D.L., Barkoh, A., Brooks, B.W., Grover, J.P., Hambright, K.D., LaClaire, J.W., Moeller, P.D.R., Patino, R., 2016. A chronicle of a killer alga in the west: ecology, assessment, and management of *Prymnesium parvum* blooms. Hydrobiologia 764, 29–50.

Saxton, M.A., Morrow, E.A., Bourbonniere, R.A., Wilhelm, S.W., 2011. Glyphosate influence on phytoplankton community structure in Lake Erie. J. Great Lakes Res. 37, 683–690. https://doi.org/10.1016/j.jglr.2011.07.004.

Schindler, D., 2006. Recent advances in the understanding and management of eutrophication. Limnol. Oceanogr. 51, 356–363. https://doi.org/10.4319/lo.2006.51.1_part_2.0356.

Schindler, D.W., 1987. Detecting ecosystem responses to anthropogenic stress. Can. J. Fish. Aquat. Sci. 44, s6–s25.

Solomon, K.R., Thompson, D.G., 2003. Ecological risk assessment for aquatic organisms

from over-water uses of glyphosate. J. Toxicol. Environ. Health Part B 289–324. https://doi.org/10.1080/10937400306468.
Southard, G.M., Fries, L.T., Barkoh, A., 2010. *Prymnesium parvum*: the Texas experience. J. Am. Water Resour. Assoc. 46, 14–23. https://doi.org/10.1111/j.1752-1688.2009.00387.x.
Svendsen, M.B.S., Andersen, N.R., Hansen, P.J., Steffensen, J.F., 2018. Effects of harmful algal blooms on fish: insights from Prymnesium parvum. Fishes 3, 11. https://doi.org/10.3390/fishes3010011.
Thompson, D.G., Solomon, K.R., Wojtaszek, B.F., Andrea, N., Stephenson, G.R., Relyea, R.A., 2006. The impact of insecticides and herbicides on the biodiversity and productivity of aquatic communities: [with response]. Ecol. Appl. 16, 2022–2034.
Tsui, M.T.K., Chu, L.M., 2003. Aquatic toxicity of glyphosate-based formulations: comparison between different organisms and the effects of environmental factors. Chemosphere 52, 1189–1197. https://doi.org/10.1016/S0045-6535(03)00306-0.
Uronen, P., Lehtinen, S., Legrand, C., Kuuppo, P., Tamminen, T., 2005. Haemolytic activity and allelopathy of the haptophyte *Prymnesium parvum* in nutrient-limited and balanced growth conditions. Mar. Ecol. Prog. Ser. 299, 137–148. https://doi.org/10.3354/meps299137.
Van Bruggen, A.H.C., He, M.M., Shin, K., Mai, V., Jeong, K.C., Finckh, M.R., Morris, J.G., 2018. Environmental and health effects of the herbicide glyphosate. Sci. Total Environ. 616–617, 255–268. https://doi.org/10.1016/j.scitotenv.2017.10.309.
VanLandeghem, M.M., Farooqi, M., Southard, G.M., Patiño, R., 2015. Spatiotemporal associations of reservoir nutrient characteristics and the invasive, harmful alga Prymnesium parvum in West Texas. J. Am. Water Resour. Assoc. 51, 487–501. https://doi.org/10.1111/jawr.12261.
Vera, M.S., Di Fiori, E., Lagomarsino, L., Sinistro, R., Escaray, R., Iummato, M.M., Juárez, A., De Molina, M.D.C.R., Tell, G., Pizarro, H., 2012. Direct and indirect effects of the glyphosate formulation Glifosato Atanor® on freshwater microbial communities. Ecotoxicology 21, 1805–1816. https://doi.org/10.1007/s10646-012-0915-2.
Vera, M.S., Lagomarsino, L., Sylvester, M., Pérez, G.L., Rodríguez, P., Mugni, H., Sinistro, R., Ferraro, M., Bonetto, C., Zagarese, H., Pizarro, H., 2010. New evidences of Roundup® (glyphosate formulation) impact on the periphyton community and the water quality of freshwater ecosystems. Ecotoxicology 19, 710–721. https://doi.org/10.1007/s10646-009-0446-7.
Wang, C., Lin, X., Li, L., Lin, S., 2016. Differential growth responses of marine phytoplankton to herbicide glyphosate. PLoS One 11, 1–20. https://doi.org/10.1371/journal.pone.0151633.
Wehtje, G., Altland, J.E., Gilliam, C.H., 2009. Interaction of glyphosate and pelargonic acid in ready-to-use weed control products. Weed Technol. 23, 544–549. https://doi.org/10.1614/WT-08-044.1.
Wong, P.K., 2000. Effects of 2,4-D, glyphosate and paraquat on growth, photosynthesis and chlorophyll-a synthesis of *Scenedesmus quadricauda* Berb 614. Chemosphere 41, 177–182. https://doi.org/10.1016/S0045-6535(99)00408-7.
Wood, A.M., Everroad, R.C., Wingard, L.M., 2005. Measuring growth rates in microalgal cultures. Algal Cult. Tech. 18, 269–288.
Yates, B.S., Rogers, W.J., 2011. Atrazine selects for ichthyotoxic *Prymnesium parvum*, a possible explanation for golden algae blooms in lakes of Texas, USA. Ecotoxicology 20, 2003–2010. https://doi.org/10.1007/s10646-011-0742-x.