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Herbicide toxicity to non-target aquatic organisms does not increase in mixtures with surfactants

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Abstract

Agricultural herbicides enter aquatic environments after rain events where they affect microscopic aquatic plants (phytoplankton) and animals (zooplankton) that form the base of aquatic food webs. Atrazine, an herbicide with low solubility in water, is often mixed with surfactants such as alkyl polyglucoside (APG) to improve effectiveness. Although APG has low-toxicity, a potential drawback to increased atrazine solubility is greater adverse effects on aquatic organisms. I hypothesized that atrazine and APG decrease phytoplankton abundance more than atrazine alone. Specifically, I predicted phytoplankton abundance should 1) decrease with increasing concentrations of the mixture compared to the same atrazine concentrations individually, and 2) decrease more with zooplankton grazers that consume phytoplankton. The responses of phytoplankton (*Chlorella* sp.) to atrazine with or without APG and with or without grazers (*Daphnia magna*) were examined over 2 weeks in freshwater microcosms. First, *Chlorella* sp. received atrazine (0, 1, 5, or 25 μgL^{-1}) with or without APG (n=5). In a second experiment, 10 *D. magna* were also added to each microcosm. Only the highest concentration of atrazine decreased phytoplankton abundance, independent of APG addition. Surprisingly, mixing APG with low and medium concentrations of atrazine either had no effect or appeared to reduce atrazine toxicity to *Chlorella* sp. As expected, grazers decreased *Chlorella* sp. abundance. However, increasing atrazine concentration adversely affected *D. magna* abundance both alone and in mixture with APG. These results suggest that high concentrations of atrazine decrease the abundance of non-target planktonic organisms and mixing atrazine with APG does not appear to increase atrazine toxicity.

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Introduction

Chemicals used to kill weeds (herbicides) are commonly used in agriculture (Hersh and Crumpton 1989). Globally, 70,000-90,000 tons yr^{-1} of the triazine herbicide atrazine are applied to corn, sugarcane, and other vegetable and cereal crops for weed control (Graymore et al. 2001; Singh and Cameotra 2014). Atrazine kills weeds by prohibiting electron transport between photosystems I and II (DeNoyelles et al. 1982; Hersh and Crumpton 1989; Seguin et al. 2001; DeLorenzo and Serrano 2003). Although atrazine application targets weeds in agricultural fields, the herbicide also enters aquatic environments via surface runoff following rain events (Hersh and Crumpton 1989; Seguin et al. 2001; DeLorenzo and Serrano 2003). Average atrazine concentrations in the surface waters range from 0.1-30 μgL^{-1} (DeNoyelles et al. 1982; Solomon et al. 1996; DeLorenzo and Serrano 2003), but concentrations as high as 500 μgL^{-1} have been measured in surface waters adjacent to agricultural fields (DeNoyelles et al. 1982). Although aquatic plants and algae are not intended targets of atrazine, these concentrations can adversely affect aquatic primary producers (Hersh and Crumpton 1989; Graymore et al. 2001; Ulrich et al. 2017).

Atrazine inhibits photosynthesis and growth in freshwater microalgae (phytoplankton) in both field and laboratory studies (DeNoyelles et at. 1982; Hersh and Crumpton 1989; Tang et al. 1997). The phytoplankton community in ponds containing 20 μgL^{-1} of atrazine experienced decreased photosynthesis and abundance after 2 days (DeNoyelles et al. 1982), but atrazine seems to have different effects on different species of phytoplankton (Tang et al. 1997). In laboratory experiments, 10 μgL^{-1} inhibited growth of the unicellular green microalga *Chlorella* sp. but promoted growth of the diatom *Synedra acus* and green algae *Chlamydomonas* sp. at atrazine concentrations of 1 μgL^{-1} and 10 μgL^{-1} (Tang et al. 1997). Thus *Chlorella* sp., an important food source for zooplankton grazers, may be more sensitive to atrazine than other freshwater algae.

Phytoplankton are a major food source for zooplankton grazers, therefore, if algae are directly affected by a toxin, the behavior, growth, and reproduction of grazers can be indirectly affected (DeNoyelles et al. 1982; Fleeger et al. 2003; Sarma et al. 2005). Atrazine negatively affects microalgal abundance at field concentrations (0.1-30 μgL^{-1}), but is not known to have direct effects on invertebrates at these concentrations (Macek et al. 1976; DeNoyelles et al. 1982; Solomon et al. 1996; DeLorenzo and Serrano 2003). Concentrations as high as 1.15 mgL^{-1} of atrazine had no direct effect on *Daphnia magna* survival and reproduction (Macek et al. 1976). Phytoplankton susceptibility to atrazine presents a potential for reduced food availability to zooplankton grazers and subsequently to higher trophic levels (Graymore et al. 2001; Sarma et al. 2005). In a field experiment, 500 μgL^{-1} of atrazine almost completely inhibited algal growth resulting in reduced growth and reproduction of *Daphnia pulex* (DeNoyelles et al. 1982).

The potential for atrazine to persist in the environment at concentrations that reduce phytoplankton abundance depends on degradation rate, environmental factors and mixture

effects (Solomon et al. 1996; Graymore et al. 2001; Rohr and McCoy 2009). Degradation of atrazine is generally slow; in laboratory experiments, concentrations did not change after 30 days, and half-lives of two weeks to three months were observed in surface waters (Solomon et al. 1996; Graymore et al. 2001). Temperature and pH also affect degradation time; atrazine persists longer in dry, cool areas with consistent pH conditions (Graymore et al. 2001). Optimal pH conditions for atrazine in soil range from 5.5-6.5 (Islam et al. 1980). In addition, atrazine does not dissolve well in water, therefore it is often mixed with surfactants to increase solubility (Singh and Cameotra 2014). Surfactants decrease surface tension through formation of micelles, circular, enclosed structures formed by molecules containing both polar and nonpolar regions (Mulligan 2005; Qin et al. 2006; Sachdev and Cameotra 2013) that improve dispersal and spreading of pesticides and herbicides. While the use of surfactants increases herbicide dispersal onto crops, it also creates a mixture that can readily disperse into adjacent aquatic environments (Mulligan 2005).

Alkyl polyglucoside (APG), is a low-toxicity surfactant derived from glucose and fatty alcohols that is increasingly used in mixture with herbicides and commercial household products (Balzer and Luder 2000) because it degrades rapidly (73-100% in 14-28 days), thereby reducing its persistence in the environment (Toshima et al. 1995; Garcia et al. 1997). This non-ionic surfactant has adverse effects on phytoplankton and zooplankton communities at concentrations of 2.5-10 mgL^{-1}, specifically on copepods and *Daphnia magna* (Riera and Cohen 2016). In a laboratory experiment, the EC_{50} value for the green algae *Selenastrum capricornutum* was 0.509 mgL^{-1} after exposure for 72 hours to the non-ionic surfactant Genapol OX-80 (Anástacio et al. 2000), suggesting that low concentrations of surfactants can decrease algal density. Exposure to APG also appears to reduce size and number of *D. magna* (Garcia et al. 1997; Benevente and

Cohen 2013). Given that APG is used at its critical micelle concentration (CMC) when mixed with herbicides (Shinoda et al. 1961; Toshima et al. 1995), there is potential for the adverse effects of the mixture on plankton to be worse than those of each chemical alone.

Currently, there is a paucity of literature investigating the toxicity of herbicide-surfactant mixtures on aquatic organisms despite the potential for damaging effects on phytoplankton and zooplankton. Several studies indicate that atrazine is used alongside nonionic surfactants, but mainly focus on the effects surfactants have on atrazine sorption in soils (Abu-Zreig et al. 1999; Chappell et al. 2005; Tao et al. 2006) I hypothesized that mixtures of APG and atrazine negatively affect *Chlorella* sp. density more than atrazine alone. Furthermore, I predicted that *Chlorella* sp. density would: 1) decrease with increasing atrazine concentration, 2) be reduced more when APG was added, and 3) have the largest reduction in mixture with grazers. Direct effects of atrazine on growth and reproduction of *D. magna* were not expected.

Methods

Study Organisms

Cultures of *D. magna* and *Chlorella* sp. were obtained from Carolina Biological Supply Company, Burlington, NC. The *D. magna* cultures were reared in a Georgia Southern University (Statesboro, GA, USA) laboratory under daylight fluorescent lighting at an irradiance of 80-100 µmol m^2 s^{-1} at 22°C on a 16:8 light:dark cycle. *Chlorella* sp. cultures were grown in an Alga-Gro® medium in an environmental chamber at 21°C on a 16:8 light:dark cycle.

Experimental Design

Experiment 1:

In order to determine whether atrazine toxicity to *Chlorella* sp. increases with increasing concentration and is worsened in the presence of APG, a laboratory experiment was conducted 11 to 25 July 2018 at Georgia Southern University. The objective was to detect effects of the treatments (0, 1, 5, or 25 µgL^{-1} atrazine with or without APG) on *Chlorella* sp. cell density. Each microcosm contained *Chlorella* sp. at a density of $2x10^4$ cells ml^{-1} (Kelly and Cohen 2018) and was verified using flow cytometry (Figure 1).

Experiment 2

The second experiment was conducted to establish the effects of both chemical treatments and grazers on *Chlorella* sp. density. This experiment was conducted from 11 to 25 August 2018. In addition to the atrazine and APG treatments and algal density used in experiment 1, each microcosm in this experiment contained 10 *D. magna*. The 14-day duration of the experiment allowed for observation of *D. magna* reproduction; *D. magna* typically reproduce 5-10 days after birth (Ebert 2005). Microcosms in both experiments were randomized

by location under daylight fluorescent lights at an irradiance of 80-100 µmol m^2 s^{-1} at 22°C on a 16:8 light:dark cycle (Duff et al. 2017).

Treatment Solutions

For each experiment, freshwater microcosms were dosed with one of four concentrations of atrazine (0, 1, 5, or 25 μgL^{-1}) with or without APG for a total of 10 treatments with 5-fold replication (N=50). Each 2 L glass microcosm contained 1 L of treatment solution. Treatment solutions were made by dissolving atrazine (CAS# 1912-24-9, Carbosynth, San Diego, CA) and/or APG 0810 (CAS# 68515-73-1, Chemistry Connection LLC, Conway, Arkansas), a type of APG used in agricultural applications (Zhang et al. 2011), in natural spring water collected from Millen, GA, USA (GPS: 32.7110,-81.8791). Atrazine treatment concentrations were selected within the average range measured in surface waters (0.1-30 μgL^{-1}) in the United States (DeLorenzo and Serrano 2003). The concentration of APG in each mixture treatment varied depending on the atrazine concentration. Treatments were made using 15 mgL^{-1} atrazine stock solutions either with or without 0.705gL^{-1} APG. This concentration of APG was the critical micelle concentration (CMC) of APG 0810, which is the minimum concentration of APG required to increase solubility of a substance (Shinoda et al. 1961). The concentrations used in the experiments were made using appropriate volumes of the stock solution, thus the resulting concentrations of atrazine and APG in the low, medium, and high mixtures were: 1 μgL^{-1} atrazine/0.2 mgL^{-1} APG, 5 μgL^{-1} atrazine/0.5 mgL^{-1} APG, and 25 μgL^{-1} atrazine/1.2 mgL^{-1} APG.

Nominal atrazine concentrations were verified at the University of Georgia Crop and Environmental Quality Laboratory (Table 1) using a modified version of EPA method 507 (Engels and Graves 1989). Briefly, ethyl acetate was used to extract atrazine from water

followed by analysis using gas chromatography (Table 1). The highest APG concentration (1.2 mgL^{-1}) was also verified using a colorimetric assay modified from Buschmann et al. (1996) where solutions were treated with anthrone reagent, sulfuric acid, and formic acid, formed a yellow-colored solution, and was compared to a standard curve measured using spectrophotometer. The limit of detection of the method is 1 mgL^{-1} (Buschmann et al. 1996). The measured concentrations of APG in the high APG only and the high mixture treatments was 1.48 mgL^{-1} and 1.36 mgL^{-1}, respectively.

Plankton Abundance and Water Quality Sampling

Abundance of *Chlorella* sp. was measured in two ways. Cell density was determined at the beginning and end of the experiment. Water samples (2 ml) were pipetted from the bottom of each microcosm after homogenization of the algae by mixing with a stirring rod (Givan 2001). Then, 27µL of the sample analyzed using flow cytometry (Accuri C6, Becton-Dickinson, CA, USA) (Givan 2001; Figure 1). Pigment concentration (chlorophyll *a*) as a proxy for *Chlorella* sp. abundance was quantified using fluorometry. Solutions in each microcosm were homogenized by vigorous shaking to resuspend algae and a 100 ml subsample was filtered onto Whatman GF/F glass fiber filters (nominal pore size 0.7µm) to collect algal cells (Duff et al. 2017). Pigments from collected cells were extracted in 90% acetone in the dark at -20°C for 24 hours followed by fluorescence measurement using a Trilogy fluorometer (Turner Designs, Sunnyvale, CA) according to EPA method 445.0 (Arar and Collins 1997). Number of *D. magna* were counted visually at the end of the experiment to determine abundance in the grazer experiment. Water quality (pH, DO, and conductivity) was also measured in both experiments initially and at the end of the experiment to monitor treatment effects on water quality.

Statistical Analysis

Cell density, chlorophyll *a* concentration, and *D. magna* abundance data were tested for equal variances using Levene's test and for normality using Shapiro-Wilk *W* test. All data from experiment 1 met the assumptions of parametric tests. In the second experiment with *D. magna*, cell density and chlorophyll *a* values were log transformed in order to meet the assumptions. The effects of atrazine concentration alone and in mixture with APG on all variables were tested using two-way ANOVA in JMP software (SAS Institute Inc., Cary, NC).

Results

Atrazine and APG effects on *Chlorella* sp. density differed with concentration, and with the presence of grazers. When *Chlorella* sp. was tested alone, there were no negative effects of atrazine on *Chlorella* sp. cell density at any concentration (Table 2a; Figure 2a,b,c). APG alone appeared to have a beneficial effect on cell density at low (>50%) and medium (40%) concentrations (Figure 2a,b) but not at the highest concentration (Figure 2c). There was an interaction between atrazine and APG at medium concentrations, likely due to opposing effects of atrazine and APG on cell density (Table 2a). APG alone seemingly increased cell density, but not in mixture with atrazine, while atrazine alone appeared to have a beneficial effect, nearly doubling cell density (Figure 2b). Cell densities in treatments with the highest concentration of atrazine and/or APG did not differ from the control (Table 2a; Figure 2c). When grazers were added to the microcosms, cell density decreased in all treatments by an order of magnitude relative to the *Chlorella* sp. only experiment (Figure 2). There were no effects of either atrazine or APG on cell density at low and medium concentrations (Table 2b; Figure 2d,e). At the high

concentration, atrazine decreased cell density (Table 2b) by 50% both with and without the addition of APG (Figure 2f).

Atrazine and APG effects on chlorophyll *a* concentration were generally concentration-dependent. In the *Chlorella* sp. only experiment, no effect of atrazine on chlorophyll *a* concentration occurred at low or high concentrations (Table 3a; Figure 3a,c). In contrast, the medium concentration of atrazine increased chlorophyll *a* concentration (Table 3a), but adding APG had no effect (Table 3a; Figure 3b). The low concentration of APG alone stimulated chlorophyll *a* concentration by 50%, but not in mixture with atrazine (Table 3a; Figure 3a). Adding grazers generally appeared to decrease chlorophyll *a* concentration in the control (50%) and the APG-only treatments (0-40%) (Figure 3d,e,f). There were no effects of atrazine or APG on chlorophyll *a* concentration at the low concentration (Table 3b; Figure 3d), but the medium concentration of atrazine more than doubled chlorophyll *a* concentration (Table 3b; Figure 3e). At the high concentration, a significant interaction between atrazine and APG occurred (Table 3b). When APG was added alone, chlorophyll *a* concentration appeared to increase, but APG in mixture with atrazine seemed to decrease chlorophyll *a* concentration (Figure 3f).

D. magna abundance declined with increasing atrazine concentration (Figure 4). At the low concentration, neither atrazine nor APG affected *D. magna* abundance (Table 4; Figure 4a). The medium concentration of atrazine resulted in an apparent 50% decrease in *D. magna* abundance when in mixture with APG (Table 4; Figure 4b). A significant interaction occurred between high concentrations of atrazine and APG on *D. magna* abundance (Table 4; Figure 4c). Atrazine virtually eliminated *D. magna* with or without APG present, but APG alone apparently stimulated a doubling in *D. magna* abundance relative to the control (Figure 4c).

DO never decreased below 5.9 mgL^{-1} in both experiments (Table 5). Final DO concentrations showed a higher increase in the *D. magna* experiment than in the *Chlorella* only (Table 5). Overall, the pH was similar in both experiments, staying between 8 and 9 (Table 6). Conductivity decreased from initial values in the *Chlorella* sp. only experiment, with the largest decreases in the control and APG-only treatments (20-30%) relative to the treatments containing atrazine (8-25%) (Table 7a). In the *Chlorella* sp. + *D. magna* experiment, the conductivity of the water did not change over 2 weeks, and remained similar across all treatments (Table 7b).

Discussion

Chlorella sp. cell density was predicted to decrease with increasing concentration of atrazine. However, neither atrazine alone or in mixtures with APG adversely affected *Chlorella* sp. cell density. Instead, addition of these chemicals either seemed to increase *Chlorella* sp. abundance at low and medium concentrations or had no effect at the high concentration. This response to atrazine was not expected for *Chlorella* sp. because a previous study discovered that 10μgL^{-1} inhibited *Chlorella* sp. growth (Tang et al. 1997). Another study also indicated that atrazine is inhibitory to green algal growth at low concentrations ranging from 1-10 μgL^{-1} (Torres and O'Flaherty 1976). However, studies focusing on the possible stimulatory effects of atrazine concentrations less than or equal to 5μgL^{-1} on *Chlorella* sp. are missing.

When using chlorophyll *a* as a proxy for abundance, the medium concentration of atrazine increased chlorophyll *a* concentration in both experiments, and the high concentration increased chlorophyll *a* concentration in the grazer experiment. Several studies have shown atrazine and other similar herbicides stimulate chlorophyll *a* concentration in green algae (El-Dib et al. 1989; Tang et al. 1997). Low concentrations of gardoprim (10 and 20 μgL^{-1}), an herbicide

similar to atrazine, increased chlorophyll *a* concentration of *Scenedesmus* sp. after 7 days of exposure (El-Dib et al. 1989). Atrazine (10 μgL^{-1}) also increased chlorophyll *a* concentration of *Chlamydomonas* sp. after 14 days of exposure (Tang et al. 1997). Adaption of photosynthetic pigments to low concentrations of atrazine may account for increased chlorophyll *a* concentration (Gustavson and Wangberg 1995).

The addition of APG to atrazine treatments did not appear to increase atrazine toxicity. Mixture treatments generally had cell densities either similar to or greater than the control. The only indication that APG increased atrazine toxicity was at the medium concentration; the mixture had a lower cell density than the atrazine-alone treatment, but had a higher cell density than the control. In some instances, surfactants have decreased the negative effects atrazine has photosynthetic organisms; atrazine toxicity to *Anabaena* CPB4337 decreased when *Anabaena* was pre-exposed to 5 mgL^{-1} of either the surfactant perfluorooctano sulphonate (PFOS) or surfactant perfluorooctanoic acid (PFOA) (Rodea-Palomares et al. 2015).

In addition to chemical effects, I anticipated that grazers would decrease *Chlorella* sp. cell density more than chemicals alone due to trophic interactions. Overall, cell density decreased by an order of magnitude when grazers were added to the microcosms across all concentrations, with the greatest reduction in the high concentration. A decline in *D. magna* abundance also occurred at this treatment, despite other studies showing that atrazine concentrations greater than 250 μgL^{-1} are needed to reduce food availability and reproduction of *D. magna* (Macek et al. 1976; DeNoyelles et al. 1982). This decline in abundance shows that grazing was not the sole cause of the decreased cell density. Also, these data suggest that reduced food availability to higher trophic levels in food webs can occur at concentrations an order of magnitude lower than previously thought. Additionally, a study found that succession of non-

resistant (green) algae occurred by resistant species of phytoplankton (diatoms) after exposure to 20 μgL^{-1} atrazine (DeNoyelles et al. 1992), indicating altered community composition.

The instances concerning increased algal growth in mixture can be associated with the stimulatory effects APG can have on algal growth (Duff et al. 2017). When APG degrades, it cleaves the bonds between glucose and fatty alcohols in the molecule, followed by oxidation of fatty alcohols to fatty acids (Eichhorn and Knapper 1998). These products may be used for heterotrophic growth of green algae (Droop 1974). Sun et al. (2008) indicated that *Chlorella zofingiensis* had the highest specific growth rate when glucose was used as its main carbon source and energy source when in the dark. Therefore, the excess glucose from APG degradation in the microcosms may have been used to stimulate *Chlorella* sp. growth.

On the whole, these results express the detrimental effects 25μgL^{-1} of atrazine can have on aquatic planktonic organisms that inhabit water sources neighboring agricultural areas. Decreased algal density following exposure to agrochemicals limits food for zooplankton grazers, thus causing their growth and reproduction to decline. Furthermore, decreased grazer abundance can reduce food availability to higher trophic levels as well. Food webs also may be altered if dominate species are more susceptible to the toxin than other species in the ecosystem. As the results in this experiment are compelling because they imply that atrazine concentrations below 25 μgL^{-1} do not adversely affect *Chlorella* sp. and *D. magna*, further testing on other microscopic green algae and zooplankton grazer species is essential because agrochemicals display different effects on different species.

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Table 1. Nominal and measured atrazine concentrations for each treatment.

Treatment	**Nominal [Atrazine] (μgL^{-1})**	**Measured [Atrazine] (μgL^{-1})**
Low Atrazine	1	1.31
Low Atrazine + APG	1	1.13
Medium Atrazine	5	5.25
Medium Atrazine + APG	5	5.38
High Atrazine	25	26.19
High Atrazine + APG	25	26.56

Table 2. Two-way ANOVA results showing the effects of atrazine and APG on *Chlorella* sp. cell density (cells ml^{-1}) after 14 days of exposure to treatments a) alone or b) with *D. magna*.

a) *Chlorella* sp. only				
Experiment	**Treatment**	**df**	**F**	***p***
Low (n=5)	APG	1,19	31.53	**<.0001**
	Atrazine	1,19	0.0003	0.99
	APG*Atrazine	1,19	0.66	0.43
Medium (n=5)	APG	1,19	1.55	0.23
	Atrazine	1,19	3.19	0.09
	APG*Atrazine	1,19	12.18	**0.003**
High (n=5)	APG	1,19	0.64	0.44
	Atrazine	1,19	0.84	0.37
	APG*Atrazine	1,19	0.86	0.37
b) *Chlorella* sp. + *D. magna*				
Experiment	**Treatment**	**df**	**F**	***p***
Low (n=5)	APG	1,19	0.02	0.89
	Atrazine	1,19	0.45	0.51
	APG*Atrazine	1,19	0.10	0.75
Medium (n=5)	APG	1,19	0.02	0.88
	Atrazine	1,19	0.98	0.34
	APG*Atrazine	1,19	1.47	0.24
High (n=5)	APG	1,19	0.03	0.87
	Atrazine	1,19	21.55	**0.0003**
	APG*Atrazine	1,19	0.007	0.94

Table 3. Two-way ANOVA results showing effects of atrazine and APG on *Chlorella* sp. chlorophyll *a* concentration (μgL^{-1}) after 14 days of exposure a) alone or b) with *D. magna*.

a) *Chlorella* sp. only				
Experiment	**Treatment**	**df**	**F**	***p***
Low (n=5)	APG	1,19	5.11	**0.04**
	Atrazine	1,19	0.03	0.86
	APG*Atrazine	1,19	0.86	0.37
Medium (n=5)	APG	1,19	1.92	0.19
	Atrazine	1,19	9.13	**0.008**
	APG*Atrazine	1,19	3.18	0.09
High (n=5)	APG	1,19	2.22	0.16
	Atrazine	1,19	2.89	0.11
	APG*Atrazine	1,19	2.46	0.14
b) *Chlorella* sp. + *D. magna*				
Experiment	**Treatment**	**df**	**F**	***p***
Low (n=5)	APG	1,19	1.26	0.28
	Atrazine	1,19	0.93	0.35
	APG*Atrazine	1,19	2.06	0.17
Medium (n=5)	APG	1,19	2.60	0.13
	Atrazine	1,19	34.90	**<0.0001**
	APG*Atrazine	1,19	2.98	0.10
High (n=5)	APG	1,19	1.10	0.31
	Atrazine	1,19	18.63	**0.0005**
	APG*Atrazine	1,19	8.70	**0.009**

Table 4. Two-way ANOVA results showing the effects of atrazine and APG on *D. magna* abundance after 14 days of exposure.

Experiment	**Treatment**	**df**	**F**	***p***
Low (n=5)	APG	1,19	0.006	0.94
	Atrazine	1,19	0.93	0.35
	APG*Atrazine	1,19	2.19	0.16
Medium (n=5)	APG	1,19	0.41	0.53
	Atrazine	1,19	5.74	**0.03**
	APG*Atrazine	1,19	0.26	0.61
High (n=5)	APG	1,19	5.90	**0.03**
	Atrazine	1,19	68.79	**<0.0001**
	APG*Atrazine	1,19	6.12	**0.02**

Table 5. Mean initial and final water quality measurements for dissolved oxygen (DO) ± one standard error of the mean (SEM) for a) *Chlorella* sp. only or b) *Chlorella sp. + D. magna* experiment.

	Low		**Medium**		**High**	
a. *Chlorella* sp. only	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**
Control	7.47 ±0.05	8.84 ±0.04	7.47 ±0.05	8.84 ±0.04	7.47 ±0.05	8.84 ±0.04
APG	7.78 ±0.02	9.10 ±0.09	7.54 ±0.02	8.57 ±0.05	7.48 ±0.04	8.68 ±0.04
Atrazine	7.55 ±0.04	9.59 ±0.06	7.68 ±0.03	8.76 ±0.07	7.46 ±0.02	8.73 ±0.08
APG + Atrazine	7.55 ±0.04	9.20 ±0.06	7.60 ±0.04	8.71 ±0.09	7.20 ±0.03	8.68 ±0.04
	Low		**Medium**		**High**	
b. *Chlorella sp. + D. magna*	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**
Control	5.94 ±0.01	8.13 ±0.11	5.94 ±0.01	8.13 ±0.11	5.94 ±0.01	8.13 ±0.11
APG	6.81 ±0.08	8.10 ±0.20	5.96 ±0.32	8.12 ±0.27	6.03 ±0.03	8.28 ±0.07
Atrazine	6.39 ±0.06	8.30 ±0.28	7.37 ±0.02	9.41 ±0.33	6.87 ±0.21	8.49 ±0.10
APG + Atrazine	6.65 ±0.40	8.54 ±0.24	5.62 ±0.04	8.90 ±0.24	6.27 ±0.03	8.24 ±0.04

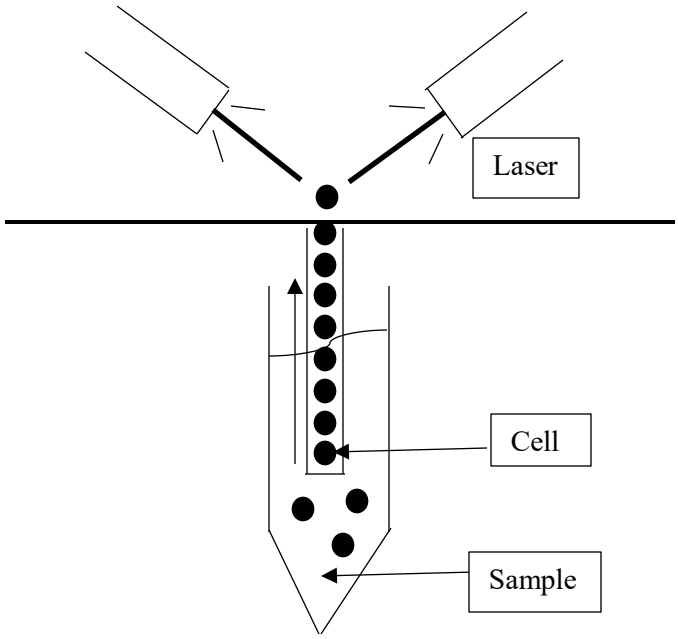
Table 6. Mean initial and final water quality measurements for pH ± one SEM for a) *Chlorella* sp. only or b) *Chlorella sp. + D. magna* experiment.

	Low		**Medium**		**High**	
a. *Chlorella* sp. only	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**
Control	8.4 ±0	8.7 ±0.02	8.4 ±0	8.7 ±0.02	8.4 ±0	8.7 ±0.02
APG	8.4 ±0.02	8.8 ±0.02	8.4 ±0	8.6 ±0.02	8.4 ±0.02	8.7 ±0.02
Atrazine	8.4 ±0.02	8.9 ±0.02	8.4 ±0	8.7 ±0.03	8.4 ±0	8.8 ±0.06
APG + Atrazine	8.4 ±0	8.9 ±0.02	8.4 ±0	8.7 ±0.03	8.3 ±0.02	8.8 ±0.03
	Low		**Medium**		**High**	
b. *Chlorella sp. + D. magna*	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**
Control	8.20 ±0	8.50 ±0.07	8.20 ±0	8.50 ±0.07	8.20 ±0	8.50 ±0.07
APG	8.16 ±0.02	8.46 ±0.08	8.10 ±0	8.52 ±0.08	8.10 ±0	8.50 ±0.05
Atrazine	8.10 ±0	8.52 ±0.10	8.10 ±0	8.84 ±0.10	8.10 ±0	8.50 ±0.03
APG + Atrazine	8.12 ±0.02	8.54 ±0.05	8.10 ±0	8.80 ±0.06	8.10 ±0	8.48 ±0.02

Table 7. Mean initial and final water quality measurements for conductivity (μS cm^{-1}) ± one SEM for a) *Chlorella* sp. only or b) *Chlorella sp. + D. magna* experiment

	Low		**Med**		**High**	
a. *Chlorella* sp. only	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**
Control	250 ±0	176 ±4	250 ±0	176 ±4	250 ±0	176 ±4
APG	250 ±0	178 ±7	250 ±0	186 ±2	250 ±0	204 ±4
Atrazine	250 ±0	214 ±10	250 ±0	188 ±7	250 ±0	234 ±4
APG + Atrazine	250 ±0	212 ±7	250 ±0	192 ±3	250 ±0	234 ±4
	Low		**Med**		**High**	
b. *Chlorella* sp. + *D. magna*	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**
Control	250 ±0	252 ±2	250 ±0	252 ±2	250 ±0	252 ±2
APG	250 ±0	250 ±0	250 ±0	246 ±2	250 ±0	246 ±2
Atrazine	250 ±0	246 ±4	250 ±0	244 ±2	250 ±0	250 ±0
APG + Atrazine	250 ±0	250 ±0	250 ±0	244 ±2	250 ±0	250 ±0

Figure 1. Flow cytometry diagram. Cells in the sample travel upward through a thin metal pipe (indicated by arrow) into the machine where lasers hit the cells one by one, counting each cell individually.



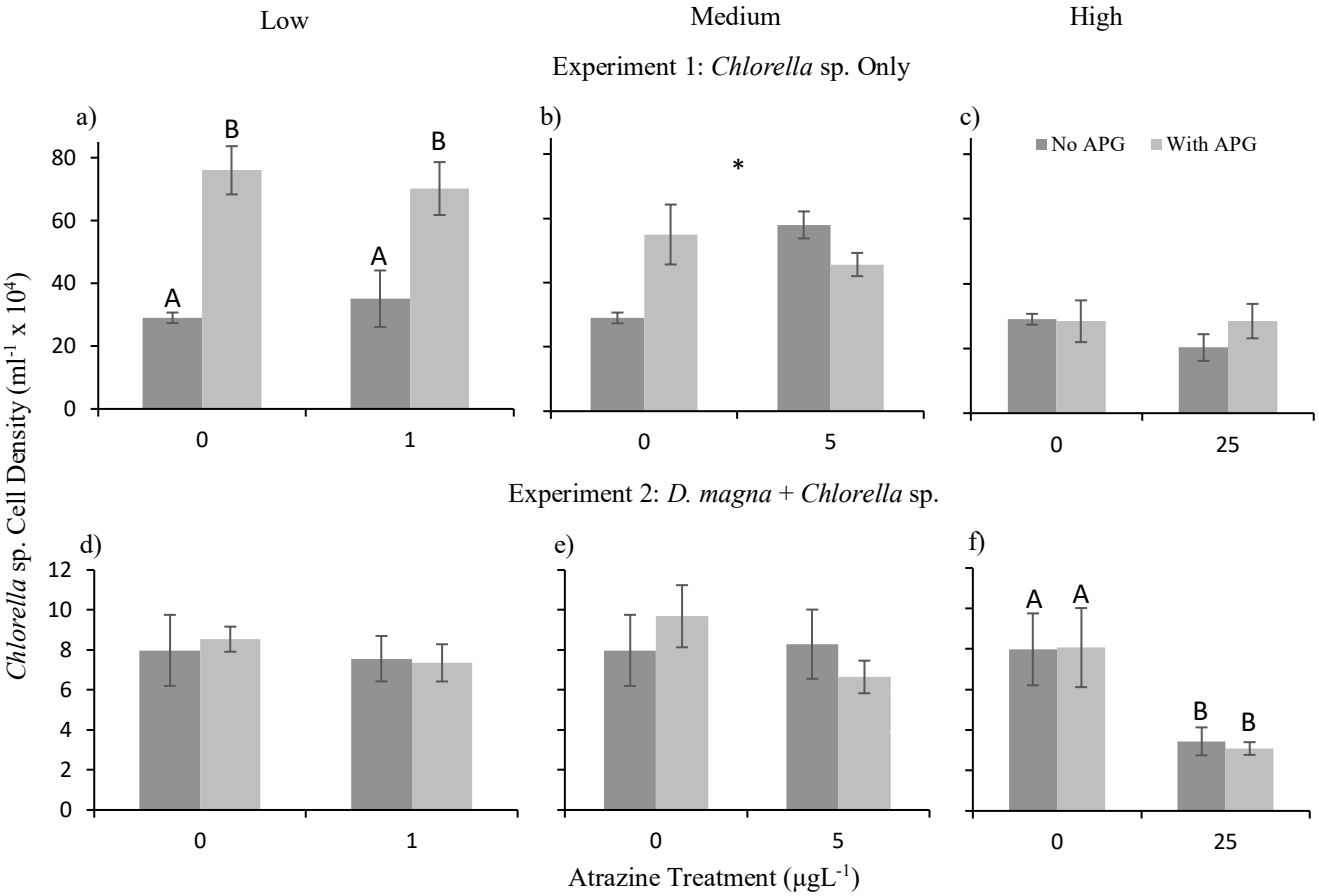


Figure 2. Mean *Chlorella* sp. cell density after 14 days of exposure to low (0.2 mgL^{-1} APG and 1 μgL^{-1} atrazine), medium (0.5 mgL^{-1} APG and 5 μgL^{-1} atrazine), or high (1.2 mgL^{-1} APG and 25 μgL^{-1} atrazine) treatments alone (a,b,c) and with *D. magna* (d,e,f). Error bars are ± one standard error of the mean (SEM) and n=5. Different letters above the bars indicate significant differences. Asterisks indicate significant interactions.

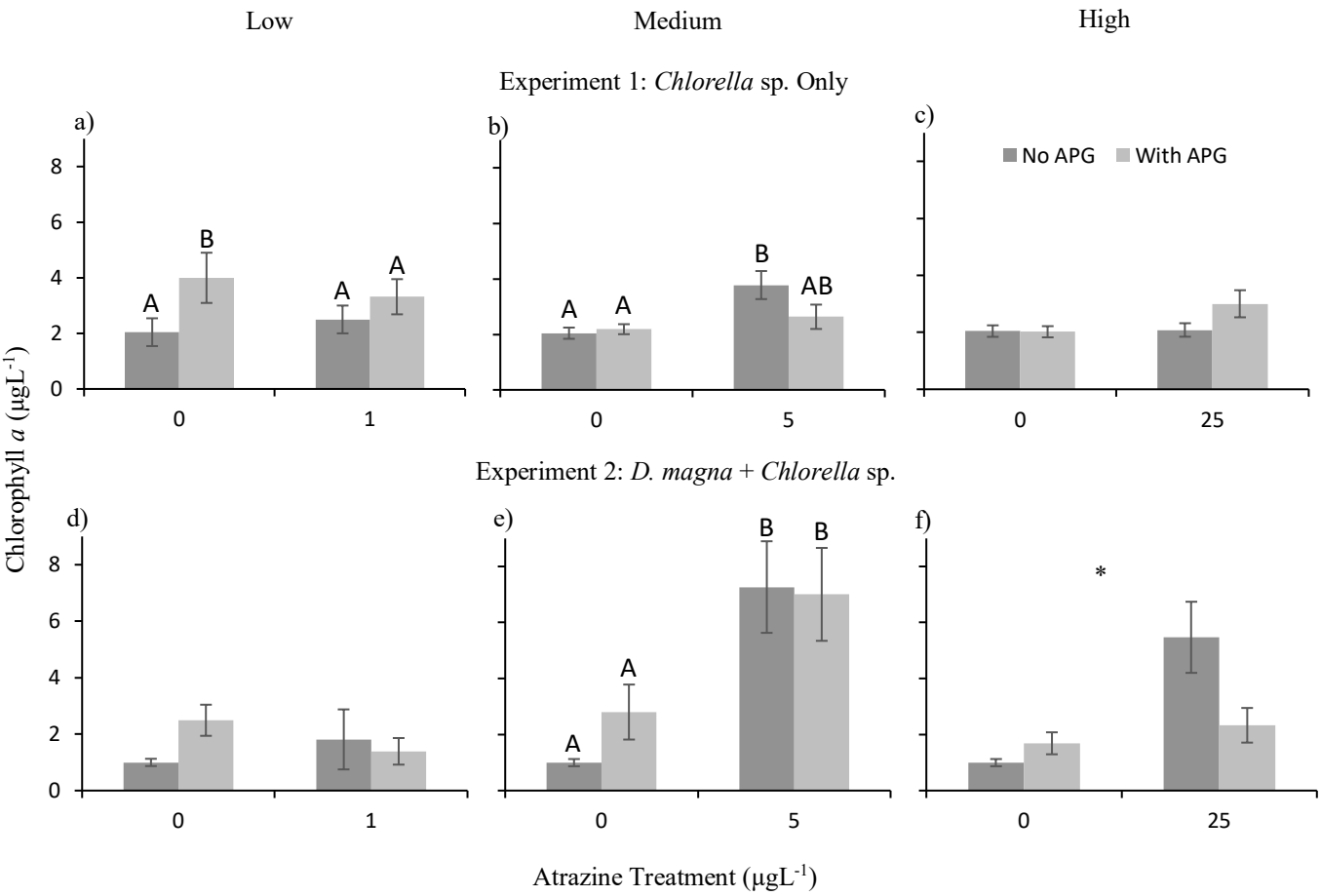


Figure 3. Mean chlorophyll *a* concentration after 14 days of exposure to low (0.2 mgL^{-1} APG and 1 μgL^{-1} atrazine), medium (0.5 mgL^{-1} APG and 5 μgL^{-1} atrazine), or high (1.2 mgL^{-1} APG and 25 μgL^{-1} atrazine) treatments alone (a,b,c) and with *D. magna* (d,e,f). Error bars are ± one SEM and n=5. Different letters above the bars indicate significant differences. Asterisks indicate significant interactions.

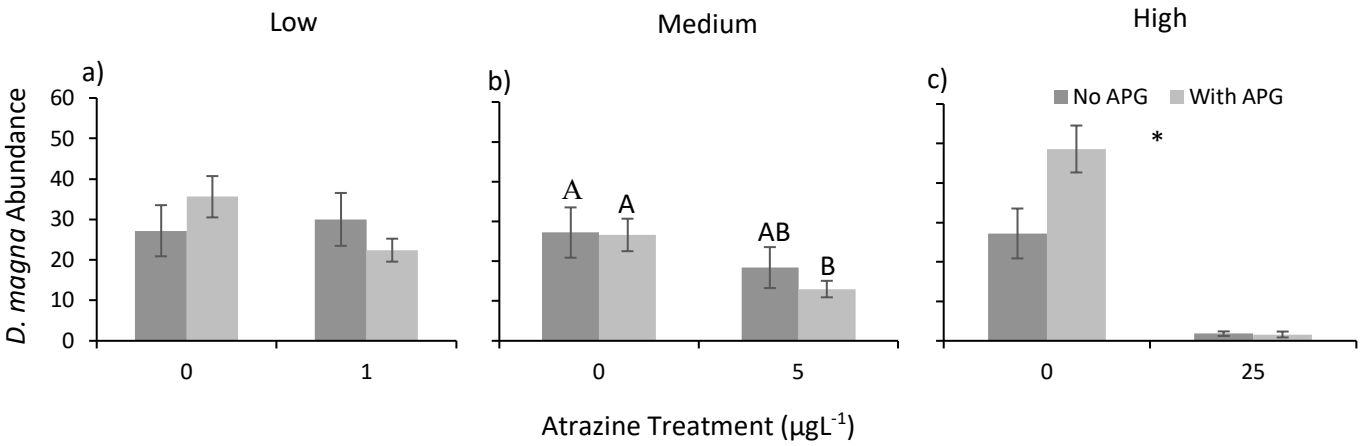


Figure 4. Mean *D. magna* abundance after 14 days of exposure to low (0.2 mgL^{-1} APG and 1 μgL^{-1} atrazine), medium (0.5 mgL^{-1} APG and 5 μgL^{-1} atrazine), or high (1.2 mgL^{-1} APG and 25 μgL^{-1} atrazine) treatments. Error bars are ± one SEM and n=5. Different letters above the bars indicate significant differences. Asterisks indicate significant interactions.