



The role of predation and pesticides in shaping phytoplankton dynamics in a short microcosms experiment

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Abstract

Aquatic organisms are subject to various forcing factors that affect their structure, some of which are natural, while others result from human activities, both having variable effects. This study aimed to determine the importance of a natural stressor (zooplankton) and an herbicide (atrazine) on phytoplankton density and morphological composition in a microcosm experiment. A natural phytoplankton assemblage was exposed to two zooplankton predators: a copepod (*Argyrodiaptomus falcifer*) and a cladoceran (*Ceriodaphnia dubia*), and to atrazine ($27 \mu\text{g L}^{-1}$), in three combinations of factors (zooplankton treatments (Z), atrazine treatment (A), the combination of both (ZA)) plus a Control. The experiment lasted 48 h. Samples were taken at the beginning and the end of the experiment, and relevant limnological variables, including inorganic nutrient concentrations, were considered. Results indicated differences in phytoplankton densities when treatments were compared with Control. In this respect, Chlorophyceae, Euglenophyceae, and Bacillariophyceae exhibited more changes than other phytoplankton classes. Chlorophyceae densities tended to be higher in the Control than in the treatments; the combination of zooplankton and atrazine favored Euglenophyceae, while atrazine favored Bacillariophyceae densities. Regarding morphological groups, unicellular and small colonies ($<35 \mu\text{m}$), showed differences between the Control and particularly with Z treatment, colonial-cenobia forms were negatively affected by atrazine and silica forms were favored by both stressors combined. It is concluded that interactions among natural and anthropogenic stressors could be complex, influencing factors such as phytoplankton taxonomical affinities, morphological groups, and the nature of the stressor applied.

Keywords Predators · Atrazine · Phytoplankton · Zooplankton · Microcosms experiment

Introduction

Phytoplankton consists of a heterogeneous group of photosynthetic microorganisms that live suspended in the water column of almost all known water ecosystems worldwide (Reynolds 2006). As primary producers, phytoplankton influence the trophic dynamics of aquatic ecosystems and impact water quality (Edwards et al. 2013). Phytoplankton are essential for ecosystem services related to carbon fixation and the biogeochemical cycles of all inorganic

elements. Additionally, they form the foundation for crucial trophic interactions such as predation, competition for resources, and mutualism, which shape the functioning of aquatic environments (Naselli-Flores and Padisák 2024).

Phytoplankton respond to environmental changes like temperature, pH, conductivity, nutrient concentration, and light penetration, which also vary across space and time (Edwards et al. 2013). In addition, human alterations like pesticides, eutrophication, metals contamination, and climate change also affect phytoplankton dynamics, and in consequence, the water ecosystems, modifying the quantity and quality of services that they provide (Myers et al. 2013; Malhi et al. 2020). Therefore, understanding and explaining how phytoplankton communities respond to these changes is a central goal in ecology with implications for water management strategies.

Two central stressors of phytoplankton assemblages in natural ecosystems are predation and pesticide intrusion (Wijewardene et al. 2021; Frau 2022). Regarding predation,

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Benndorf et al. (2002) concluded that grazing impacts on phytoplankton could be expected only in short-term experiments, shallow lakes, and mesotrophic or slightly eutrophic deep lakes. In another review study, Frau (2022) demonstrated that other relevant factors like phytoplankton shape, size, and the nature of predators (planktivorous fish, filter-feeding bivalves, or herbivorous zooplankton) also influence the effect of predation as a controlling factor of phytoplankton structure. Indeed, for zooplankton as the main predator, Colina et al. (2016) found that small phytoplankton organisms ($<35\ \mu\text{m}$) with high surface/volume ratios, lacking specialized traits, have a high grazing rate. In contrast, unicellular flagellates of medium to large size ($>35\ \mu\text{m}$) have medium grazing susceptibility. Frau et al. (2017) found that macrozooplankton species (*Argyrodiaptomus* sp. (Copepoda) and *Daphnia obtusa* Kurz (Cladocera)) effectively graze on the phytoplankton of a shallow eutrophic lake in the absence of fish predation. They also found that grazing by large zooplankton mainly affected small-sized algae ($<35\ \mu\text{m}$), such as small cyanobacteria colonies, small silica cell-wall algae, or large groups flexible enough to be handled (e.g., metabolic flagellates like *Euglena*). Similar results have been obtained by Eskinazi-Sant'Anna et al. (2002) and Rietzler et al. (2002). Eskinazi-Sant'Anna reported that 80% of the total ingested food by *Daphnia laevis* Birge in the Pampulha reservoir (Brazil) was colonial chlorophytes. Rietzler et al. (2002) found that *Argyrodiaptomus furcatus* Sars G.O. and *Notodiaptomus iheringi* Wright S. preferred small-sized filamentous diatoms ($\sim 20\ \mu\text{m}$) and single-cell chlorophytes in Broa Reservoir (Brazil).

Pesticides may also influence phytoplankton structure in natural ecosystems (Wijewardene et al. 2021). In rural areas, agriculture is widely recognized as a major contributor to water resource degradation (Gutierrez et al. 2020; Frau et al. 2021a; Frau et al. 2023; Frau and Pineda 2024). Various agricultural practices release pollutants, such as pesticides, which are transported into water bodies compromising their quality (Lushchak 2011; Mateo Sagasta et al. 2018; Patel et al. 2020). Atrazine is a common herbicide used to selectively control annual grasses and broadleaf weeds before they emerge in crop areas in countries like the United States, China, and Argentina (Sass and Colangelo 2006; Frau et al. 2021a). Atrazine hinders photosynthesis in phytoplankton by obstructing electron transfer and disrupting the cytomembrane. This disruption leads to alterations in the transmembrane transport of macromolecules (Graymore et al. 2001). Previous studies have reported the negative impact of this herbicide on planktonic organisms at highly variable concentrations (ranging from $10\ \mu\text{g L}^{-1}$ to $182\ \mu\text{g L}^{-1}$), with effects depending on the phytoplankton group exposed (Jüttner et al. 1995; Seguin et al. 2001; Frau and Gutierrez 2024).

As we have seen, in natural ecosystems, phytoplankton are subject to a combination of several stressors, some natural and others regulated by human interventions. The effects of these stressors can be unpredictable, making experimentation a valuable tool for understanding phytoplankton responses (Frau and Gutierrez 2024). In this study, we examined responses to a natural stressor like predation and a human-induced stressor like atrazine exposure in a microcosm experiment. Our objective was to explore the effect of these two stressors (zooplankton and atrazine) on a natural phytoplankton assemblage. We hypothesized that a) zooplankton effectively decrease phytoplankton density, especially of those more palatable groups; b) atrazine affects only certain phytoplankton groups, favoring the proliferation of others more resistant; and c) the combination of both stressors has antagonistic effects which depend on the group analyzed.

Materials and methods

Phytoplankton samples and water for the experimental vessels were collected from an urban eutrophic shallow lake in Santa Fe city (see Table 1 from Frau et al. 2019 for more details about limnological characteristics) and then transported to the lab for acclimation over four days before the experiment began. For the zooplankton treatment, two species were included together: the copepod *Argyrodiaptomus falcifer* Daday and the cladoceran *Ceriodaphnia dubia* Richard, with a total of five individuals each per vessel. Both species were collected from artificial culture tanks at the Instituto Nacional de Limnología (INALI). The decision to use both species was based on their different food preferences and feeding strategies. *A. falcifer* is a selective feeder with a preference for large phytoplankton algae, while *C. dubia* is a filter-feeder microcrustacean capable of consuming small algae cells and organic particles (Vijverberg 1989; Barnett et al. 2007). Thus, their joint consumption activity encompasses a wide size and algae type range. For the treatments with atrazine, a concentration of $27\ \mu\text{g L}^{-1}$ of the commercial brand Atratop 90 WG was used, based on the maximum concentration of $44\ \mu\text{g L}^{-1}$ observed in natural environments linked to agricultural areas (Frau et al. 2021a). In a previous experiment (Frau & Gutierrez 2024) we found that this concentration was enough to see an effect.

During the experiment, the collected phytoplankton assemblage was exposed to the two zooplankton species used as predators and to the known concentration of atrazine, considering all possible treatment combinations: just zooplankton (treatment Z), just atrazine (treatment A), the combination of both (AZ), and a Control with only phytoplankton. The experiment lasted 48 h, and each treatment

Table 1 Morphological groups assigned by cluster analysis, main characteristics and examples of some species represented in the experiment

MF groups	Cell-wall	Motility	Organization	MLD	Some examples
G1	Pellicle	Flagellum	Unicellular	<35	<i>Chlamydomonas microspiraera</i> ; <i>Cryptomonas ovata</i> ; <i>Phacus orbicularis</i>
G2	Mucilaginous	Aerotopes or oscillation	Colonies-cenobia or filaments	>35	<i>Microcystis aeruginosa</i> ; <i>Lynghya sordida</i> ; <i>Anathece minutissima</i>
G3	Mucilaginous	No motile	Colonies-cenobia	<35	<i>Oocystis elliptica</i> ; <i>Aphanocapsa delicatissima</i> ; <i>Chlorogoeopsis fritschii</i>
G4	Cellulose	No motile	Colonies-cenobia	>35	<i>Micractinium pusillum</i> ; <i>Desmodesmus communis</i>
G5	Cellulose	No motile	Colonies-cenobia	<35	<i>Golenkinia radiata</i> ; <i>Tetraëdron trigonum</i>
G6	Silica	No motile	Unicellular	>35	<i>Ulmaria ulna</i> ; <i>Stephanocyclus meneghinianus</i> ; <i>Fragilaria sp.</i>

MDL maximum linear dimension (µm)

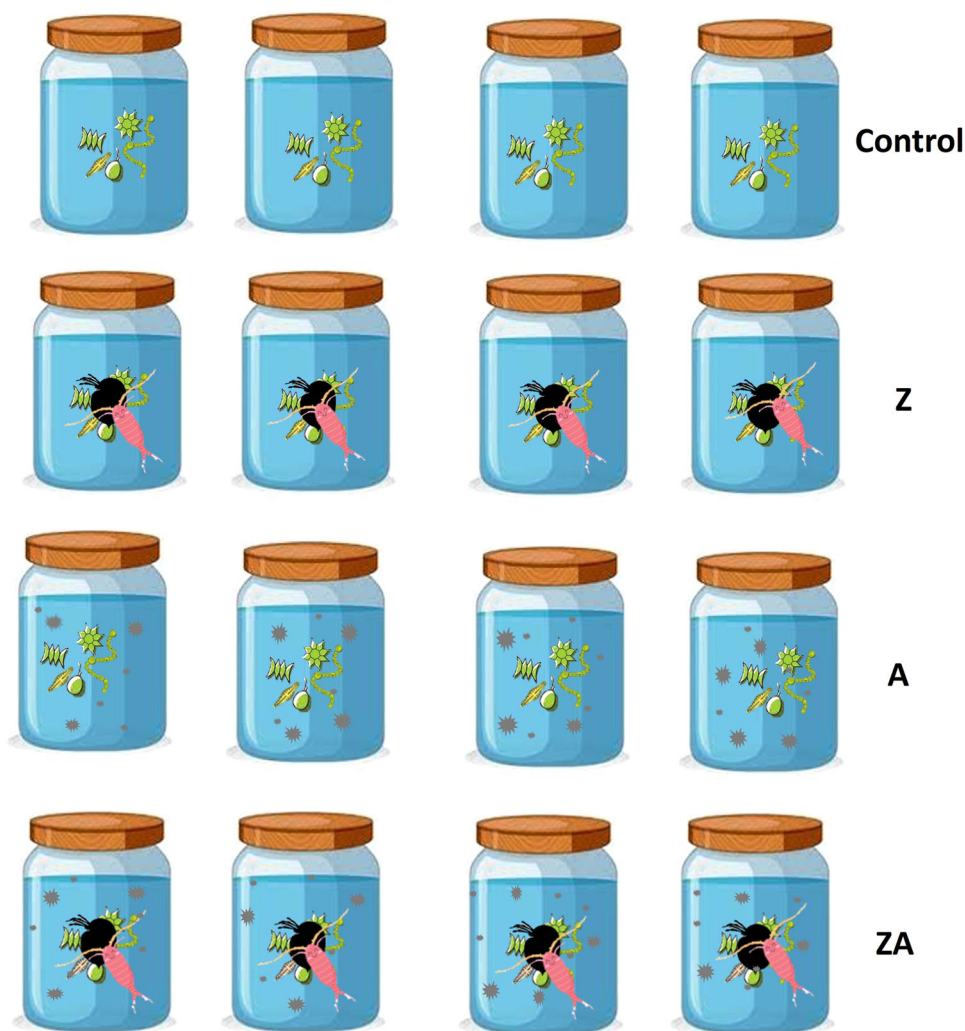
was replicated four times (n = 16). Each treatment vessel consisted of a 500 ml glass container, and the experiment was conducted at 21 °C under a constant photoperiod of 16:8 light-dark conditions (Fig. 1). Samples were collected at the beginning to check if initial densities among treatments were similar and at the end of the experiment to test the proposed hypotheses.

The physical and chemical variables measured at the beginning and end of the experiment included temperature (°C), dissolved oxygen concentration (DO, mg l⁻¹), oxygen saturation (DO % sat.), pH, and conductivity (µS cm⁻¹), which were obtained using a HACH multiparameter probe. Samples of 50 ml for nutrient analysis were taken at the beginning and the end of each vessel in plastic bottles and filtered through 0.7 µm Whatman GF/F glass-fiber filters. Soluble reactive phosphorus (SRP) was measured using the ascorbic acid method, ammonium (NH₄⁺) was determined using the indophenol blue method, while nitrate plus nitrite (N-NO₂ + N-NO₃) was quantified by reducing N-NO₂ with metallic cadmium and subsequent colorimetric determination of N-NO₂ following APHA (2005) recommendations. Both forms of inorganic nitrogen were considered as Dissolved Inorganic Nitrogen (DIN). One-way ANOVA was applied to each environmental variable at the beginning and at the end of the experiment to secure no effect of environmental variations among treatments and the control.

Phytoplankton samples were taken using glass containers and immediately fixed with acidified Lugol's solution for preservation. Quantitative analysis of phytoplankton was done following the Utermöhl (1958) method, counting organisms as they appear in nature (single cells, cenobia, colonies, or filaments) at 400X magnification. Counting error was estimated according to Venrick (1978), accepting a maximum error of 20%, and density was expressed as ind mL⁻¹. Taxonomic identifications were carried out at the species level using various algae keys, including Krammer and Lange-Bertalot (1991), Zalocar de Domitrovic and Maidana (1997), Tell and Conforti (1986), Komarek and Fott (1983), Komarek and Anagnostidis (1998, 2005), and Komarek (2013).

Phytoplankton major classes (Chlorophyceae, Euglenophyceae, Bacillariophyceae, Dinophyceae, Cyanobacteria, and Cryptophyceae) were compared among the Control and the treatments at the end of the experiment using one-way ANOVA analyses with Log₁₀ (x + 1) data transformation to secure normality requirements (Zar 1996). Additionally, phytoplankton species were grouped according to morphological characteristics: cell wall (mucilaginous, cellulose, pellicle, or silica), cellular organization (single cell, colonies-cenobia, or filaments), motility (non-mobile, by raphe, or by flagellum), and maximum linear dimension (MLD) (> 35 µm and < 35 µm). Algae groups were created by considering the results obtained in a cluster analysis with

Fig. 1 Experimental setup design. Control with just phytoplankton, treatment with zooplankton (Z) by adding two species of zooplankton, treatment with atrazine (A), and a treatment combining both stressors (ZA)



Euclidean distance and Ward's transformation. With this analysis, and by considering the variables explained above, we obtained six phytoplankton morphological groups, which were denominated as G1, G2, G3, G4, G5 and G6. Their densities were compared also by using one-way ANOVA analyses with $\text{Log}_{10}(x + 1)$ data transformation (Table 1, Fig. 2).

Results

Environmental parameters variations

The environmental mean variable values at the beginning and the end of the experiment on each treatment remained constant when compared to each other. For pH, values ranged between 7.3 and 7.9, temperature from 19.3 to 19.5 °C, DO from 9.4 to 9.6 mg L⁻¹, DO% sat from 103.2 to 156.5 mg L⁻¹, and conductivity from 1597.8 to

1645.3 $\mu\text{S cm}^{-1}$. Inorganic nutrient concentrations remained above limiting concentrations for DIN (2679.7 $\mu\text{g L}^{-1}$) and SRP (298.3 $\mu\text{g L}^{-1}$) among the treatments and the control. No statistically significant differences were found among treatments at the beginning or the end of the experiment ($p > 0.05$ for all).

Treatment effects on phytoplankton classes

A total of 34 phytoplankton species were recorded during the experiment, belonging to Bacillariophyceae, Chlorophyceae, Cyanobacteria, Euglenophyceae, Cryptophyceae, and Dinophyceae. In terms of density, *Chlamydomonas reinhardtii* P.A. Dangeard (Chlorophyceae) was the dominant species, followed by *Kirchneriella lunaris* (Kirchner) Möbius and *Scenedesmus ecornis* (Ehrenberg) Chodat (both Chlorophyceae). In terms of species richness, 14 taxa were recorded for Chlorophyceae, 7 for Bacillariophyceae, 7 for Cyanobacteria, 4 for Euglenophyceae, and 1 each for

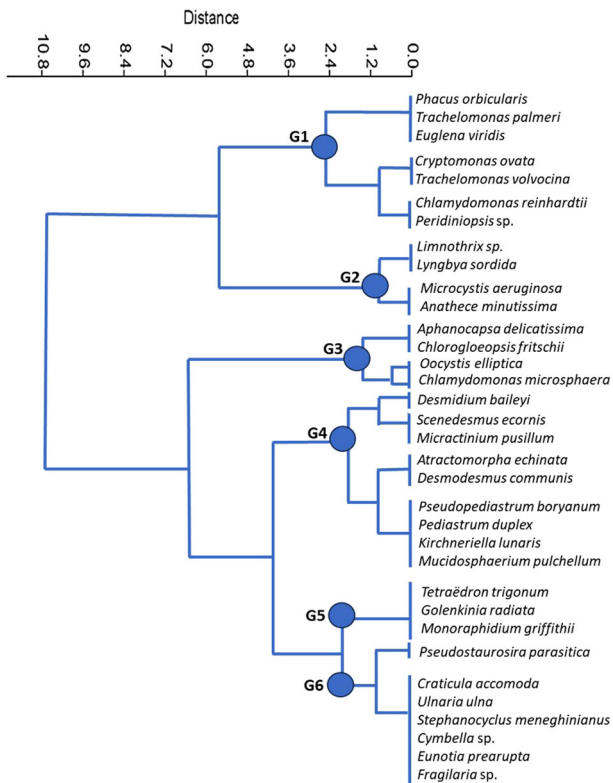


Fig. 2 Cluster analysis plot based on Euclidean distance with species and morphological group assigned (G1 to G6). The latter considers characteristics of the cell wall, mobility, maximum linear dimension, and cellular organization

Cryptophyceae and Dinophyceae (Supplementary Material, Supplementary Table S1).

At the beginning of the experiment, the density reported in the vessels assigned to Control and treatments was above 301 ind mL^{-1} ($\pm 86 \text{ ind mL}^{-1}$). At the end of the experiment, when total phytoplankton density was compared among treatments and the Control, density was higher in the Control ($458 \pm 128 \text{ ind mL}^{-1}$) compared to treatments, which showed similar densities among them (between 246 and 295 ind mL^{-1}). These differences were statistically significant (ANOVA, $F = 9.69$, $p = 0.001$), with the Control showing differences with all treatments (Tukey test, $p < 0.001$ for all paired comparisons). No differences were recorded among treatments Z, A, and ZA (Tukey test, $p > 0.05$ for all combinations) (Fig. 3a).

Regarding the major phytoplankton classes, the results obtained at the end of the experiment were variable. For Cyanobacteria, despite lower densities among treatments compared to the Control was observed (Fig. 3b), no statistically significant differences were reported (ANOVA, $F = 0.48$, $p = 0.69$). The same pattern was seen with Cryptophyceae plus Dinophyceae (ANOVA, $F = 2.38$, $p = 0.12$). Both groups were poorly represented in the

assemblage in both density and species richness therefore, their densities were combined for the statistical analyses (Fig. 3c).

For the other classes, differences were observed at the end of the experiment between the Control and the treatments, particularly for Chlorophyceae (ANOVA, $F = 31.52$, $p < 0.005$), which reported lower densities in all treatments when they were compared to the Control (Tukey test, $p < 0.001$ in all paired comparisons) (Fig. 3d). Additionally, atrazine treatment (A) had higher densities than Z (Tukey test, $p = 0.04$). For Euglenophyceae, the Control had the lowest densities (above 1 ind mL^{-1}) compared to ZA, which had the highest density at the end of the experiment (14 ind mL^{-1}). Statistically significant differences were reported for this phytoplankton class ($F = 10.18$, $p = 0.001$), particularly when Control and ZA treatment were compared (Tukey test, $p < 0.001$). Differences were also detected at the end of the experiment when ZA and A treatment were compared (Tukey test, $p < 0.01$). In both cases, ZA had higher densities (Fig. 3e). Finally, Bacillariophyceae showed similar and low densities among Control, Z, and ZA treatments (above 2 ind mL^{-1}), with the highest values reported in A treatment (6 ind mL^{-1}). These differences were statistically significant (ANOVA, $F = 3.78$, $p = 0.04$) particularly when comparisons were done between the Control and A treatment ($p < 0.001$) at the end of the experiment (Fig. 3f).

Treatment effects on phytoplankton morphological groups

The G1 morphological group was dominant in the assemblage across treatments and Control vessels, accounting for almost 50% of total density, followed by G2 (20% of total density) and G4 (10%). G3, G5, and G6 were the least represented in density ($< 5\%$ of total density) (Fig. 4). When morphological group densities were compared among treatments and the Control at the end of the experiment, results reported were also variable. For G1, differences were found between the Control and all treatments, which reported similar densities among themselves (above 100 ind mL^{-1}) (Fig. 5a). These differences were statistically significant (ANOVA, $F = 21.78$, $p < 0.001$) particularly when the Control was compared to all treatments (Tukey test $p < 0.01$ for all paired comparisons). For G2, similar densities were found among treatments and the Control (above 70 ind mL^{-1}), showing no statistically significant differences (ANOVA, $F = 0.29$, $p = 0.83$) (Fig. 5b). For G3, the Control and ZA treatment showed similar densities (above 25 ind mL^{-1}) compared to Z and A treatments, which also showed similar but lower densities (above 13 ind mL^{-1}). Differences were obtained (ANOVA, $F = 6.04$, $p < 0.01$) especially between Z and A (Tukey test, $p = 0.01$) and Z and Control (Tukey test, $p = 0.02$), with Z always

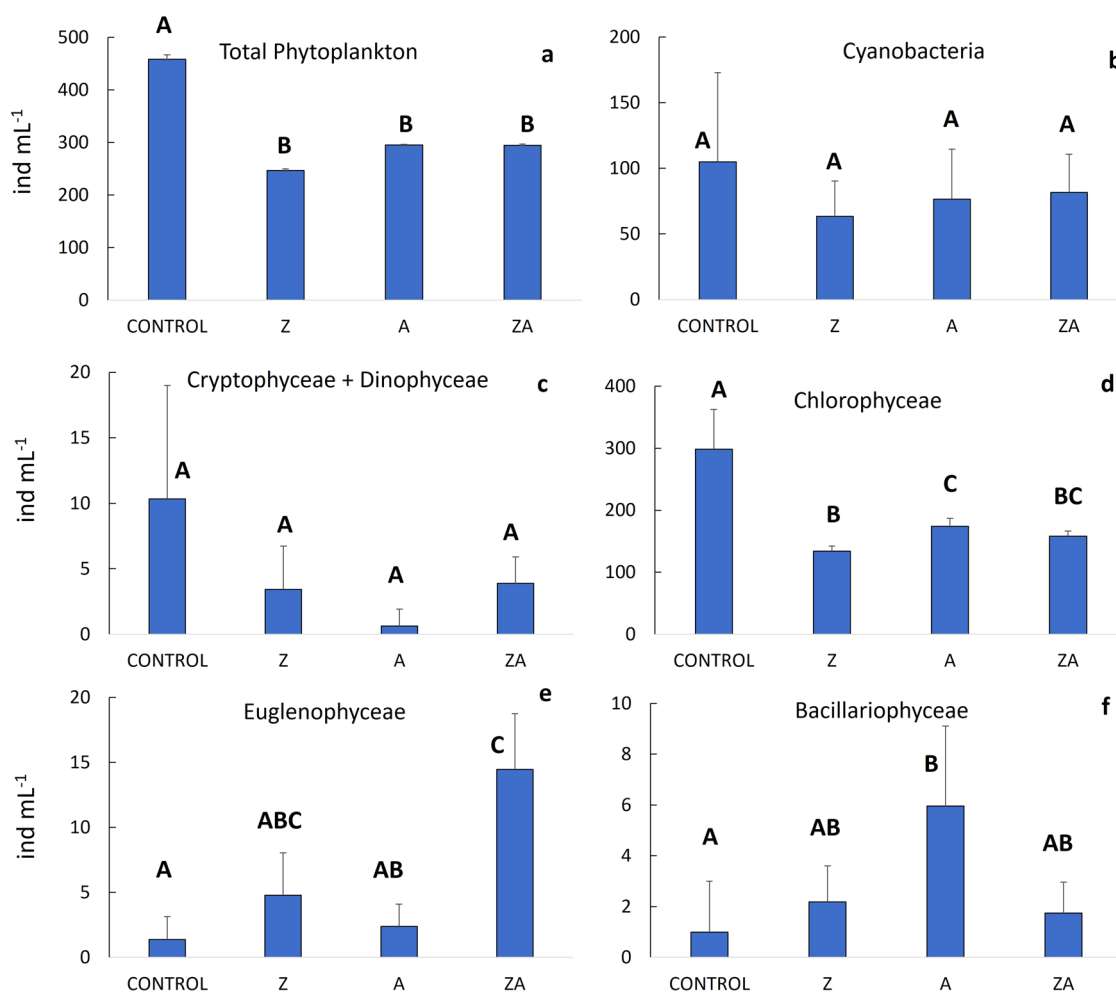


Fig. 3 Phytoplankton total density in the control and treatments at the end of the experiment (a) and densities registered for each phytoplankton class considered (b–f). Treatments: Z = zooplankton, A = atrazine, ZA =

zooplankton + atrazine. Tiny bars represent standard deviations while capital letters indicate statistically significant differences (Tukey test pairwise comparisons, $p < 0.05$) for those paired bars with uncommon letters

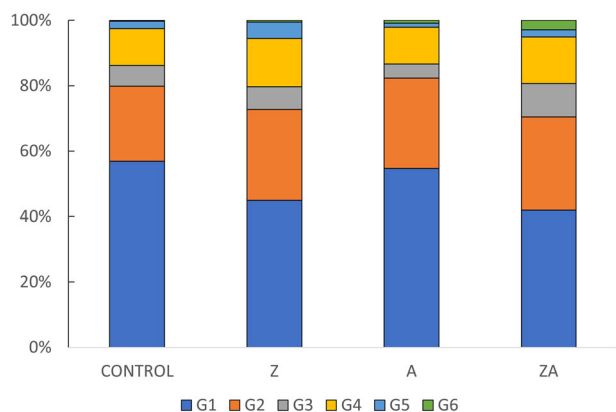


Fig. 4 Phytoplankton morphological groups density percentage distribution across control and treatments at the end of the experiment

having lower densities (Fig. 5c). With G4, similar densities were reported among treatments and the Control (above 40 ind mL⁻¹), and no statistically significant differences were reported (ANOVA, $F = 1.3$, $p = 0.41$) (Fig. 5d). For

G5 differences in densities were clear among Control *versus* A and ZA treatments (ANOVA, $F = 3.06$, $p < 0.01$); however, the Tukey test showed only statistically significant differences between Control and A treatment ($p = 0.04$). For this morphological group, Z reported the highest densities (Fig. 5e). Finally, for G6 the maximum densities at the end of the experiment appeared in ZA treatment (7 ind L⁻¹) (ANOVA, $F = 52.85$, $p < 0.01$), showing the Tukey test differences among ZA with all the rest (Tukey test, $p < 0.01$ for all paired comparisons) (Fig. 5f).

Discussion

Our experiment revealed differential responses of phytoplankton to two stressors of natural and anthropogenic origins, with the effects reported highly dependent on the phytoplankton groups exposed. Specifically, Chlorophyceae emerged as the most affected group by both predation and

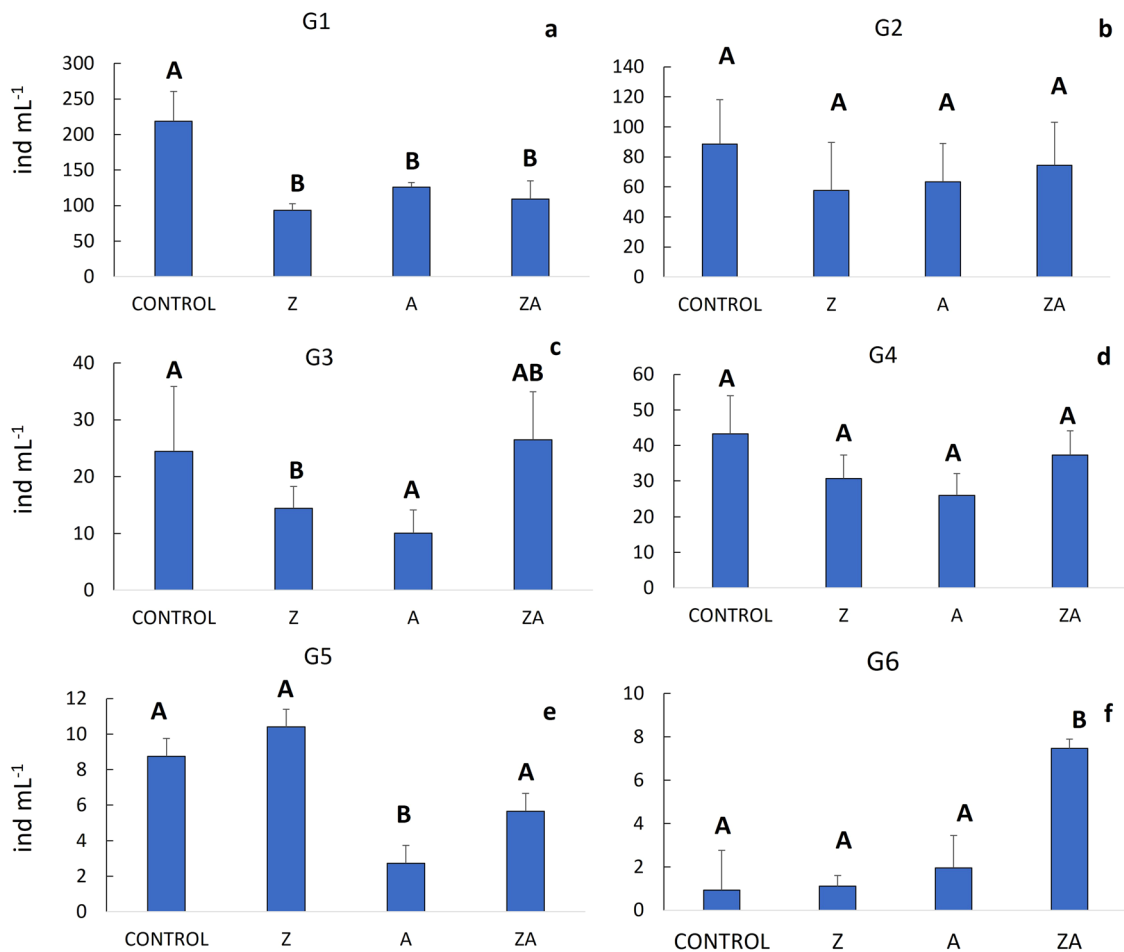


Fig. 5 Phytoplankton morphological groups density (a-G1, b-G2, c-G3, d-G4, e-G5, f-G6) in the control and treatments at the end of the experiment. Treatments: Z = zooplankton, A = atrazine, ZA =

zooplankton + atrazine. Tiny bars represent standard deviations while capital letters indicate statistically significant differences (Tukey test pairwise comparisons, $p < 0.05$) for those paired bars with uncommon letters

atrazine exposure. This finding is consistent with previous studies like Wang et al. (2010) or Frau et al. (2017) who demonstrated the high palatability of this group for zooplankton and some other studies which also reported negative impacts of atrazine at similar concentrations on Chlorophyceae (Tang et al. 1997; Lockert et al. 2006; Frau and Gutierrez 2024). Interestingly, in this experiment, predation exerted a more pronounced effect than atrazine exposure on this algal class. However, it is crucial to note that these effects may be influenced by experimental conditions, including the specific consortium of species used, the absence of planktivorous fish that could mitigate zooplankton predation, or seasonal variations affecting Chlorophyceae dominance. Nevertheless, the most significant aspect for this group is the evidence of a direct negative effect of atrazine, which decreases chlorophyte density being however predation a more relevant factor.

Euglenophyceae also exhibited statistically significant differences, benefiting from the combination of atrazine and predator presence (ZA treatment). This result is in

accordance with previous evidence reporting enhancing or null effects of atrazine on euglenoids (Lockert et al. 2006; Frau and Gutierrez 2024). However, our experiment showed inconsistency with earlier findings regarding predation on this group since some studies (e.g., Eskinazi-Sant’Anna et al. 2002; Frau et al. 2017) have demonstrated effective predation by large zooplankton on large Euglenophyceae from genus *Euglena*, capable of altering their shape due to their flexible cell walls. Euglenoid species in our experiment mainly belonged to less palatable genera, such as genera *Trachelomonas* and *Phacus*, which are non-metabolic. Particularly in *Trachelomonas*, the presence of an inorganic envelope (lorica) may protect against predation, as suggested for other filter-feeding predators (Frau et al. 2016). *Phacus* does not have a metabolic cell wall, but the large size of most species may prevent predation by zooplankton.

Bacillariophyceae exhibited a distinct pattern, showing higher densities in the treatment with atrazine (A) compared to the Control and the other treatments at the end of the experiment. This pattern aligns with the findings of previous

studies like Frau and Gutierrez (2024) and Bodean et al. (2024), where diatom densities benefited in the presence of atrazine. The explanation for this pattern could be complex and we still need more evidence. For example, Wood et al. (2017) showed that pollution history affects diatoms' response to atrazine, with those assemblages coming from polluted environments being more resistant; while Nelson et al. (1999) indicate that inhibitory effects of atrazine on diatoms have been reported only to high concentrations ($>80 \mu\text{g L}^{-1}$).

No significant effects were observed for Cyanobacteria, Dinophyceae, or Cryptophyceae under any treatment. Previous studies have documented effective predation by large zooplankton on these groups. For Cyanobacteria, although some evidence shows no predation effects (e.g., Lazzaro et al. 2003; Von Rückert and Giani 2008; Lacerot et al. 2013), several other authors have reported predation by large zooplankton, particularly on filamentous Cyanobacteria forms (Panosso et al. 2003; Dos Santos Severiano et al. 2017; Diniz et al. 2019), including similar zooplankton predators like use in this experiment (Frau and Gutierrez 2024). However, the predation effect may depend significantly on algal morphology (colonies, single cells, or filaments) and the nature of the cell wall and size (for a review consult Frau (2022)). For Cryptophyceae and Dinophyceae, several authors have demonstrated their susceptibility to feeding by zooplankton species (Rietzler et al. 2002; Perbiche-Neves et al. 2016; Frau et al. 2021b), but in our case, their negligible impact could be due to the low number of individuals registered during the experiment.

When our analyses were based on major morphological groups identified through cluster analysis, significant patterns emerged. Morpho-functional classification appears promising as an alternative to taxonomic approaches, grouping organisms based on their ecological responses rather than phylogenetic relationships, which may not always reflect real interactions between groups and their environment (Zwart et al. 2015; Mammola et al. 2021). In this regard, G1, which dominated the experiment, consisted mainly of single-celled algae with flagella mobility and size $<35 \mu\text{m}$. Previous studies (Colina et al. 2016; Frau et al. 2017; Frau 2022) have reported positive feeding responses of zooplankton to phytoplankton with these characteristics, consistent with our findings. This group was predominantly represented by *C. reinhardtii*, highly palatable to zooplankton. Also, *C. reinhardtii* is a chlorophyte, which was demonstrated to be susceptible to atrazine, explaining why G1 was negatively affected by atrazine in our experiment. Other responsive groups were G3 and G5, dominated by mucilaginous and cellulose cell wall species, immobile, and small ($<35 \mu\text{m}$). G3 was particularly affected by predation, consistent with findings by Frau et al. (2017). Additionally, G3 and G5 were susceptible to atrazine, both primarily comprising chlorophytes, which responded with a reduction in density. Finally, G6 was shown to be only responsive to the

combination of both stressors. G6 was dominated by large diatoms, that could be less palatable to large zooplankton due to their size (Rietzler et al. 2002; Frau et al. 2017; Diniz et al. 2019). In the presence of atrazine, densities reported for G6 increase over the other treatments, but more inquiries are necessary to determine which processes affect diatoms positively in the presence of this herbicide.

Conclusions

This experiment demonstrated the differential impacts of predation and atrazine exposure on phytoplankton assemblages. Predation predominantly diminished densities across most classes and morphological groups of small size ($<35 \mu\text{m}$), whereas atrazine negatively affected Chlorophyceae but showed potential positive effects on diatoms and euglenoids. It is important to note that our conclusions based on experimental conditions may not fully extrapolate to natural ecosystems due to the complex interactions of several factors that could influence the observed responses. Nonetheless, this study contributes to the understanding that the effects of natural and human-mediated stressors on microbial communities are often specific to phytoplankton groups.

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1007/s10646-024-02805-4>.

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Author contributions DF and MFG designed the experiment, SKZ performed the experiment and processed phytoplankton samples, DF analyzed data and wrote the manuscript, SKZ, MFG, and DF revised the manuscript. All the authors agreed with the final version of the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

References

- APHA (2005) Standard methods for the examination of water and wastewater, 21st ed. American Public Health Association, USA
- Barnett AJ, Finlay K, Beisner BE (2007) Functional diversity of crustacean zooplankton communities: towards a trait-based classification. *Freshw Biol* 52:796–813

- Benndorf J, Böing W, Koop J, Neubauer I (2002) Top-down control of phytoplankton: the role of time scale, lake depth and trophic state. *Freshw Biol* 47:2282–2295
- Bodean MF, Regaldo L, Mayora G, Mora C, Giri F, Gervasio S, Popielarz A, Repetti MR, Licursi M (2024) Effects of herbicides and fertilization on biofilms of Pampean lotic systems: A microcosm study. *Sci Total Env* 917:170238
- Colina M, Calliari D, Carballo C, Kruk C (2016) A trait-based approach to summarize zooplankton–phytoplankton interactions in freshwaters. *Hydrobiologia* 767:221–233
- Diniz AS, Dos Santos, Severiano JS, Melo Júnior M, Dantas ÊW, Moura AN (2019) Phytoplankton–zooplankton relationships based on phytoplankton functional groups in two tropical reservoirs. *Mar Freshwater Res* 70:721–733
- Dos Santos Severiano J, Almeida-Melo VLS, Melo-Magalhães EM, Bittencourt-Oliveira MD, do Nascimento Moura A (2017) Effects of zooplankton and nutrients on phytoplankton: an experimental analysis in a eutrophic tropical reservoir. *Mar Freshwater Res* 68:1061–1069
- Edwards KF, Litchman E, Klausmeier CA (2013) Functional traits explain phytoplankton responses to environmental gradients across lakes of the United States. *Ecology* 94:1626–1635
- Eskinazi Sant’Anna EM, Maia-Barbosa PM, Barbosa FAR (2002) On the natural diet of *Daphnia laevis* in the eutrophic Pampulha Reservoir (Belo Horizonte, Minas Gerais). *Braz J Biol* 62:445–452
- Frau D, Rojas Molina F, Mayora G (2016) Feeding Selectivity of the Invasive Mussel *Limnoperna fortunei* (Dunker, 1857) on a Natural Phytoplankton Assemblage: What Really Matters? *Limnology* 17:47–57
- Frau D, Battauz Y, Sinistro S (2017) Why predation is not a controlling factor of phytoplankton in a Neotropical shallow lake: a morpho-functional perspective. *Hydrobiologia* 788:115–130
- Frau D, Battauz Y, Alvarenga P, Scarabotti P, Mayora G, Sinistro R (2019) Assessing the relevance of top-down and bottom-up effects as phytoplankton structure drivers in a subtropical hypereutrophic shallow lake. *Aquatic Ecol* 53:265–280
- Frau D, Gutierrez MF, Regaldo L, Saigo M, Licursi M (2021a) Plankton community responses in Pampean lowland streams linked to intensive agricultural pollution. *Ecol Indic* 120:106934
- Frau D, Gutierrez MF, Rojas Molina F, Texeira de Melo F (2021b) Drivers assessment of zooplankton grazing on phytoplankton under different scenarios of fish predation and turbidity in an in situ mesocosm experiment. *Hydrobiologia* 848:485–498
- Frau D (2022) Grazing impacts on phytoplankton from South American water ecosystems: a synthesis. *Hydrobiologia* 849:833–860
- Frau D, Pineda A, Mayora G, Gutierrez MF (2023) Eutrophication as a Homogenizer Process of Phytoplankton β -Diversity in Lowland Streams. *Limnologica* 99:126058
- Frau D, Gutierrez MF (2024) Plankton Response to a Mix of Environmental Stressors. *Wat Air Soil Poll* 235:316
- Frau D, Pineda A (2024) Phytoplankton Species and Traits Response to a Gradient of Urbanization in Subtropical Lowland Streams. *Ecology* <https://doi.org/10.1002/eco.2675>
- Graymore M, Stagnitti F, Allinson G (2001) Impacts of Atrazine in aquatic ecosystems. *Environ Int* 26:483–495
- Gutierrez MF, Simões NR, Frau D, Saigo M, Licursi M (2020) Responses of stream zooplankton diversity metrics to eutrophication and temporal environmental variability in agricultural catchments. *Environ Monit Assess* 192:792
- Jüttner I, Peither A, Lay JP, Kettrup A, Ormerod SJ (1995) An outdoor mesocosm study to assess ecotoxicological effects of atrazine on a natural plankton community. *Arch. Environ. Contam. Toxicol.* 29:435–441
- Komárek J (2013) Cyanoprokaryota. Teil/3rd part: heterocytous genera. In: Büdel B, Gärtner G, Krienitz L, Schagerl M (Eds.) Süßwasserflora von Mitteleuropa (Freshwater flora of Central Europe). Springer Spektrum, Germany
- Komárek J, Anagnostidis K (1998) Cyanoprokaryota. Teil 1: Chroococcales. In: Ettl H, Gärtner G, Heynig H, Mollenhauer D (Eds.) Süßwasserflora von Mitteleuropa 19/1. Jena. Gustav Fisher Verlag, Germany
- Komárek J, Anagnostidis K (2005) Cyanoprokaryota. Teil 2: Oscillatoriales. In: Büdel B, Gärtner G, Krienitz L, Schagerl M (Eds.) Süßwasserflora von Mitteleuropa 19/ 2. Elsevier, Germany
- Komárek J, Fott B (1983) Chlorophyceae, chlorococcales. In: Huber-Pestalozzi G (Ed) Das Phytoplankton des Süßwassers. Die Binnengewässer, Vol. 16(5). Schweizerbart’sche Verlagsbuchhandlung, Germany
- Krammer K, Lange-Bertalot H (1991) Bacillariophyceae. 3. Teil Centrales, Fragilariaceae, Eunotiaceae. In: Ettl H, Gerloff J, Heynig H, Mollenhauer D (Eds.) Süßwasserflora von Mitteleuropa. Gustav Fischer Verlag, Germany
- Lacerot G, Kruk C, Luerling M, Scheffer M (2013) The role of subtropical zooplankton as grazers of phytoplankton under different predation levels. *Freshw Biol* 58:494–503
- Lazzaro X, Bouvy M, Ribeiro-Filho RA, Oliveira VS, Sales LT, Vasconcelos ARM, Mata MR (2003) Do fish regulate phytoplankton in shallow eutrophic Northeast Brazilian reservoirs? *Freshw Biol* 48:649–668
- Lockert CK, Hoagland KD, Siegfried BD (2006) Comparative Sensitivity of Freshwater Algae to Atrazine. Faculty Publications: Department of Entomology, 66. University of Nebraska - Lincoln (U.S)
- Lushchak VI (2011) Environmentally induced oxidative stress in aquatic animals. *Aquatic Toxicol* 101:13–30
- Malhi Y, Franklin J, Seddon N, Solan M, Turner MG, Field CB, Knowlton N (2020) Climate change and ecosystems: threats, opportunities and solutions. *Phil Trans R Soc B* 375:20190104
- Mammola S, Carmona CP, Guillerme T, Cardoso P (2021) Concepts and applications in functional diversity. *Functional Ecol* 35:1869–1885
- Mateo Sagasta J, Marjani Zadeh S, Turrall H (2018) More people, more food, worse water? a global review of water pollution from agriculture. FAO Colombo, Rome, Italy
- Myers SS, Gaffikin L, Golden CD, Ostfeld RS, Redford KH, Ricketts TH, Turner WR, Osofsky SA (2013) Human health impacts of ecosystem alteration. *Proc Natl Acad Sci USA* 110:18753–18760
- Naselli-Flores L, Padišák J (2024) Analysis of morphological traits as a tool to identify the realized niche of phytoplankton populations: what do the shape of planktic microalgae, Anna Karenina and Vincent van Gogh have in common? *Hydrobiologia* 851:733–749
- Nelson KJ, Hoagland KD, Siegfried BD (1999) Chronic effects of atrazine on tolerance of a benthic diatom. *Environ Toxicol Chem* 18:1038–1045
- Panosso R, Carlsson P, Kozłowski-Suzuki S, Azevedo MFO, Granéli E (2003) Effect of grazing by a neotropical copepod, *Notodiaptomus*, on a natural cyanobacterial assemblage and on toxic and non-toxic cyanobacterial strains. *J Plankton Res* 25:1169–1175
- Patel N, Khan MD, Shahane S, Rai D, Chauhan D, Kant C, Chaudhary VK (2020) Emerging Pollutants in Aquatic Environment: Source, Effect, and Challenges in Biomonitoring and Bioremediation- A Review. *Pollution* 6:99–113
- Perbiche-Neves G, Lago Portinho J, Romero Ferreira RA, Gomes Nogueira M (2016) Increases in microcrustaceans (Cladocera and Copepoda) associated with phytoplankton peaks in tropical reservoirs. *Trop Ecol* 57:523–532
- Reynolds C (2006) Ecology of Phytoplankton. University Press, United Kingdom
- Rietzel AC, Matsumura-Tundisi T, Tundisi JG (2002) Life cycle, feeding and adaptive strategy implications on the co-occurrence of *Argyrodiaptomus furcatus* and *Notodiaptomus iheringi* in Lobo-Broa reservoir (SP, Brazil). *Braz J Biol* 62:93–105

- Sass JB, Colangelo A (2006) European Union bans atrazine, while the United States negotiates continued use. *Int J Occup Med Environ* 12:260–267
- Seguin F, Leboulanger C, Rimet F, Druart JC, Bérard A (2001) Effects of Atrazine and Nicosulfuron on Phytoplankton in Systems of Increasing Complexity. *Arch Environ Contam Toxicol* 40:198–208
- Tang JX, Hoagland KD, Siegfried BD (1997) Differential toxicity of atrazine to selected freshwater algae. *Bull Environ Contam Toxicol* 59:631–637
- Tell G, Conforti V (1986) Euglenophyta pigmentadas de Argentina. *Bibliotheca Phycologica* 75:1–301
- Utermöhl H (1958) Zur Vervollkommnung der quantitative Phytoplankton: methodik. *Int Assoc Theoret Appl Limnol* 9:1–38
- Venrick EL (1978) How many cells to count? In: Sournia InA (Ed.) *Phytoplankton manual*. UNESCO, Paris
- Vijverberg J (1989) Culture techniques for studies on the growth, development and reproduction of copepods and cladocerans under laboratory and in situ conditions: A review. *Freshw Biol* 21:317–373
- Von Rückert G, Gianni A (2008) Biological interactions in the plankton community of a tropical eutrophic reservoir: is the phytoplankton controlled by zooplankton? *J Plankton Res* 10:1157–1168
- Wang X, Qin B, Gao G, Paerl HW (2010) Nutrient enrichment and selective predation by zooplankton promote *Microcystis* (Cyanobacteria) bloom formation. *J Plankton Res* 32:457–470
- Wijewardene L, Wu N, Qu Y, Guo K, Messyas B, Lorenz S, Riis T, Ulrich U, Fohrer N (2021) Influences of pesticides, nutrients, and local environmental variables on phytoplankton communities in lentic small water bodies in a German lowland agricultural area. *Sci Total Environ* 780:146481
- Wood RJ, Mitrovic SM, Lima RP, Kefford BJ (2017) Chronic effects of atrazine exposure and recovery in freshwater benthic diatoms from two communities with different pollution histories. *Aquat Toxicol* 189:200–208
- Zalocar de Domitrovic Y, Maidana NI (1997) Taxonomic and ecological studies of the Parana River diatom flora (Argentina). In: Lange-Bertalot F, Kociolek P (Eds.) *Bibliotheca Diatomologica*. J. Cramer, Berlin
- Zar JH (1996) *Biostatistical analysis*. Prentice Hall, New York, p 918
- Zwart JA, Solomon CT, Jones SE (2015) Phytoplankton traits predict ecosystem function in a global set of lakes. *Ecology* 96:2257–2264
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