



# Effects of bifenthrin on microcrustaceans grazing behavior on a phytoplankton assemblage dominated by Cyanobacteria

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## Abstract

Insecticides are widely used for pest control and frequently reach aquatic systems, imposing a risk to the biota. In this work, the effect of environmental concentrations of bifenthrin on the grazing capacity of *Simocephalus vetulus* (Cladocera) and *Argyrodiaptomus falcifer* (Copepoda) on phytoplankton was evaluated. Fifteen microcrustacean individuals and a natural phytoplankton assemblage dominated by Cyanobacteria were exposed during 46 h to three concentrations of bifenthrin (C0 0  $\mu\text{g L}^{-1}$ , C1 0.02  $\mu\text{g L}^{-1}$ , and C2 0.05  $\mu\text{g L}^{-1}$ ). A significant decrease in both microcrustaceans grazing rates on total phytoplankton was observed in C2 compared to C0 and C1. The filtration rate ( $\text{ml ind}^{-1} \text{h}^{-1}$ ) of *S. vetulus* decreased significantly for the cyanobacteria *Anabaenopsis arnoldii*, *Dolichospermum circinale*, and *Glaucospira* sp. in C2 compared to C0 and C1. The ingestion rate ( $\text{org ind}^{-1} \text{h}^{-1}$ ) of *A. falcifer* decreased significantly in C1 and C2 compared to C0 only for *A. arnoldii*. Regarding phytoplankton morphological groups, the filtration rate of *S. vetulus* decreased in C1 and C2 compared to C0 for Colonies and Coenobiums in C2 concerning C0 and C1 for Filaments and in C2 compared to C0 for Silicified. For *A. falcifer*, the ingestion rate was reduced in C2 compared to C0 for Silicified, Flagellated, and Sessile. The results showed that bifenthrin affected both microcrustaceans grazing capacity on phytoplankton, especially at the highest insecticide concentration.

**Keywords** Grazing rate · Zooplankton · Insecticide · Cyanobacteria

## Introduction

Globally, agricultural land covers about five billion hectares, approximately 38% of the world's land surface. Around one-third of this surface is used for crop cultivation, while the remaining two-thirds are meadows and pastures used for livestock (FAO 2022). The indiscriminate use of agrochemicals to maximize crop yield has resulted in adverse effects on the environment, including air, water, and soil pollution,

as well as harm to non-target organisms and human health (Elahi et al. 2019). This phenomenon is in increase, considering that the pesticides applied have increased from 4.0 to 8.7 million tons between 2019 and 2020 (FAO 2022). Based on the data regarding ecological effects, we know that agricultural insecticide use, in combination with nutrient and habitat degradation, is likely a significant driver of biodiversity loss in aquatic ecosystems affected by agriculture (Stehle and Schulz 2015).

Pesticides have various routes of entry into surface waters, including runoff by precipitations, one of the primary pathways, leaching through soil to groundwater, air transport and deposit by wind or precipitation, or direct application. Ultimately, the extensive use of pesticides in agriculture leads to widespread contamination of surface waters, which can damage biota (Hladik et al. 2015; Sanchez-Bayo and Goka 2014; Sy et al. 2022).

Pesticides are known to harm non-target species at various levels of organization, including individuals, populations, and ecosystems. For instance, at the individual level, pesticides can lead to direct toxicity, which may cause death, stunted growth, or impaired reproduction. Similarly, at the population

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level, the use of pesticides can lead to a decrease in the number of individuals, causing a decline in population size or even local extinction. Additionally, at the ecosystem level, pesticides can impact community structure, disturb food webs, and reduce biodiversity. Hence, the adverse effects of pesticides can be extensive, leading to significant consequences at multiple levels of organization (Stehle and Schulz 2015). However, the toxicity of pesticides on zooplankton differs depending on various factors, including species, genotype, life stage, and size. Populations in the growth phase are particularly susceptible to the harmful effects of pesticides, but they also can recover promptly from the damage. Pesticides can affect population dynamics by impeding individual survival and reproduction and disrupting the sex ratio (Hanazato 2001).

Zooplankton is a valuable community in aquatic trophic networks as it is a primary food source for numerous aquatic organisms, including fish and larger invertebrates. They consume phytoplankton and bacteria, facilitating the transfer of energy and nutrients between various trophic levels. Additionally, they act as regulators of phytoplankton populations by limiting their growth and preventing the occurrence of algae blooms. Zooplankton also participates in nutrient cycling within aquatic ecosystems by excreting and releasing nutrient-rich fecal pellets that other organisms can utilize as a source of nutrients (e.g., Hutchins et al. 1995; Pace and Cole 2002; Sommer and Lengfellner 2008). Previous studies suggest that the impact of pesticides on zooplankton may result in changes to their swimming behavior and body morphology, which could have a ripple effect on the ecosystem dynamics, such as the elongation of the food chain and the reduction of energy transfer efficiency from primary producers to top predators (Hanazato 2001; Mendoca et al. 2018).

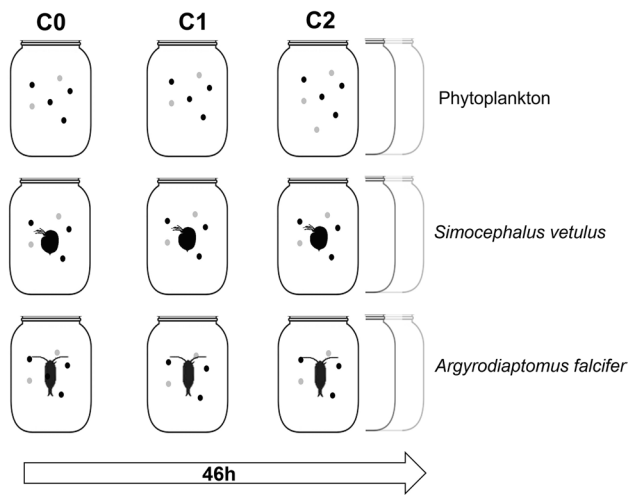
The grazing effects of zooplankton on Cyanobacteria, a bacteria group characterized by having oxygen-evolving photosynthesis as algae and Plantae (Reynolds 2006), are highly variable, and current literature suggests that it may be species-dependent (Frau 2022). For example, some studies like Lazzaro et al. (2003), Von Rückert and Giani (2008), and Lacerot et al. (2013) concluded that zooplankton grazing was null or inefficient in assemblages dominated by Cyanobacteria. In contrast, some studies, such as Nadin-Hurley and Duncan (1976), found that larger *Daphnia* species could consume filamentous cyanobacteria in bundles resembling spaghetti. Similarly, Panosso et al. (2003) reported positive outcomes when studying the effects of *Notodiaptomus iheringi* (Wright S.) (Copepoda) on filamentous, single-cell, and small colonies of cyanobacteria, although they preferred other food items. Additionally, Bouvy et al. (2001) and Kâ et al. (2012) demonstrated that certain tropical rotifers such as *Brachionus* spp., *Keratella* spp., and some copepods such as *Notodiaptomus cearensis* (Wright S.) and *Thermocyclops decipiens* (Kiefer), were able to cut and consume large filamentous cyanobacteria. Dos Santos Severiano et al. (2018)

and Diniz et al. (2019) reported that calanoid copepods and cladocerans were effective grazers on colonial and filamentous cyanobacteria.

Cyanobacteria blooms should not be overlooked due to their potential to cause environmental, health, and economic issues, as evidenced by Huisman et al. (2005), Merel et al. (2013), Drobac et al. (2013), and Carmichael et al. (2016), among others. However, little is known about insecticides' effect on zooplankton capacity of feeding and controlling Cyanobacteria blooms. In this study, we tested the effect of several environmental concentrations of the insecticide bifenthrin on the grazing rates of two microcrustaceans over a natural phytoplankton assemblage dominated by Cyanobacteria. We hypothesized that both microcrustaceans feed on Cyanobacteria, but their grazing capacity is diminished in the presence of bifenthrin, in a concentration-dependent manner.

## Material and methods

To achieve the proposed aim, we designed a microcosm experiment with two microcrustacean species: *Simocephalus vetulus* (O.F. Müller) (Cladocera) and *Argyrodiaptomus falcifer* (Daday) (Copepoda) feeding on a natural phytoplankton assemblage dominated by Cyanobacteria. Microcrustaceans were collected from shallow isolated lakes in the Paraná River floodplain. The organisms were gradually adapted to develop laboratory stock cultures with 16:8h light darkness photoperiod,  $22 \pm 1$  °C in dechlorinated tap water, and were fed with *Tetrademus obliquus* (Turpin) M.J. Wynne prior to the experiment began. The phytoplankton assemblage was obtained from a shallow urban lake that recurrently presents Cyanobacteria blooms (Frau et al. 2019). Microcrustaceans were gradually acclimated to the experimental medium for a week. The experimental design consisted of a control (C0:  $0 \mu\text{g L}^{-1}$ ), a low (C1:  $0.02 \mu\text{g L}^{-1}$ ), and a high concentration of bifenthrin (C2:  $0.05 \mu\text{g L}^{-1}$ ). A commercial formulation (Bifentrin10®, Formulagro S.R.L., Argentina, bifenthrin 10%) was employed. Exposure concentrations were selected based on preliminary mortality tests and environmental reports of bifenthrin in surface water (e.g., Ensminger et al. 2013; Weston et al. 2015; Budd et al. 2020). Bifenthrin concentrations were determined with C18 solid phase extraction (SPE C18) and liquid chromatography coupled to triple quadruple mass spectrometry (LC–MS/MS). The insecticide concentrations were set up in three conditions: phytoplankton alone, *S. vetulus* addition (15 adults), and *A. falcifer* addition (15 adults) per replicate. All treatments were replicated three times ( $n = 27$ ) in 250 ml transparent glass vessels, and the experiment lasted 46 h. The experiment was performed at  $22 \pm 1$  °C and photoperiod of 16:8h light darkness. Samples for phytoplankton quantification were taken at the experiment's beginning and the end (Fig. 1).



**Fig. 1** Experimental design. Medium water containing the phytoplankton assemblage was the same in all vessels, being variable the Bifenthrin concentration used (C0 0  $\mu\text{g L}^{-1}$ , C1 0.02  $\mu\text{g L}^{-1}$ , and C2 0.05  $\mu\text{g L}^{-1}$ ) and the microcrustacean tested (*S. vetulus* and *A. falcifer*)

Every 24 h, neonates were removed, and microcrustacean mortality was recorded at the end of the experiment. At the beginning and the end of the experiment, relevant limnological variables like temperature ( $^{\circ}\text{C}$ ), pH, conductivity ( $\mu\text{S cm}^{-1}$ ), and dissolved oxygen (DO,  $\text{mg L}^{-1}$ ) were measured with a HACH multiparameter probe. In addition, samples for inorganic nutrient analyses (nitrite-nitrate, ammonium, and soluble reactive phosphorus) were taken and processed following APHA (2005) methods. Samples for bifenthrin quantification were also taken at the beginning and end of the experiment and sent to a specialized lab for its quantification. Phytoplankton counting was performed following the method proposed by Utermöhl (1958) using an inverted

microscope NIKON to 400X magnification. Counting was done until 100 individuals of the dominant species were registered in the sample to secure a counting error of  $< 20\%$  (Venrick 1978). The density obtained was expressed as  $\text{ind mL}^{-1}$ .

## Data analysis

Since *S. vetulus* is a filter-feeding cladoceran, a filtration rate ( $\text{ml ind}^{-1} \text{h}^{-1}$ ) was calculated following Wetzel and Likens (2000). For *A. falcifer*, an ingestion rate was estimated according to Paffenhöfer (1971), considering that it is a raptorial copepod. Both filtration and ingestion rates, respectively, were estimated considering the total phytoplankton density, the dominant phytoplankton species of the assemblage ( $\geq 50\%$  of total density), and phytoplankton morphological groups: Sessile, Flagellates, Colonies and Coenobiums, Filaments, and Silicified species (Table 1).

Filtration and ingestion mean rates at the end of the experiment were compared between bifenthrin treatments (C0, C1, and C2) through one-way analyses of variance (ANOVA) with a previous evaluation of parametric application requirements (homoscedasticity and normality). Tukey tests were applied as *post-hoc* analyses, considering for all the comparisons an  $\alpha = 0.05$ .

## Results

### Experimental conditions

During the experiment, the environmental variables showed low variation between treatments and sampling dates, with a mean temperature of  $22^{\circ}\text{C}$ , circumneutral pH, conductivity of  $2000 \mu\text{S cm}^{-1}$ , and DO of  $8 \text{ mg L}^{-1}$  ( $P > 0.05$  for all

**Table 1** Classification of phytoplankton morphological groups found in the medium used

Groups	Characteristics	Examples of taxa found
Colonies and Coenobiums	Organisms form by more than 2 cells with or without flagellum	<i>Aphanocapsa delicatissima</i> West & G.S.West; <i>Microcystis aeruginosa</i> (Kützing) Kützing; <i>Desmodesmus communis</i> (E. Hegewald) E.Hegewald (Turpin) Brébisson; <i>Tetrastrum glabrum</i> (Y.V.Roll) Ahlstrom & L.H.Tiffany
Filaments	Organisms form by several cells disposed one besides the other	<i>Raphidiopsis curvata</i> F.E.Fritsch & M.F.Rich; <i>Jaaginema gracile</i> Anagnostidis & Komárek
Flagellates	Unicellular organisms with flagellum	<i>Euglenaria clavata</i> (Skuja) Karnkowska & E.W.Linton; <i>Euglena oblonga</i> F.Schmitz; <i>Peridinium</i> sp.; <i>Cryptomonas ovata</i> Ehrenberg
Sessiles	Unicellular organism without flagellum	<i>Monoraphidium griffithii</i> (Berkeley) Komárková-Legnerová; <i>Pseudoschroederia antillarum</i> (Komárek) Hegewald & Schnepf; <i>Golenkinia radiata</i> Chodat
Silicified	Unicellular algae with silicified cell-wall	<i>Fragilaria capucina</i> Desmazières; <i>Stephanocyclus meneghinianus</i> (Kützing) Kulikovskiy; <i>Aulacoseira distans</i> (Ehrenberg) Simonsen

of them) (Table 2). Inorganic nutrients concentrations were for ammonium  $135 \mu\text{g L}^{-1}$  ( $\pm 44$ ), for nitrite+nitrate:  $23 \mu\text{g L}^{-1}$  ( $\pm 17$ ), and for soluble reactive phosphorus the concentration was below the detection limit in all the vessels. Bifenthrin concentrations in the medium decreased through time and varied from  $0.02 \mu\text{g L}^{-1}$  at the beginning to  $0.006 \mu\text{g L}^{-1}$  at the end in C1 and from  $0.05 \mu\text{g L}^{-1}$  to  $0.008 \mu\text{g L}^{-1}$  in C2. No bifenthrin was detected in control vessels.

Regarding phytoplankton, the initial medium accounted for thirty-eight species. Sixteen were Chlorophyceae, eight Cyanobacteria, seven Bacillariophyceae, four Euglenophyceae, two Cryptophyceae, and one Dinophyceae. The total density recorded at the beginning of the experiment was  $4551 \text{ ind mL}^{-1}$ . Cyanobacteria was the dominant group accounting for 50% of total density, followed by Bacillariophyceae (28%), Chlorophyceae (11%), Cryptophyceae (9%), and Dinophyceae (2%). Dominant species were three Cyanobacteria: *Anabaenopsis arnoldii* Aptekar, *Dolichospermum circinale* (Rabenhorst ex Bornet & Flahault) Wacklin, Hoffmann & Komárek, *Glaucospira* sp., and one diatom species *Ulnaria acus* (Kützing) Aboal, accounting for more than 70% of total density in all treatments. No statistically differences were found in phytoplankton density of the three controls (C0) (control without bifenthrin, control with low bifenthrin concentration and control with high bifenthrin concentration,  $F = 2.48$ ,  $p = 0.35$ ). Microcrustacean survival at the

experiment's end was generally higher than 85%. In C0, a mean of 6% of deaths were reported for both microcrustaceans, in C1 (6% for the cladoceran and 13% for the copepod) and C2 (13% for the cladoceran and 27% for the copepod).

### Microcrustaceans grazing rates under bifenthrin effects

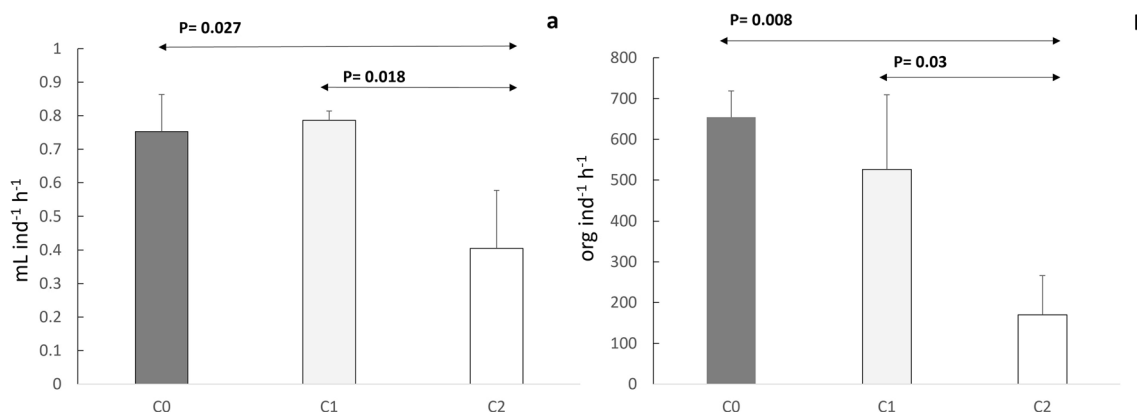
For *S. vetulus*, the maximum filtration rate was reported in C1 with  $0.78 \text{ mL ind}^{-1} \text{ h}^{-1}$  and the lowest in C2 concentration ( $0.40 \text{ mL ind}^{-1} \text{ h}^{-1}$ ). Regarding *A. falcifer*, the maximum ingestion rate was registered in the control (C0) with  $654 \text{ org ind}^{-1} \text{ h}^{-1}$ , and the lowest in C2 ( $169 \text{ org ind}^{-1} \text{ h}^{-1}$ ). The grazing rates on total phytoplankton decreased significantly in C2 compared to C0 and C1 for both, *S. vetulus* ( $F = 9.39$ ,  $P = 0.014$ ) and *A. falcifer* ( $F = 12.02$ ,  $P = 0.008$ ) (Fig. 2a and b, respectively).

Our specific analysis on the phytoplankton species dominance revealed that for *S. vetulus*, the highest filtration rates were obtained for *Glaucospira* sp., followed by *A. arnoldii* and *U. acus*, while the lowest was for *D. circinale*. *Simocephalus vetulus* filtration rates decreased significantly in C2 compared to C0 and C1 for the three cyanobacteria species: *Anabaenopsis arnoldii* ( $F = 26.95$ ,  $P = 0.001$ ), *D. circinale* ( $F = 13.09$ ,  $P = 0.006$ ), and *Glaucospira* sp. ( $F = 15.41$ ,  $P = 0.04$ ). These differences were found between C0 vs. C2 and C1 vs. C2. No differences in the filtration rate were found for *U. acus* ( $F = 0.85$ ,  $P = 0.47$ ) (Fig. 3a). In contrast, *A. falcifer* ingestion rate was higher for *A. arnoldii*, followed by *U. acus*, and *Glaucospira* sp. No grazing over *D. circinale* was registered. *A. falcifer* ingestion rates decreased significantly in C1 and C2 compared to C0 just for *A. arnoldii* ( $F = 8.06$ ,  $P = 0.02$ ) (Fig. 3b).

Regarding the morphological groups of phytoplankton, different grazing patterns were found among groups and microcrustacean species. *S. vetulus* filtrated mainly Colonies and Coenobiums, while *A. falcifer* ingested mainly Filaments,

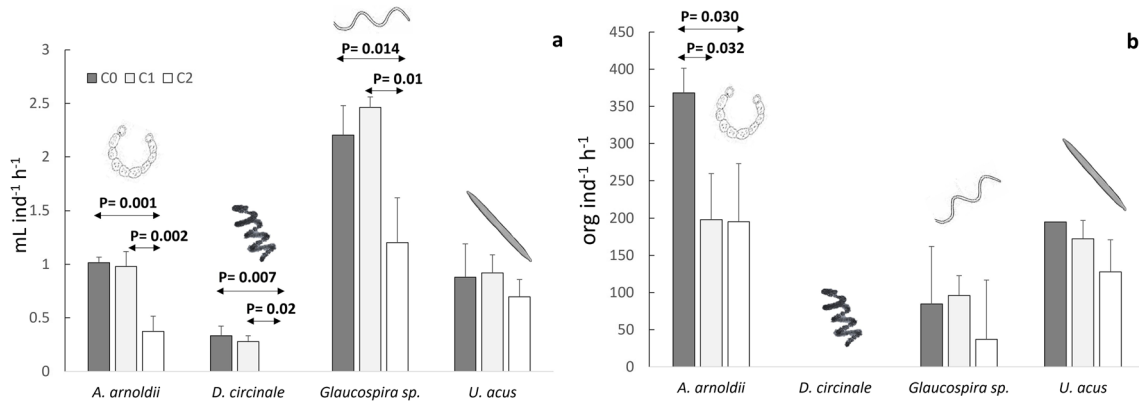
**Table 2** Mean and standard deviation of the environmental variables measured at the beginning and at the end (46 h later) of the experiment. Temp temperature, Cond conductivity, DO dissolved oxygen

	Temp (°C)	pH	Cond ( $\mu\text{s cm}^{-1}$ )	DO ( $\text{mg L}^{-1}$ )
Beginning	$22.3 \pm 0.11$	$7.3 \pm 0.22$	$2046 \pm 3.25$	$8.45 \pm 0.1$
End	$22.4 \pm 0.11$	$7.2 \pm 0.04$	$2104 \pm 23.7$	$7.57 \pm 0.36$



**Fig. 2** Mean values and standard deviations of *S. vetulus* (a) and *A. falcifer* (b) filtration and ingestion rates, respectively, at the end of the experiment, by considering the total phytoplankton density registered

on each treatment (C0, C1, and C2). Arrows show Tukey test significant differences between treatments



**Fig. 3** Mean values and standard deviations of the filtration and ingestion rates, respectively, of *S. vetulus* (a), and *A. falcifer* (b) at the end of the experiment by considering dominant species density

registered on each treatment (C0, C1, and C2). Arrows show Tukey test significant differences between treatments

and Silicified phytoplankton. *Simocephalus vetulus* filtration rate decreased significantly in C2 and C1 compared to C0 for Colonies and Coenobiums ( $F = 6.68, P = 0.03$ ), in C2 compared to C0 and C1 for Filamentous ( $F = 17, P = 0.03$ ) and in C2 vs. C0 for Silicified ( $F = 6.86, P = 0.02$ ) (Fig. 4a). For *A. falcifer*, the ingestion rate decreased significantly in C2 compared to C0 for Silicified ( $F = 10.09, P = 0.012$ ), C2 vs. C0 and C1 for Flagellates ( $F = 21.52, P = 0.02$ ), and C2 vs. C1 for Sessile ( $F = 27.18, P = 0.001$ ) (Fig. 4b).

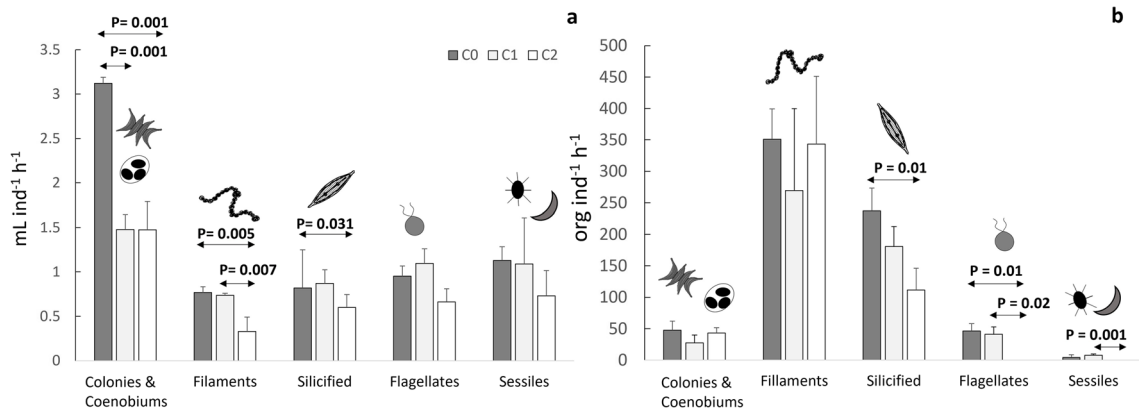
environmental concentrations, affects the grazing capacity of two zooplankton species on phytoplankton. Numerous studies have reported the impact of insecticides on life history traits and behavior of zooplankton, especially those particularly designed to alter the nervous system such as pyrethroids. Specifically, these chemicals can diminish zooplankton ability to escape fish and other invertebrates. Examples of such studies include Groner and Relyea (2011), Hayasaka et al. (2012), and Gutierrez et al. (2020). However, little is known about the effect of insecticides on the grazing capability of zooplankton, and particularly on Cyanobacteria assemblages.

**Discussion**

In natural ecosystems, zooplankton populations must cope with natural and anthropogenic structuring pressures acting simultaneously (Hanazato 2001). In this experiment, we proved that the pyrethroid bifenthrin, even at low

**Microcrustaceans’ grazing preferences in the absence of bifenthrin**

Both zooplankton species demonstrated an active grazing effect on the phytoplankton assemblage offered in the



**Fig. 4** Mean values and standard deviations of the filtration and ingestion rate, respectively, of *S. vetulus* (a), and *A. falcifer* (b) at the end of the experiment by considering phytoplankton morphological

groups density registered on each treatment (C0, C1, and C2). Arrows show Tukey test significant differences between treatments

absence of bifenthrin (C0). However, some patterns probably more related to their feeding strategies and palatability preferences were found. Indeed, filter-feeders Cladocera use their appendages with sieve-like structures to create water flow, which traps particles larger than the filter mesh size for consumption. In contrast, diaptomid Copepods like *A. falcifer* are selective raptorial feeders and select food items by considering several morphological and physiological characteristics (Titocci et al. 2022).

In our experiment, *S. vetulus* efficiently fed on filamentous Cyanobacteria, especially *Glaucospira* sp. following previous evidence supporting the idea that cladocerans may effectively graze on medium size phytoplankton species (<40 µm of maximum linear dimension, MLD) (Frau, 2022) and particularly on small filamentous cyanobacteria (Kâ et al. 2012). Despite their ability to feed on filamentous organisms has been questioned, with this experiment, we contribute to the idea that they effectively feed on filamentous forms in the absence of the insecticide. Their feeding rate, however, seems to be lower in larger and more complex forms of filamentous species, like *A. arnoldii* (> 40 µm of MLD), and much larger species, like *D. circinale* (>> 40 µm MLD). The three species of Cyanobacteria dominant in our experiment have a filament morphological structure, but compared with *Glaucospira* sp., in *D. circinale* and *A. arnoldii*, cells appear linked to each other in bundles (much condensed and larger in *D. circinale*), possibly affecting grazing. We have a low comprehension of the feeding mechanisms used by *S. vetulus* to ingest these filaments, and new, more specific experimental designs exploring their feeding mechanism should be performed to contribute to this topic. Both species of Cyanobacteria (*A. arnoldii* and *D. circinale*) have been reported as potentially toxic (Bernard et al. 2016), and this may also harm cladoceran feeding (Nandini and Sarma 2023). However, for the shallow lake where we collected the phytoplankton assemblage, although several events of Cyanobacteria blooms have been reported in the past (Frau et al. 2019), no toxins production has been detected recently (Frau et al. 2022, unpublished data). *Simocephalus vetulus* feeding on *U. acus* was lower than Cyanobacteria species and was not statistically significant in the absence of bifenthrin. Although several studies performed with marine cladocerans have already proved that they may effectively feed on pennate diatoms like *U. acus* (e.g., Kim et al. 1989, Wong et al. 2006, Katechakis and Stibor 2004), the evidence for freshwater cladocerans is inconclusive (Frau et al. 2017).

Regarding feeding on morphological groups, results were consistent with those found with dominant species, adding information from other phytoplankton groups not represented in the previous classification of dominant species. This is, *S. vetulus* shown to feed predominantly on Colonies and Coenobiums, a morphological group represented by other phytoplankton taxa, like *A. delicatissima*,

*T. glabrum*, and *D. communis*. These results are consistent with previous reports (e.g., Frau et al. 2017). In this respect, it has been reported that Cladocera may feed efficiently in a range between 20 and 50 µm of MLD (Moustaka-Gouni and Sommer 2020). The non-statistically significant feeding on single cells (flagellates and sessile forms) is also consistent with previous studies. Because of their small size and high surface-to-volume ratio, these algae can rapidly counteract the zooplankton grazing impact by absorbing nutrients and displaying high growth rates (Litchman et al. 2010; Colina et al. 2016).

For *A. falcifer*, grazing pattern effects in the absence of bifenthrin were radically different. For this species, only significant ingestion rates were obtained for *A. arnoldii*. Previous studies reported efficient grazing effects of Copepoda on filamentous Cyanobacteria, like Panosso et al. (2003), who reported that *Notodiaptomus iheringi* Wright S. fed on filamentous cyanobacteria. Bouvy et al. (2001) and Kâ et al. (2012) also reported that some copepod species like *N. cearensis* (Wright S.) and *Thermocyclops decipiens* (Kiefer) can cut large filamentous cyanobacteria into smaller pieces and consume them. Indeed, in our experiment, we found a similar result in those treatments with *A. falcifer* at the end of the experiment, where *A. arnoldii* individuals appeared as shorter filaments with some single cells dispersed in the sample.

When phytoplankton morphological groups were considered, the pattern of grazing observed contrasted with *S. vetulus*. Indeed, this copepod demonstrated to graze efficiently on groups that *S. vetulus* did not, such as Silicified, Sessile, and Flagellates. *A. falcifer* has a selective feeding strategy, which means that it can detect and capture single preys. Previous studies have already reported the feeding capability of this copepod on the morphological groups reported here. Rietzler et al. (2002) reported grazing on Silicified species (diatoms) and some other Chlorophyceae, showing a wide range of sizes and phytoplankton species in the gut content. In the same line, Titocci et al. (2022) also reported for *Eudiaptomus* sp. an effective grazing effect on small-size phytoplankton species like those represented in the Sessile and Flagellate groups. Frau et al. (2017) also reported that *Argyrodiaptomus* sp. might feed on single cells and diatoms.

### The effects of bifenthrin on microcrustacean's grazing behavior

Bifenthrin is an artificial compound from pyrethrins present in extracts from chrysanthemum flowers. It acts as a neurotoxin by binding to voltage-gated sodium channels in neurons. This insecticide is extensively used in both agricultural and urban settings. However, its high toxicity significantly threatens aquatic life, including fish and invertebrates (Riar 2014). Previous studies reported pyrethroids' effects,

particularly bifenthrin, in aquatic biota (Werner and Moran 2008). However, this may be the first study demonstrating the effect of bifenthrin on zooplankton grazing capability on phytoplankton. In this regard, we found a decrease in the grazing capability for both microcrustacean species used in this experiment, especially with the highest concentration of bifenthrin (C2: 0.05  $\mu\text{g L}^{-1}$ ).

For *S. vetulus*, we found that the filtration rate was diminished in the highest concentration of bifenthrin (C2) compared with the control (C0) when dominant species and morphological phytoplankton groups were considered. Similar results were found by Pestana et al. (2010) with *Daphnia magna* Straus feeding on *Chlorella vulgaris* Beijerinck. In their experiment, Pestana (*op. cit.*) tested the effect of another neurotoxic insecticide, the imidacloprid, at a higher test concentration (between 2.2 and 8.8  $\text{mg L}^{-1}$ ). Similarly, Fernandez et al. (1994) proved the negative effect of sublethal concentrations of endosulfan (0.44  $\text{mg L}^{-1}$ ) and diazinon (0.47  $\text{mg L}^{-1}$ ) on the filtration rate of *D. magna* on a monoculture of *Nannochloropsis oculata* (Droop) D.J. Hibberd. In this experiment, we test a complete phytoplankton assemblage and several times lower insecticide concentrations representing environmental conditions, and a significant effect on the grazing capacity of this Cladocera was also observed.

The ingestion rate of *A. falcifer* on *A. arnoldii* decreased in the presence of the highest concentration of bifenthrin (C2). No changes were observed regarding the other dominant phytoplankton species, which is probably related to the high food selectivity that these diaptomids show. On the contrary, when dominant morphological groups were contemplated, the effect of bifenthrin was more conspicuous. *Argyrodiaptomus falcifer* decreased its grazing capacity in the presence of the higher bifenthrin concentration with Silicified, Flagellates, and Sessile phytoplankton groups. As was indicated in the previous section of the discussion, our results suggests that *A. falcifer* has a low preference for Cyanobacteria as a food resource, and its preference for grazing over other phytoplankton groups is negatively affected by the presence of bifenthrin. We could not find much evidence of other pesticides acting on copepod grazing rates. However, the tendency observed seems similar, as denoted by Donghui and Guangxing (2014), who found that *Sinocalanus tenellus* (Kikuchi K.) (marine copepod) showed a decrease in its filtration rates with increased DDT concentrations.

Previous evidence indicates that copepods are less sensitive to pesticides than cladocerans. For instance, Relyea (2005) showed that two copepods (*Eurytemora* and *Mesocyclops*) exposed to carbaryl and malathion, as well as common herbicides such as 2,4-D and glyphosate, were less responsive than other zooplankton groups that exhibited high mortality rates. Groner and Relyea (2011)

found that malathion and carbaryl had a lower effect on copepods than cladocerans. Similarly, in a review study, Sanchez-Bayo (2006) revised the effect of 468 organic pollutants on planktonic crustaceans and found that cladocerans are much more sensitive than copepods. In our experiment, copepod mortality was slightly higher, especially at the highest bifenthrin concentration (C2). This suggests that the highest mortality observed for *A. falcifer* in the treatments with bifenthrin could be more related to the effects that this insecticide has on copepods feeding behavior. This is the presence of the insecticide affected its feeding behavior, suggesting a possible secondary effect of starvation.

## Conclusions

With this experiment, we contribute to the knowledge that insecticides may affect microcrustaceans grazing capability over phytoplankton, even considering environmental concentrations that may be found and persist for long periods in water. Bifenthrin effects showed to be more relevant for Cladocera than for Copepoda grazing rates over Cyanobacteria, and this may be attributed to the fact that cladocerans are generalist filter feeders. Particularly for *A. falcifer*, results showed a decreased selectivity over Cyanobacteria (only on *A. arnoldii*), but bifenthrin effects appear as equal as relevant by negatively affecting feeding on other phytoplankton groups that may be more palatable for this copepod.

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**Data availability** Data available under reasonable request.

## Declarations

**Ethical approval** Not applicable.

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