

Feeding responses of cladocerans (Cladocera) exposed to microplastics, imiprothrin and their combinations

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Abstract

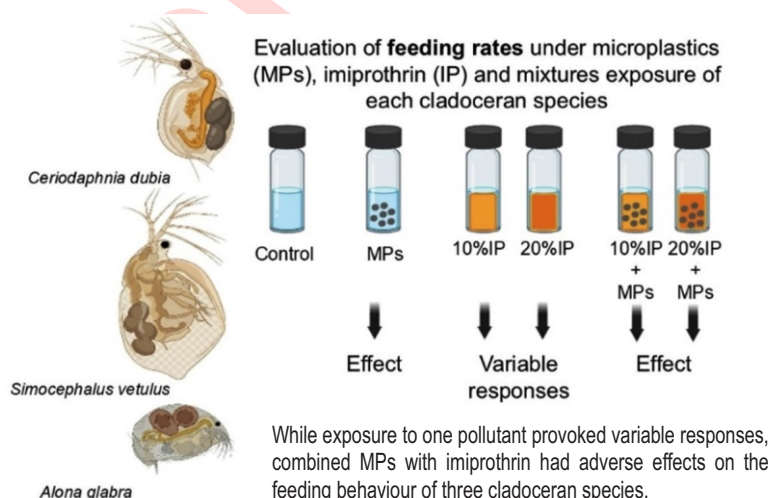
Aim: In this work, we determined the ingestion and filtration rates of three cladoceran species (*Ceriodaphnia dubia*, *Simocephalus vetulus* and *Alona glabra*) exposed to microplastics or pesticide imiprothrin separately and together on the green alga *Tetradismus obliquus*.

Methodology: We tested the acute toxicity (24 hr) of imiprothrin for *C. dubia*, *S. vetulus* and *A. glabra* and the values were 0.248, 0.307, and 0.336 mg l⁻¹, respectively. For feeding studies two concentrations of the pesticide (equivalent to 10% and 20% of LC₅₀ for each species) and one density of microplastics, 20 mg l⁻¹ for *C. dubia* and 40 mg l⁻¹ for *S. vetulus* and *A. glabra* were used.

Results: With microplastics alone the mortality rate did not exceed 40% for any cladoceran species. The results showed that, depending on the treatment, the ingestion and filtration rates varied considerably, depending on the test species. For *C. dubia*, the controls differed significantly from other treatments. *C. dubia* and *S. vetulus*, when exposed to pesticide at 10% LC₅₀+MPs, significantly higher ingestion rates compared to controls were observed, but this was not the case for *A. glabra*. At higher concentrations of pesticide+microplastics, both the ingestion and filtration rates were adversely affected for all the tested cladoceran species.

Interpretation: The results further showed that imiprothrin and microplastics interacted to cause significant changes in the feeding responses of the chosen cladoceran species. These changes included stress responses, such as hormesis, which have been discussed in this work.

Key words: Cladocerans, Feeding responses, Microplastics, Pesticide, Zooplankton



Introduction

For all animal species, adequate food consumption is a prerequisite for successful reproduction. Species are often omnivores; few are exclusively herbivores or carnivores (Attayde et al., 2010; Wootton, 2017). For heterotrophic organisms, that consume other organisms, it is critical to maintain their basal metabolism, survival, and reproduction (Boyce and Jenking, 1980; Salt, 1987). Contaminants, especially at sub-lethal concentrations, have an adverse effect on organisms, often by reducing their food intake (Hanazato, 2001). Cladocerans, which form an important group in the aquatic ecosystems, contribute to the complexity of food web structure and function (Smirnov, 2018; Wetzel, 2023). These crustaceans are crucial for energy transfer in the aquatic ecosystems, in addition to actively filtering algae, detritus, and various heterotrophs; they also participate in nutrient regeneration and serve as food for planktivorous fish and decapods (Martens et al., 1998; Taipale et al., 2018). Thus, cladocerans support ecosystem services by controlling algae and bacteria through their filtration and transfer energy to higher trophic levels (Nevalainen and Luoto, 2016; Lomartire et al., 2021).

Cladocerans are also sensitive indicators of water quality and respond rapidly to changes in the water column including multiple types of environmental stressors, thus participating in the ecosystem stability (Adamczuk, 2024). Therefore, any change in the process for obtaining energy (feeding) can affect the aquatic systems. Since 1980s the feeding mechanisms of cladocerans have been studied. They are mostly primary consumers, moving their thoracic limbs in the water column, and the food particles collected on the filtration apparatus (Smirnov, 2018). During this process, particles larger than their mesh openings are retained for consumption. Thus, cladocerans select their diet size and can consume a broad range of particles (Stern, 2009). Feeding behaviour of zooplankton can be evaluated by the quantity of particles gathered from a food suspension in a specified period of time (ingestion rate), while filtration or clearance rates indicate the quantity of medium filtered to gather food items (Downing and Rigler, 1984). Quantitative studies on these aspects are important in cladoceran bioenergetics since the quantity of food intake measured by feeding rates is reflected in their fecundity (Nandini et al., 2007). Changes in the feeding rates affect both survival and reproductive rates of cladocerans.

Mainly due to anthropogenic activities, freshwaters are contaminated by numerous chemicals that interact in the water column. Contaminants such as microplastics and pesticides affect the aquatic fauna in different ways (Khatri and Tyagi, 2015; Wu et al., 2024). Feeding processes, especially filtration, in cladocerans are affected when exposed to contaminants (Smirnov, 2018). Among different types of contaminants, plastic pollution has received considerable attention from the researchers (Nava et al., 2023). Unlike conventionally known contaminants such as heavy metals, plastics are purely man-made and their production and consumption are directly related to human activities (Thompson et al., 2009).

Microplastics (< 5 mm) can be ingested by organisms from different trophic levels and interfere in the feeding process (Desforges et al., 2015). Microplastics are also known to adsorb substances such as heavy metals and pesticides that are frequently found in the contaminated waterbodies (Llorca et al., 2018). Many synthetic pyrethroids are routinely used as pesticides. Synthetic pyrethroids have been continuously improved to obtain higher effectiveness at low doses against pests such as insects and enhanced thermal and photo-stability (Ahmad and Kumar, 2023). For example, imiprothrin, a synthetic pyrethroid has been improved for lower volatility and higher residual effectiveness. Due to these and other commercially viable presentations, imiprothrin is commonly used in the agriculture sectors and domestic applications (Ruberti, 2024). During these processes, it affects non-target organisms, including cladocerans when agricultural and domestic effluents reach freshwater bodies, including lake and reservoirs. Further, microplastics adsorb contaminants from agricultural effluents such as pesticides, thereby causing enhanced damage to biological processes, such as filtration of cladocerans in waterbodies receiving effluents (Debnath et al., 2024).

More than 600 species of Cladocera are known worldwide (Forró et al., 2008). In Mexico, the common species of Cladocera belong to the genera *Daphnia*, *Ceriodaphnia*, *Moina*, and chydorids such as *Alona* (Elías-Gutiérrez et al., 1999). Cladoceran species including *Ceriodaphnia dubia*, *Simocephalus vetulus* and *Alona glabra* are common inhabitants of shallow lakes in Mexico (Chaparro-Herrera et al., 2022). Various ecological such as including feeding and filtration rates have been studied on *Daphnia*; less is known about other cladocerans (Ebert, 2022). Emerging contaminants, including microplastics and synthetic pyrethroids, interact synergistically in the aquatic ecosystems causing enhanced adverse effects on zooplankton (Zhao et al., 2024). Although the negative effects of microplastics and pesticides have been separately tested on cladocerans (Devi et al., 2025), their combined effects are less known, especially the filtration process in zooplankton. Therefore, the aim of this work was to quantify the feeding and filtration rates of three cladoceran species (*Ceriodaphnia dubia*, *Simocephalus vetulus* and *Alona glabra*) exposed to microplastics and imiprothrin alone, and together.

Materials and Methods

Three cladoceran species used in this study (*Ceriodaphnia dubia*, *Simocephalus vetulus* and *Alona glabra*) occur in Lake Xochimilco (Mexico City, Mexico) (Gayosso-Morales et al., 2017; Chaparro-Herrera et al., 2022). These species were cultured in the laboratory under controlled conditions for over a decade. Starter cultures of all these species were initiated with a single parthenogenetic female. In this study, the green alga *Tetradismus obliquus* (previously known as *Scenedesmus acutus*) (Wynne and Hallan, 2015) was used as food for the cladoceran species cultured separately. Regularly, the cultures of cladocerans received algae at a density of 1×10^6

cells ml^{-1} and were maintained in moderately hard water (EPA medium). EPA medium was prepared by dissolving 1.9 g NaHCO_3 , 1.2 g CaSO_4 , 1.2 g MgSO_4 , and 0.04 g KCl in 20 l of distilled water (Weber, 1993). *Tetrademus obliquus* was cultured in 2 l bottles using Bold Basal medium (Borowitzka and Borowitzka, 1988). To maintain favourable conditions for the stock cultures of cladocerans, we changed them every 3 days and maintained at pH 7.0, $25 \pm 1^\circ\text{C}$, and continuous but diffuse illumination.

Polystyrene microspheres measuring 30 μm diameter (MPs) used for the experiments, were obtained from Sigma-Aldrich (Batch: #BCCF6424). The stock solution was prepared based on the microplastic concentration. Before use in any test, it was sonicated (Ultrasonic Processor, CP 130PB-1) at a frequency of 20 kHz and 10 watts for 3 min to homogenize. The concentrations used in the experiments were based on the preliminary tests. Analytical grade pesticide imiprothrin was purchased from Sigma-Aldrich (USA). A stock solution of 1 g l^{-1} was prepared in double-distilled water, and 0.05% w/w of dimethylsulfoxide (DMSO) to completely dissolve the pesticide. First, a range-finding test was conducted. Subsequently, five concentrations of imiprothrin ranging from 0.072 – 1.28 mg l^{-1} were selected. The LC_{50} – 24 hr was determined by the Probit method was used (Finney, 1971). Once these data were obtained, two sublethal concentrations of imiprothrin (IP) were selected (10 and 20% of LC_{50} for each cladoceran species) and one of microplastics (MPs). The ingestion and filtration rate of three cladoceran species on each contaminant were evaluated individually and in combination. Two concentrations of imiprothrin (separately or in combination with microplastics) used for each cladoceran species were: 10%IP (= 0.0248, 0.0307 and 0.0336 mg l^{-1}) and 20%IP (= 0.0496, 0.0614, 0.0672 mg l^{-1}) for *C. dubia*, *S. vetulus*, and *A. glabra*, respectively. For microplastics (MPs), only one concentration (separately or in combination with IP) was tested: for *C. dubia* it was 20 mg l^{-1} and for *S. vetulus* or *A. glabra* the concentration was 40 mg l^{-1} .

Tests were conducted in 40 ml capacity glass vials. Each vial contained 20 ml of EPA medium with 10 individuals of one of the three chosen cladoceran species, 0.5×10^6 cells ml^{-1} of *T. obliquus*, a given concentration of imiprothrin, a selected concentration of microplastics, or the combination of contaminants. The juveniles of each species were previously exposed for 24 hr to the selected sublethal concentration of imiprothrin. Prior to starting the experiments, the cladocerans were left for 30 min in starvation. Subsequently, the individuals were allowed to feed for 20 min, and thereafter the uningested algae were counted in a Neubauer chamber. The feeding and filtration rate were calculated from the data recorded using the following formulas (Rigler, 1971):

$$f = \frac{v(C_o - C_t)}{tN}$$

Where, f : feeding rate, v : medium volume, C_o : initial cell concentration, C_t : final cell concentration, t : feeding time (min), N : number of individuals.

$$F = \left(\frac{\ln C_o - \ln C_t}{tN} \right) W$$

Where, F : filtration rate, $\ln C_o$: natural logarithm of initial cell concentration, $\ln C_t$: natural logarithm, f : final cell concentration, W : medium volume, t : feeding time (min) and N : number of individuals per jar. Prior to quantifying statistically significant differences in the feeding and filtration rates, data were analysed by a normality test (Shapiro-Wilk) and a homoscedasticity test (examining residual plots), followed by One-way ANOVA and Tukey's posthoc tests using Sigma Plot version 11.

Results and Discussion

Depending on the sensitivity of the tested cladoceran species, the concentrations of imiprothrin and the density of microplastics varied. In general, *Ceriodaphnia dubia* was most sensitive while *Alona glabra* was least when exposed to either microplastics or pesticides. *Simocephalus vetulus* had intermediate values. The LC_{50} (24 hr bioassay) values of the pesticide for *C. dubia*, *S. vetulus*, and *A. glabra* were 0.248, 0.307 and 0.336 mg l^{-1} , respectively. The mortality rate never exceeded 40% for any cladoceran species with only MPs. Therefore, LC_{50} values were not obtained for microplastics, as also observed in a previous study on *Daphnia pulex* (Reyes-Santillán et al., 2025). However, the acute toxicity trends for only microplastics showed higher resistance by *C. dubia* as compared to *S. vetulus* and *A. glabra*. Therefore, for feeding studies microplastics at a density of 20 mg l^{-1} for *C. dubia* and 40 mg l^{-1} for *S. vetulus* and *A. glabra* were used. DMSO concentration used to prepare the stock solution was very low, (0.05%) and for the tests, this was further diluted (10–20%). Thus, the actual quantity of DMSO reaching the test jars would not have caused any adverse impact on cladocerans as they are known to tolerate much higher levels (Andrade-Vieira et al., 2022). Another possible source of error could be crowding from individuals in each container (0.5 ind ml^{-1}). However, this possibility is extremely unlikely as all the test species were previously cultured upto a density > 10 ind ml^{-1} without adverse crowding effects (Nandini and Sarma, 2003).

The size of the microplastics used in the ecotoxicological studies on zooplankton varied considerably, from 10 nm to 5 mm. Therefore, the response of zooplankton exposed to microplastics also varies. In general, smaller particles have more adverse effects on zooplankton than larger particles (Lawrence et al., 2023). The size of microplastics used here shows that they were ingestible by the cladocerans. Uniform particle size enables improved reproducibility of results (Reyes-Santillán et al., 2025). Data on the ingestion rates of three cladoceran species fed on *Tetrademus obliquus* under different treatments are presented in Fig. 1. For *C. dubia*, the controls differed significantly from the rest of the treatments ($p < 0.05$, One-way ANOVA) (Table 1). However, at 20% LC_{50} of imiprothrin, the presence or absence of microplastics did not cause significant differences in the ingestion rates. However, for both *S. vetulus* and *A. glabra*, the differences were significant under comparable conditions. *C. dubia* and *S. vetulus*, when exposed to imiprothrin at 10% LC_{50} +MPs, had significantly higher ingestion rates compared to controls, however, *A. glabra* did not show a similar trend. The lowest

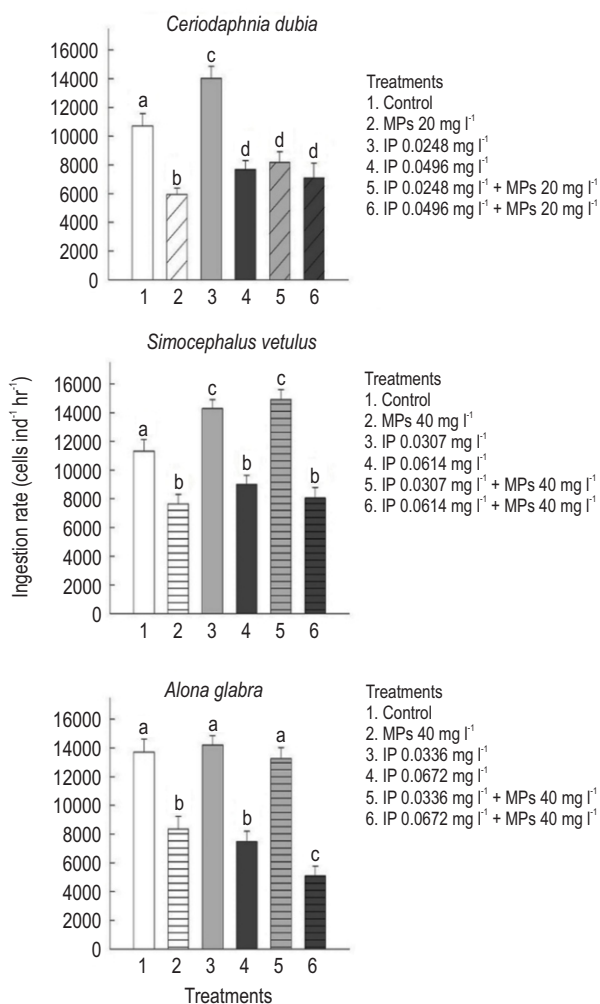


Fig. 1: Ingestion rates (*Tetrademus obliquus* cells ind⁻¹ hr⁻¹) of *Ceriodaphnia dubia*, *Simocephalus vetulus* and *Alona glabra* exposed to two sublethal concentrations of imiprothrin, one concentration of microplastics and combined. Shown are the mean $\pm$ SE based on five replicates. For each cladoceran species data bars carrying different alphabets are significantly different ($p < 0.05$ One-way ANOVA).

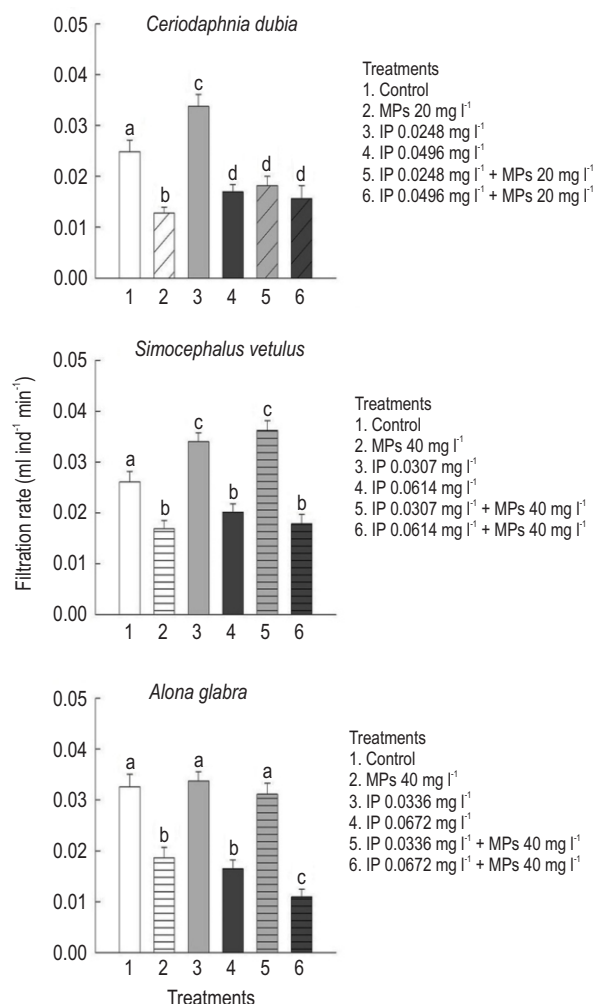


Fig. 2: Filtration rates (ml ind⁻¹ min⁻¹) of *Ceriodaphnia dubia*, *Simocephalus vetulus* and *Alona glabra* exposed to two sublethal concentrations of imiprothrin, one concentration of microplastics and combined. Shown are the mean $\pm$ SE based on five replicates. For each cladoceran species data bars carrying different alphabets are significantly different ($p < 0.05$ One-way ANOVA).

ingestion rate (ca. 5000 cells ind⁻¹ hr⁻¹) was observed for *A. glabra* when exposed to 20% LC₅₀ pesticide+MPs. The filtration rates of three cladoceran species under different treatments varied from 0.015 to 0.035 ml ind⁻¹ min⁻¹ (Fig. 2). The trends in the changes of filtration rates in relation to the treatments were similar to those of ingestion rates under comparable conditions.

Feeding and filtration rates are sensitive indicators of stress. Normally, cladocerans show a Type 1 functional response (Lampert and Sommer, 2007), where the ingestion rates remain constant at and beyond the incipient levels. In this study, a constant food level was used, and therefore, it was not possible to determine the functional responses. Nevertheless, the concentration used in this study was optimal for growth and

reproduction of the tested cladoceran species (Nandini and Sarma, 2003). The swimming behaviour of cladocerans is adversely affected by the contaminants. Reduced swimming rate leads to reduced food consumption and under very low algal levels, it is not easy to quantify the particles consumed (Clance et al., 2023). Therefore, to quantify algal consumption under stressful pollutant levels, higher algal concentrations than those observed in nature are offered. Ingestion rates quantify the amount of food particles gathered from the medium in a specific period of time. Higher algal densities and longer duration of exposure lead to rapid excretion of undigested algal cells into the medium. Therefore, the feeding duration set in this work was based on the previous studies (Murtaugh, 1985; Pérez-Morales et al., 2014). Filtration rate in zooplankton is inversely related to feeding rates on abroad

Table 1: One-way analysis of variance (ANOVA) on the feeding rate of *C. dubia*, *S. vetulus* and *A. glabra* exposed to sublethal concentrations of imiprothrin, microplastics and the combination of both pollutants. DF: Degrees of Freedom; SS: Sum of Squares; MS: Mean Squares ($\alpha=0.05$)

<i>Ceriodaphnia dubia</i>					
Source of Variation	DF	SS	MS	F	P
Between groups	5	218587491	43717498	14.539	<0.001
Residual	24	72164910	3006871		
Total	29	290752402			
<i>Simocephalus vetulus</i>					
Between groups	5	249953540	49990708	20.613	<0.001
Residual	24	58206083	2425253		
Total	29	308159623			
<i>Alona glabra</i>					
Between groups	5	372122148	74424429.682	25.095	<0.001
Residual	24	71175746	2965656.095		
Total	29	443297894			

range of algal concentrations. At higher food concentrations, the zooplankton species including cladocerans filter less volume and therefore, the filtration rate decreases (Monakov, 2003). However, under stressful conditions, this condition changes, because depending on the stress type, the swimming rates differ. For example, higher temperatures lead to higher swimming rates and, in turn, higher feeding rates. On the other hand, at higher toxicant concentrations, the swimming rates reduce, and hence the feeding rate decreases (Moruzzi *et al.*, 2025). Another factor that affects the food consumption by herbivorous zooplankton is the possible inhibition of feeding by particles already present in the gut, particularly when toxic particles are involved. Thus, when toxic substances accumulate in the gut on feeding both microplastics and imiprothrin, there may be a decrease in further food consumption. Although, in this study, the quantity of toxins accumulated in the cladoceran gut was not determined, there is some evidence that ingestion of microplastics by copepods resulted in altered feeding capacity (Cole *et al.*, 2015). In this study, the combined treatment of microplastics and pesticide, especially at higher imiprothrin level, may have caused decreased algal consumption. Toxicants have an adverse effect on the functioning of cladocerans, including their thoracic appendages (Smirnov, 2018). In addition, low levels of toxicants provoke stimulatory responses in cladocerans.

This response, known as hormesis, occurs to overcome stress with overcompensation of one or more physiological processes (Calabrese and Baldwin, 2003). This also differs from species to species and the type of stressor. Due to these reasons, the statistical significance of feeding and filtration rates varied when exposed to pesticide-microplastic combination. For example, both *C. dubia* and *S. vetulus* showed significantly higher ingestion rates when exposed to low concentrations of imiprothrin (10% LC₅₀), as compared to controls. However, for *A. glabra*, the differences between controls and treatment with low pesticide-

microplastic combination (10% LC₅₀+MPs) were not significantly different. On the other hand, at a higher level of imiprothrin (20% of LC₅₀), the feeding rates declined significantly compared to controls, suggesting the onset of toxic effects of the pesticide and absence of hormetic responses. These trends were similar for filtration rates, too. The absence of a hormetic response by *A. glabra* when exposed to a lower level of imiprothrin is possibly a species-specific reaction (Sun *et al.*, 2022). When exposed to microplastics alone, or in combination with the pesticide, the feeding and filtration responses of three cladoceran species differed widely. These results suggest that the feeding behaviour of cladocerans is highly variable, not only in relation to the test species, but also the combination and concentration of stressful agents (Debnath *et al.*, 2024).

In nature, microplastics interact with many toxic substances, such as pesticides and heavy metals. Therefore, isolated studies using only microplastics may not reveal the effects of interactions with other substances on the aquatic organisms. Further, at sublethal levels, different toxicants exhibit different responses: some show no quantifiable impact, while others may have an adverse effects (Sherborne *et al.*, 2020). On the other hand, certain toxicants at very low concentrations provoke hormesis, which leads to increased filtration and/or consumption rates. However, its impact on the zooplankton community structure can be elucidated only through field studies (Jiménez-Contreras *et al.*, 2024). The quantity of food particles gathered is generally reflected in both survivorship and reproductive parameters, which were not quantified in this study. However, it is important to note that contaminated algae when consumed in elevated quantities, could be counter productive for the survival and reproduction of cladocerans. A toxicant-loaded diet could kill the consumer earlier than uncontaminated food particles. In such cases the zooplanktons could reduce their food intake (Sarma *et al.*, 2001), which is evident in certain treatments

in this study, for example, for *A. glabra* at 20% LC₅₀ of pesticide, had the lowest ingestion rates compared to other treatments. However, this was not the trend for two other species. In addition to pesticide-contaminated food, the presence of inorganic particles in the environment such as microplastics, can have a greater impact on food consumption by zooplankton. Few studies have evaluated the capacity of MPs to adsorb other toxic substances, leading to synergistic effects (Felten *et al.*, 2020). This effect was observed in *C. dubia* with the pesticide at 10% LC₅₀+MPs when compared to only pesticide.

Regardless of treatments, in controls, the feeding and filtration rates of the tested cladocerans were within the known range reported in the literature. For example, most cladoceran species filter about 0.1 ml ind⁻¹ hr⁻¹ (Monakov, 2003). In the present study, under non-stressful conditions (controls), three cladocerans filtered from 0.025 to 0.033 ml ind⁻¹ hr⁻¹. These lower rates were due to a higher algal level (1×10⁶ cells ml⁻¹) used in this study. It is known that the filtration rates of zooplankton are dependent on the body size of the herbivore. For example, Pérez-Morales *et al.* (2014) quantified the ingestion and filtration rates of smaller (rotifers, <150 µm body size) and larger (cladocerans > 800 µm) zooplankton fed algae and cyanobacteria. Their data on the ingestion and filtration rates ranged from 4800–24000 cells ind⁻¹ hr⁻¹ and 0.02–0.22 ml ind⁻¹ min⁻¹, respectively. In this study, for controls, the cladoceran feeding rates ranged from 10,000 to 14,000 cells ind⁻¹ hr⁻¹. Many species of cladocerans feed on <10,000 to >20,000 cells ind⁻¹ hr⁻¹, depending on the particle size (Monakov, 2003). Thus, the data range obtained in this work, for both feeding and filtration rates was within the intervals reported for most cladoceran species in the literature (Monakov, 2003; Pérez-Morales *et al.*, 2014). However, the range of these parameters varied depending on the stress type, concentration, their combination, and tested cladoceran species.

In conclusion, the results of this study showed that the microplastics and pesticide imiprothrin had adverse effects on the feeding and filtration rates of three tested cladoceran species, with the extent varying depending on the zooplankton species, and the concentration and combination of toxicants. Further studies are still needed to quantify the impact of these results on the population level parameters of the tested cladoceran species.

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