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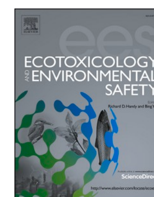

How does increasing temperature affect the toxicity of bisphenol A on *Cryptomonas ovata* and its consumer *Daphnia magna*?

Juan Manuel González-Olalla [a,*], María Vila-Duplá [a,b], Marco J. Cabrerizo [b], Irene González-Egea [a], Gema Parra [c], Juan Manuel Medina-Sánchez [b], Presentación Carrillo [a,b]

[a] *Institute of Water Research, University of Granada, Ramón y Cajal, 4, Granada 18071, Spain*
[b] *Department of Ecology, University of Granada, Campus Fuentenueva s/n, Granada 18071, Spain*
[c] *Department of Animal Biology, Plant Biology, and Ecology, University of Jaén, Campus de Las Lagunillas s/n, Jaén 23071, Spain*

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ABSTRACT

The global rise in plastic production has led to significant plastic deposition in aquatic ecosystems, releasing chemical compounds as plastics degrade. Among these, bisphenol A (BPA) is a major global concern due to its endocrine-disrupting effects and widespread presence in aquatic environments. Furthermore, the toxicity of BPA on aquatic organisms can be modulated by global change stressors such as temperature, which plays an essential role in the metabolism of organisms, including the degradation and accumulation of toxic compounds. In this study, we aimed to understand how temperature can modulate the toxic effect of BPA on a phytoplankton species (*Cryptomonas ovata*) and how this effect can be transferred to its herbivorous consumer (*Daphnia magna*). To do this, we first determined the sensitivity of *C. ovata* over a BPA gradient (0–10 mg L^{-1}). Subsequently, we experimentally determined how the increase in temperature (+5ºC) could modify the toxic effect of BPA on the physiology, metabolism and growth of the phytoplankton. Finally, we investigated how this effect transferred to the growth rate of *D. magna* through food. Our results show a negative effect of BPA on *C. ovata* from 5 mg BPA L^{-1}, affecting its photosynthetic yield of photosystem II, net primary production, respiration, and growth. This effect was accelerated when the temperature was higher. Additionally, the growth rate of *D. magna* also decreased when fed on *C. ovata* grown in the presence of BPA and high temperature. Our results indicate that high temperature can accelerate the toxic effects of BPA on organisms located at the base of the food web and this effect could be transferred to higher levels through food.

1. Introduction

The widespread production of plastics and its impact on ecosystem functioning have garnered increasing attention (Thompson et al., 2009; Godoy et al., 2022). The progressive breakdown of plastics may release chemical compounds, including plasticizers, into the environment. One of the synthetic substances most used as a plasticizer is bisphenol A (BPA), an endocrine disruptor organic compound primarily used as an intermediate in the production of polycarbonate plastic and epoxy resins (Staples et al., 1998).

The widespread use of BPA has led to its continuous release and dissemination into the aquatic environment, primarily through the discharge of BPA-enriched effluents from wastewater treatment plants (Kang and Kondo, 2006). Other secondary sources of BPA include leachates from landfills contaminating groundwater and rivers (Masoner et al., 2014). Although the half-life of BPA is short, the continuous discharge of BPA into the environment ensures ongoing exposure of aquatic organisms (Sharma et al., 2009). Furthermore, BPA has moderate potential for bioaccumulation (Staples et al., 1998), thus it could be transferred to higher trophic levels in food webs with still unknown consequences. A literature review showed that the concentration of BPA in highly contaminated waters of the Yamuna River in India or a Netherlands river, reached up to 14.8 and 21 μg BPA L^{-1}, respectively (Lalwani et al., 2020), while in river sediments in Taiwan (China), up to 10.5 mg kg^{-1} has been detected (Huang et al., 2012). BPA in municipal wastewater treatment plants receiving influents from landfills, industries or hospitals can reach concentrations over 1 mg L^{-1}, making it a major source of BPA in surface waters (Ferrer-Polonio et al., 2021). For instance, research studies have reported BPA concentrations of 2.72 mg L^{-1} in a sanitary landfill in Serbia (Narevski et al., 2021), and

* Corresponding author.
E-mail address: jmolalla@ugr.es (J.M. González-Olalla).

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even as high as 33.46 mg BPA L^{-1} in leachates from the treatment of municipal solid waste in northern Italy (Baderna et al., 2011). Despite the emergence of new bisphenol analogues to BPA such as bisphenol S (BPS), F (BPF), and AF (BPAF) (Liu et al., 2021), which are causing BPA to lose its dominant position in the market, its production is still expected to increase in the next five years (BrandEssence, 2022).

The aquatic toxicity of BPA depends on the organism and even on the species, having been measured in bacteria, micro- and macroalgae, mollusks, insects, crustaceans, amphibians, and fish (Wu and Seebacher, 2020). Particularly, due to its position at the basis of aquatic food webs, phytoplankton plays an essential role in the transfer of organic compounds, also being one of the pathways for BPA entry into food webs. On the other hand, phytoplankton appears to be capable of degrading low concentrations of BPA (<1 mg L^{-1}) (Ji et al., 2014). In fact, some studies suggest that phytoplankton could use BPA as a carbon source to enhance its growth (Wang et al., 2017; Álvarez et al., 2024). However, at higher concentrations, it is expected that BPA will negatively affect phytoplankton. Indeed, experiments conducted with *Chlorella pyrenoidosa* have shown that a concentration of 10 mg BPA L^{-1} decreased its growth rate due to damage to metabolic pathways involved in photosynthesis, the tricarboxylic acid (TCA) cycle, or glycolysis, among others (Duan et al., 2019). Although the potential acute impact of BPA on marine and freshwater aquatic organisms have been extensively studied (Wu and Seebacher, 2020), information regarding how BPA interacts with other abiotic factors, synergistically or antagonistically, altering its final effect on phytoplankton metabolism is lacking.

The increase in water temperature is a chronic stressor on lakes and oceans (IPCC, 2021) altering the metabolism of aquatic organisms. Phytoplankton, given its dependence on external temperature, could experience higher metabolic activity (respiration and photosynthesis) accompanied by a greater growth rate (Yvon-Durocher et al., 2010; Regaudie-De-Gioux and Duarte, 2012), which can affect the biodiversity and biogeochemical cycles of aquatic ecosystems (Litchman et al., 2015). Thus, higher temperatures may enhance the release, transport, and mobilization of pollutants like BPA, increasing its bioaccumulation in aquatic organisms (Kibria et al., 2021). The exposure to BPA concomitantly with the increase in temperature has been recently studied in some aquatic organisms (e.g., fish, in Wu et al. (2022); zooplankton, in Álvarez et al. (2024)). We found only one study analyzing the interactive effects of BPA and temperature on phytoplankton, which showed an antagonistic interaction of both factors on the growth of three species (Theus et al., 2023). However, the physiological and metabolic mechanisms (often stress indicators) behind these phytoplankton responses remain unexplored/understudied. Besides, most ecotoxicological studies on BPA have focused on its effects on different trophic levels, while studies examining the transfer of effects between phytoplankton and zooplankton are scarcer. Currently, there is also a growing interest in understanding how effects of BPA on lower trophic levels could be transferred along the aquatic food chain (Tang et al., 2023). Thus, given phytoplankton's position in the trophic web and its high lipid content facilitating the accumulation of hydrophilic BPA (Michałowicz, 2014), they serve as model organisms for studying the transfer of BPA effects to consumers (i.e., zooplankton). In this regard, the intake of BPA in aquatic organisms can originate from direct adsorption from the water column or through daily diet (Du et al., 2014). Recently, Álvarez et al (Álvarez et al., 2024). reported that the negative effects of BPA on *Daphnia magna* are mainly associated with the uptake of BPA contained in phytoplankton rather than the BPA dissolved in the medium, pointing to a trophic transfer mechanism. However, there is still limited information on how abiotic factors, such as temperature, may influence the trophic transfer of the effects of BPA in aquatic ecosystems (Windsor et al., 2018).

In this research, we analyze how a predicted future scenario of higher temperature (+5ºC according to IPCC, 2021) may modify the toxic effect of BPA on the physiology, metabolism, and growth of *C. ovata.* Besides, we investigated how these effects can be transferred to primary consumers (*Daphnia magna*) through diet. We hypothesize that higher temperature may enhance the toxic effect of BPA on *C. ovata* due to a higher uptake rate and potential assimilation of BPA. As a result, subcellular (maximum photosynthetic efficiency as a stress indicator) and metabolic (net primary production and respiration) damage will increase. This will have negative consequences on the population dynamics of phytoplankton (cell abundance and growth rate), transferring this effect to zooplankton.

2. Material and methods

2.1. Culture conditions

We designed an experiment to study the interactive effect of BPA and temperature on a model phytoplanktonic species, *C. ovata.* Previous to the beginning of the experiment, this organism was cultured in COMBO medium (Kilham et al., 1998) under non-axenic conditions. This species is ideal for our experiment for several reasons: i) it belongs to a functional group (cryptophyte) widely distributed in freshwater, brackish and marine environments (Hoef-Emden and Archibald, 2017); ii) *C. ovata* is a mixotrophic species able to use organic C sources (Calderini et al., 2022); and iii) it is an important food source for microzooplankton (ciliates, rotifers, small flagellates) and high-quality food for *Daphnia* complex species (see Hiltunen et al. (2017), or Taipale et al. (2014)), so it may affect the pelagic carbon flow in aquatic systems (Wirth et al., 2019). During a two-week acclimation period, this species was growing at 19ºC and exposed to ~100 μmol photon m^{-2} s^{-1} of photosynthetically active radiation (PAR, 400–700 nm) under a photoperiod of 12 h light:12 h dark.

After acclimation, the *C. ovata* culture was used to test its sensitivity to an increasing gradient of BPA (Section 2.2) and, subsequently, to analyze the interactive effect of BPA and increased temperature on *C. ovata* (Section 2.3) and its effect on consumers (*Daphnia magna*; Section 2.4) (Fig. 1).

2.2. Previous bioassays

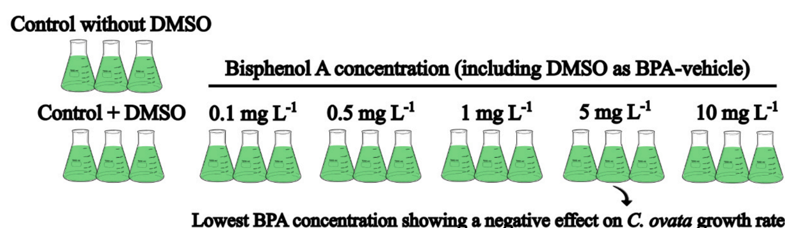
2.2.1. Selection of a non-toxic BPA vehicle

Previous studies analyzing the toxic effects of BPA on aquatic organisms reveal the need to use a vehicle that facilitates the dissolution of BPA in the water. A literature review showed that ethanol and dimethyl sulfoxide (DMSO) are the two main compounds used as BPA-vehicles (e.g., Maamar et al., 2015). Therefore, the first step was to determine the effect of these vehicles on the *C. ovata* growth. We exposed *C. ovata* to ethanol or DMSO (both at 0.002 % v/v, which was the minimum concentration of the vehicle needed to dilute BPA in water), for 7 days or until significant differences were detected between control (without BPA-vehicle addition) and BPA vehicle treatments. Cultures (in triplicate for each treatment) were grown in 100-mL glass tubes filled with COMBO medium and incubated under the same temperature and light conditions specified above. Finally, for performing the subsequent BPA-gradient and BPA × Warming experiments (see below), we chose the BPA vehicle whose values were not significantly different from that of the control treatment.

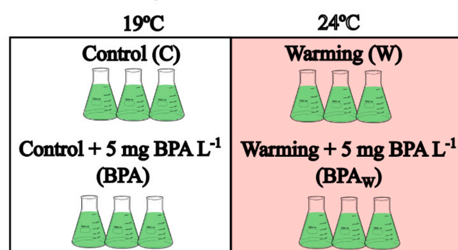
2.2.2. Determination of the C. ovata sensitivity to BPA

We exposed *C. ovata* to increasing final concentrations of BPA (0.1, 0.5, 1, 5 and 10 mg L^{-1}), prepared from an initial BPA solution (250 g L^{-1}) dissolved in dimethyl sulfoxide (DMSO; >99 % purity), to determine the minimal concentration that causes a negative effect on the algal species. Like the first assay, the cultures of *C. ovata* were grown in 100-mL glass tubes filled with COMBO medium (in triplicate) at 19ºC, under a 12:12-hour light/dark photoperiod and a light intensity of 100 μmol photons m^{-2} s^{-1} of PAR. After determining the lowest BPA concentration from those tested in this study with a significant toxic effect on the *C. ovata* population (i.e., 5 mg L^{-1}; see also Section 3.2 in the

1st experiment - Sensitivity of *Cryptomonas ovata* to an increasing BPA concentration gradient



2nd experiment - Sensitivity of *C. ovata* to BPA × Warming interaction



3rd experiment - Effect of BPA contained on *C. ovata* on *Daphnia magna*

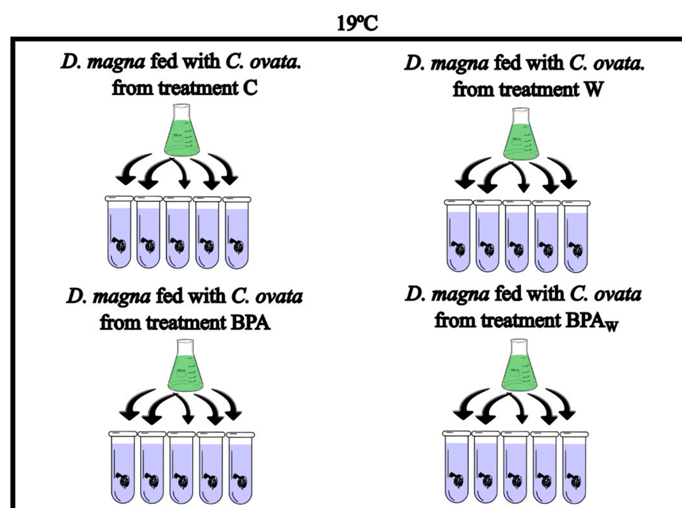


Fig. 1. Experimental design scheme. The experiment was divided into three sub-experiments where the toxicity of BPA on *Cryptomonas ovata* was detected across a BPA gradient (0.1–10 mg L^{-1}) (first experiment); a second experiment where *C. ovata* was exposed to the interactive effect of BPA and a 5ºC temperature increase; and a third sub-experiment where *Daphnia magna* was fed with cultures of *C. ovata* from the second experiment.

results section), an interaction experiment with temperature was conducted (Section 2.3; see below) to analyze how BPA toxicity on phytoplankton was altered under warming conditions.

2.3. Quantification of the BPA × Warming effects on C. ovata

To assess the combined impact of BPA (factor 1) and increased temperature (warming) (factor 2), a 2 × 2 full factorial experimental design was implemented with: (a) factor 1 including two chemical levels: control with addition of DMSO as solvent vehicle, and addition of DMSO and BPA; and (b) factor 2 including two temperature levels: ambient (19ºC) and warming (24ªC) conditions. Therefore, from the combination of levels of both factors, four treatments were defined: C (without BPA-ambient temperature), W (without BPA-warming), BPA (with BPA-ambient temperature), and BPA_W (with BPA-warming). The 5 °C temperature increase lies within the range predicted by IPCC for the late twenty-first century climate (IPCC, 2021; Scenario RCP8.5).

Each treatment consisted of 5 replicates, resulting in a total of 20 experimental units of 250 mL quartz flasks. Thus, we ensured that each treatment had a sufficient sample volume to prevent any negative differential effects when extracting samples for measuring each variable. The flasks were filled with a *C. ovata* culture at a concentration of 1×10^5 cells mL^{-1}. Then, DMSO (0.002 % v/v final concentration) or DMSO+BPA (5 mg BPA L^{-1} final concentration) were added to the corresponding treatments. The use of DMSO as a BPA vehicle, as well as the nominal concentration of BPA used in this experiment (5 mg BPA L^{-1}), were determined based on the results obtained in previous assays (see Results section, Figs. 1 and 2). Since our objective was to analyze how the increase in temperature can modify the toxic effect of BPA, it is necessary to clarify that the concentration of BPA used in this part of the experiment exceeds the environmental concentrations found in surface waters, being only similar to those detected in landfill leachates and the inflow of wastewater from hospitals or industries.

Finally, the quartz flasks were randomly placed in an aquarium system with adjustable temperature (TECO® S.R.L. tank TK 2000, EU) and illuminated with LED lights (nano LED light v.2.0, BLAU aquaristic) providing photosynthetically active radiation (PAR). The flasks were incubated for 7 days under a 12:12-hour light/dark photoperiod and a

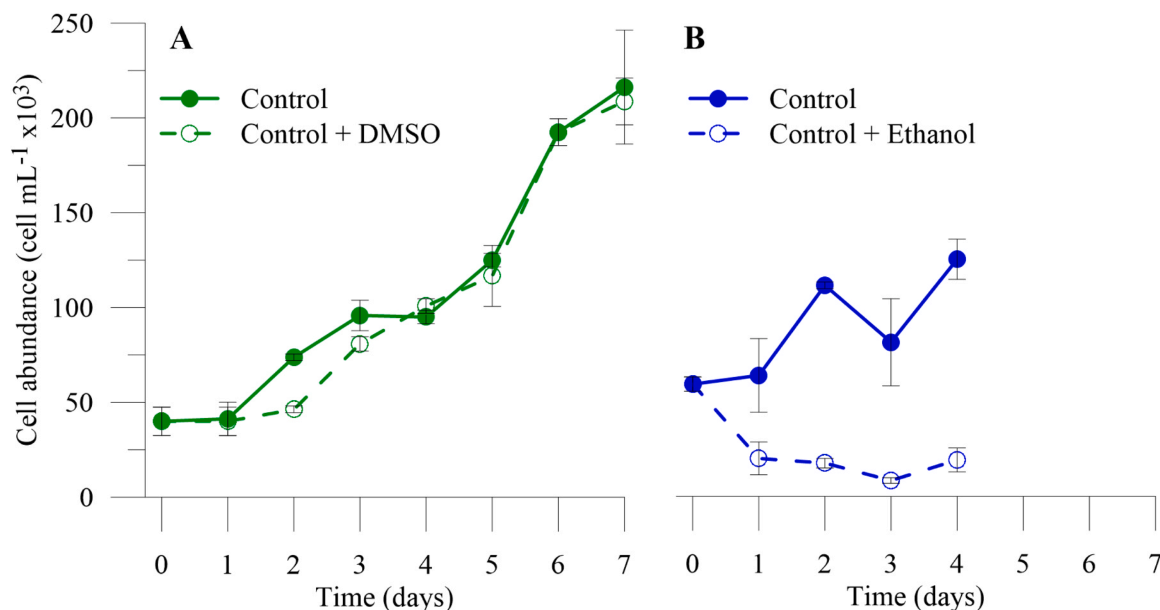


Fig. 2. Cell abundance (cell mL^{-1}) of *C. ovata* exposed to dimethyl sulfoxide (DMSO) (A), and ethanol (B) as bisphenol A vehicles. Data are represented over 7 and 4 days, respectively, and they are expressed as mean values ± SD (n = 3).

light intensity of 100 μmol photons m^{-2} s^{-1} of PAR.

2.3.1. Maximum photochemical efficiency (Fv/Fm)

The maximum photochemical efficiency of photosystem II (*Fv/Fm*) in *C. ovata*, also considered as a stress indicator in plants (Janka et al., 2015), was measured for each treatment (in triplicate) on days 1, 2, 4, and 7 of the experiment using a Pulse-Amplitude-Modulated (PAM) fluorometer (Water-ED PAM, Walz, Germany). Samples of 3 mL were extracted from the quartz flasks under dark conditions. Dark conditions are necessary for the determination of the baseline fluorescence (F_0) of the sample. Subsequently, the *Fv/Fm* was determined as:

$$F_v \Big/ F_m = \frac{(F_m - F_0)}{F_m} \quad (1)$$

where *Fm* is the maximum fluorescence induced by a saturating light pulse (approximately 5300 μmol photons m^{-2} s^{-1} in 0.8 s).

2.3.2. Net primary production and respiration

To measure net primary production and respiration, on days 1, 2, 4, and 7, three 25-mL quartz flasks equipped with O_2 sensor-spot optode (SP-PSt3-NAU-D5-YOP; PreSens GmbH, Germany) were filled from three out of the 5 randomly selected quartz flasks experimental units per treatment. The 25-mL flasks were gently filled without bubbles, sealed to avoid gas exchanges, and incubated for 12 hours of light (net oxygen production – *NPP*) and 12 hours of darkness (respiration - *R*) on days 1, 2, 4, and 7 of the experiment. Measurements were made at the beginning and at the end of the light period, and the same for the dark period. We used an oxygen transmitter (OXY-4 mini, PreSens GmbH, Germany) connected to Oxyview 6.02 software to register data. This system was previously calibrated at 19 °C and 24 °C using two-point calibration (0 % and 100 % oxygen saturation) together with temperature and atmospheric pressure data.

NPP and *R* rates (in μg O_2 L^{-1} h^{-1}) were calculated as the slope of the linear portion of the curve representing O_2 concentration vs. time. Oxygen values were converted to C units assuming a respiratory quotient of 1 (del Giorgio and Cole, 1998).

2.3.3. Cell abundance

A volume of 1 mL per experimental unit was taken daily for a week to estimate phytoplankton abundance. Samples of organisms were fixed with Lugol's solution at 2.5 % (final concentration) and 10 μL were pipetted into a Neubauer chamber (American Optical, Buffalo, NY, USA) and counted at 250× using an inverted microscope (Carl Zeiss AX10, LCC, USA).

Accumulated growth rate was calculated from cell abundance as follows:

$$\mu = \frac{LnC_f - LnC_i}{T_f - \quad T_i} \quad (2)$$

Where C is the cell abundance (cell mL^{-1}), at the beginning (day 0, T_i) and at the end of each experimental day (T_f).

2.4. Bioassay with Daphnia magna

One day after the setup of the BPA×Warming experiment with *C. ovata*, we initiated a coupled-growth bioassay using *Daphnia magna* to measure the effect of BPA in zooplankton through food. The strain of *D. magna* used in our experiment originated from an uniclonal stock culture provided by the University of Jaén (Spain), sampled from Laguna Grande (Jaén, Spain). *D. magna* was grown in APHA-reconstituted hard water and maintained under constant conditions of 19ºC, light intensity of 100 μmol photons m^{-2} s^{-1} of PAR, and under a 14:10-hour light/dark photoperiod in the laboratory of the Water Research Institute (University of Granada). This strain was fed with BPA-free *C. ovata* for two months before the start of the experiment.

At the beginning of the zooplankton experiment, neonates of *D. magna* hatched within the previous 24 hours were collected and placed in 3 glass tubes of 100 mL each (1 neonate per tube, 3 tubes per treatment) containing free-BPA APHA medium.

The initial biomass of neonates was measured from five individuals, after being washed with distilled water, in pre-weighed aluminum capsules. The capsules were then placed in an oven at 60°C for 24 hours to evaporate the water and re-weighed (Mettler ultramicrobalance (±0.1 μg, Mettler, Germany)) to determine the individual dry mass of each neonate. Every day, a variable volume of the *C. ovata* culture was harvested from each treatment (C, W, BPA, and BPA_W) to guarantee a consistent daily provision of 1 mg C L^{-1} as sustenance for *Daphnia*. To do so, we estimated the biovolume of *C. ovata* from the geometric shape (prolate spheroid) most similar to the shape of the cell (Hillebrand et al., 1999), and then converted it to carbon units by assuming a

multiplication factor of 0.860 (Menden-Deuer and Lessard, 2000). Additionally, we also included a control treatment without DMSO (C*) to test the absence of a significant effect of the BPA vehicle (DMSO). To determine the effect of BPA in food, each volume of *C. ovata* culture extracted from the experimental units was centrifuged and quickly washed 3 times with APHA medium. Thus, we ensured that the zooplankton's exposure to BPA came exclusively from the food (BPA content in *C. ovata*) and not from the BPA dissolved in the *C. ovata* culture medium or adhered to the phytoplankton cells. Temperature and light conditions were kept constant and identical to the growth conditions of *D. magna* (i.e., 19ºC, 100 μmol photons m^{-2} s^{-1} of PAR, and under a 14:10-hour light/ dark photoperiod) over the zooplankton experiment. The free-BPA APHA medium and the food (*C. ovata* with and without BPA) were replaced daily and gently stirred with a pipette twice a day to resuspend the food. After 5 days, each *D. magna* individual was rinsed in distilled water and placed in a pre-weighed aluminum capsule. As with the initial biomass of neonates, the capsules were dried at 60 °C for 24 hours until reaching a constant weight. Finally, somatic growth rate (*g*) was calculated as follows:

$$g = (\ln M_f - \ln M_0) / t \tag{3}$$

where M_f is the dry mass of each individual at the final time, M0 is the individual dry mass of the neonates, and t is the time (5 days) elapsed between both measurements.

2.5. Numerical methods and statistical analysis

To test the effect of the interaction between BPA and temperature on *C. ovata*, experiments were carried out in triplicate (similar number of replicates for BPA experiments to Theus et al (Theus et al., 2023). and Atengueño-Reyes et al (Atengueño-Reyes et al., 2023)., while the treatments with zooplankton were performed with five replicates, following Álvarez et al (Álvarez et al., 2024).

To assess the magnitude and direction of single and interactive effects of the studied factors, the effect size of BPA, Warming, and their interaction on the biological variables (*Fv/Fm*, *NPP*, *R*, *C. ovata* abundance, and growth rate of *Daphnia magna*) were calculated as follows:

$$BPA\ effect\ size(\%) = \frac{(X_{BPA} - X_C)}{X_C} \times 100 \tag{4}$$

$$Warming\ effect\ size(\%) = \frac{(X_W - X_C)}{X_C} \times 100 \tag{5}$$

$$BPA \times Warming\ effect\ size(\%) = \frac{(X_{BPA_W} - X_C)}{X_C} \times 100 \tag{6}$$

Where X is the response variable measured in each experimental unit, and C, W, BPA, and BPA_W represent the corresponding treatments.

The BPA concentration that inhibits the growth rate of *C. ovata* by 50 % after a 72-hours exposure, Median Effective Concentration ($EC50_{72\ h}$), was determined by nonlinear regression analysis from the growth rate inhibition curve relative to the control (OECD, 2011). To test the effects of the different treatments over time (dependent samples) on the response variables, we chose to perform RM-ANOVA analysis to avoid temporal pseudo replication, whereas for independent samples, we used a t-test analysis (for samples from 2 groups) or ANOVA (for samples from 3 or more groups). This is described below. A one-way RM-ANOVA was used to assess the effect of BPA vehicles (ethanol and DMSO) on the abundance of *C. ovata*, as well as to evaluate the effect of the BPA concentration gradient (0.1–10 mg BPA L^{-1}) on the abundance and growth rate of *C. ovata*. The effects of BPA, temperature (warming), and their interaction over time, as well as their effect size, were assessed for each response variable measured in the phytoplankton experiment (abundance, *NPP*, *R*, and *Fv/Fm*) using a two-way repeated measures analysis of variance (RM-ANOVA). For each RM-ANOVA, the assumption of sphericity was verified through the Mauchly test, and in cases where it was not met, the Geisser-Greenhouse correction was applied. The assumption of normality of residuals was checked using the Kolmogorov-Smirnov test. When interactive effects were found, differences between treatments were evaluated using the Fisher Least Significant Difference (LSD) *post hoc* test.

A *t*-test analysis was conducted to verify the absence of an effect of dimethyl sulfoxide (DMSO) on the growth rate of *D. magna*. A two-way ANOVA was carried out to determine the effect of BPA, warming, and their interaction, as well as their effect size, on *D. magna* growth rate. Homoscedasticity of variances (using Levene's tests) and normality of residuals (by Kolmogorov-Smirnov test) were verified. All statistical analyses were conducted using STATISTICA v7.0 software (Statsoft Inc., 2005).

3. Results

3.1. Selection of a non-toxic BPA vehicle for C. ovata

C. ovata showed a differential sensitivity to the exposure to two BPA vehicles (DMSO and ethanol). Namely, the results showed that *C. ovata* exhibited similar abundances in the control and the DMSO treatment over 7 days (Fig. 2 A; Table S1; p-value = 0.121 post hoc Fisher's LSD for each day). However, when ethanol was used as the vehicle, the abundance of *C. ovata* decreased rapidly from day 1, showing significant differences compared to the control (Fig. 2B; Table S1; p-value < 0.001 post hoc Fisher's LSD for each time). Therefore, DMSO was selected as BPA vehicle for our subsequent experiments with *C. ovata.*

3.2. Sensitivity of C. ovata to an increasing BPA concentration gradient

Our results showed that treatments with BPA concentrations up to 1 mg L^{-1} did not negatively affect *C. ovata.* In fact, treatment of 0.5 mg BPA L^{-1} exerted a positive effect on *C. ovata* cell abundance (p-value = 0.011, post hoc Fisher's LSD test) on day 4 compared to the Control or Control-DMSO treatments (Fig. 3A; Table S2). However, treatments with 5 and 10 mg of BPA L^{-1} showed lower growth rate values and cellular abundance compared to the Control+DMSO treatment from day 2, inducing even negative accumulated growth rates in the case of the 10 mg BPA L-1 treatment every day of the experiment (Fig. 2B). Therefore, the treatment with 5 mg BPA L^{-1} was identified as the minimum concentration studied in this work necessary to induce negative effects on *C. ovata*, (Fig. 3A,B; Table S2; From day 2 onwards, all p-values were < 0.001, post hoc Fisher's LSD test).

3.3. Sensitivity of C. ovata to BPA × Warming interaction

The maximum photosynthetic efficiency (*Fv/Fm*) of *C. ovata* initially exhibited values between 0.65 and 0.69 across all experimental treatments (day 1, Fig. 4A). Both the control (C) and warming (W) treatments maintained similar *Fv/Fm* values throughout the experiment, indicating the absence of a significant warming effect on photosynthetic efficiency (p-value > 0.05, post hoc Fisher's LSD; Fig. 4A,B). However, both BPA treatments (BPA and BPA_w) had a detrimental effect on the *Fv/Fm* of *C. ovata* (p-value < 0.0001 for BPA, and < 0.001 for BPA_W; see Table S3). This negative effect was initially more pronounced under warming conditions (BPA_W treatment), with *Fv/Fm* values of 0.3 on day 2, compared to values of 0.57 in the ambient temperature BPA treatment (p-value = 0.0001, post hoc Fisher's LSD; Fig. 4A). However, both BPA treatments showed very low values of *Fv/Fm* at the end of the experiment (p-value > 0.05, post hoc Fisher's LSD; Fig. 4A). Consequently, the effect size revealed an inhibitory effect > 20 % of the BPA and BPA × Warming interaction from day 2 until the end of the experiment, when the inhibitory effect surpassed 80 % (Fig. 4B).

During the 7-day experiment, the *NPP* in the control (C) and warming (W) treatments consistently showed positive rates, while, in

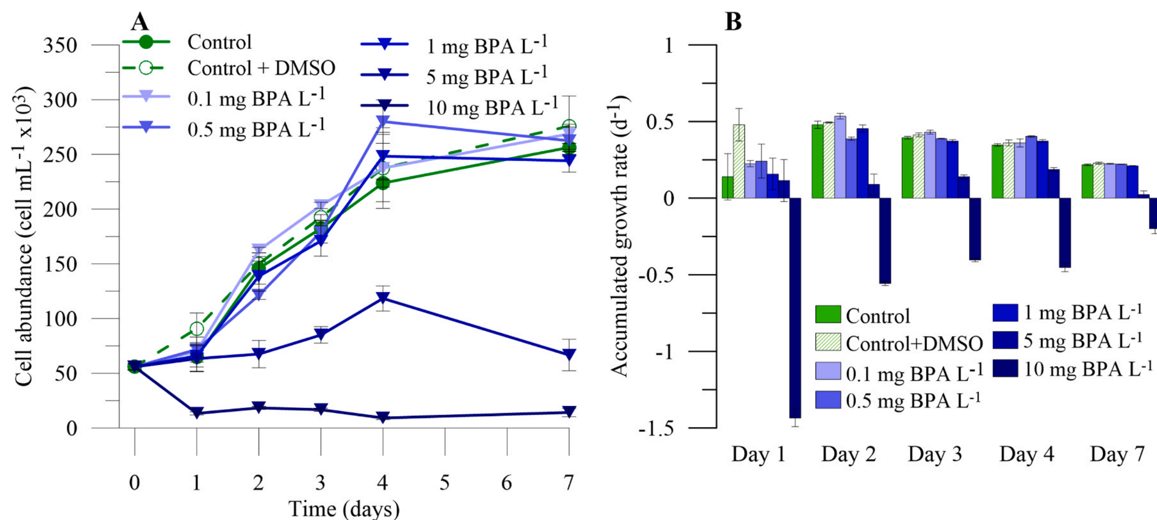


Fig. 3. (A) Cell abundance (cell mL^{-1}), and (B) accumulated growth rate (d^{-1}) of *C. ovata* exposed to bisphenol A gradient (0–10 mg L^{-1}) over 7 days of experiment. Data are expressed as mean values ± SD (n = 3).

general, both BPA treatments exhibited negative ones (Fig. 4C). The W treatment showed values similar to the C treatment throughout the experiment, except on day 2, where W was slight- but significantly higher than C (p-value = 0.019, post hoc Fisher's LSD; Fig. 4C). Both BPA treatments exerted a clear negative effect on *NPP* (p-value < 0.0001, see Table S3; Fig. 4 C). The BPA_W treatment showed higher, even positive, production values on the first day compared to the BPA treatment (p-value < 0.0001, post hoc Fisher's LSD). However, these rates dropped significantly on days 2 and 4, reaching the lowest values across the entire experiment (p-value < 0.0001, post hoc Fisher's LSD; Fig. 4C).

The analysis of the effect size revealed that Warming had a stimulatory effect on net primary production during the first two days, which then reversed to an inhibitory effect on days 4 and 7 (Fig. 4D). Regarding the BPA factor, its effect was always inhibitory, but it was lower when BPA interacted with Warming (BPA × Warming) at the beginning of the experiment (day 1). However, it increased drastically on days 2 and 4. By the end of the experiment (day 7), the inhibitory effect of BPA and BPA × Warming became analogous (Fig. 4D).

The respiration of *C. ovata* remained similar throughout the experiment in the treatments without BPA, i.e. C and W (p-value > 0.05, post hoc Fisher's LSD; Fig. 4E), although the effect size analysis revealed a general increase in respiration under warming (W) conditions (Fig. 4F). The BPA treatment displayed the highest respiration values of all treatments on day 2 (p-value < 0.01, post hoc Fisher's LSD; Fig. 4E) but gradually decreased to reach the lowest values, alongside the BPA_W treatment, on day 7. In contrast, respiration values in the BPA_W treatment were similar to those of the other treatments on day 1 but were the lowest from day 2 until the end of the experiment.

The effect size analysis showed that BPA stimulated respiration until day 4 but strongly inhibited it on day 7 (Fig. 4F). The BPA × Warming interaction also stimulated respiration on day 1 but then began to inhibit it from day 2, showing inhibitory values on day 2 and 4 (p-value < 0.05, post hoc Fisher's LSD), and the highest inhibition, similar to BPA treatment, at the end of the experiment (Fig. 4F).

The abundance of *C. ovata* in the BPA-free treatments (C and W) remained between 1×10^5 and 2×10^5 cells mL^{-1} throughout the experiment (Fig. 5A), whereas treatments with BPA (BPA and BPA_W) showed lower abundance values compared to the BPA-free treatments (p-value < 0.0001, see Table S3; Fig. 5A). Although BPA and BPA_W showed similar abundance values at the end of the experiment, the temperature increase accelerated by two days the negative effect of BPA (in BPA_W treatment) with very low abundance values from day 3 of the experiment (Fig. 5A).

The analysis of effect size on cell abundance showed that W and BPA_W treatments exerted a stimulatory effect on day 1, but it was reverted to inhibitory from day 2 onwards (Fig. 5B). Despite the constant inhibitory effect of BPA on cell abundance, the BPA_W treatment showed a constant and powerful (>80 %) inhibitory effect from day 3 onwards (Fig. 5B).

The accumulated growth rate showed a similar pattern to that of cell abundance, with both BPA treatments exhibiting decreasing rates, while BPA-free treatments consistently showed positive or near-zero rates (Fig. 6). The 5ºC temperature increase led to higher growth rates at the beginning of the experiment in both BPA and BPA-free treatments, i.e. in W and BPA_W (p-value < 0.001, see Table S3). However, from day 2 until the end of the experiment, warming (W and BPA_W) resulted in lower growth rates, with the BPA_W treatment showing the lowest growth values for all treatments from day 3 onward (p-value < 0.001, post hoc Fisher's LSD, see Table S3, Fig. 6).

3.4. *Effect of BPA contained in C. ovata on Daphnia magna*

Our results show that there were not significant differences in the growth rate of *D. magna* fed with *C. ovata* from cultures with (C) or without DMSO (C*), thus ruling out a toxic effect of the BPA vehicle (p-value = 0.75, t-test analysis). Also, the growth rates of *D. magna* indicate that there were no significant differences between C, W and BPA treatments (p-value > 0.05, post hoc Fisher's LSD; Fig. 7A; Table S4). However, the BPA_W treatment showed a marginally significant lower growth rate compared to treatment C (p-value = 0.053, post hoc Fisher's LSD) and significantly lower than treatment W (p-value = 0.003, Fig. 7A). Effect size analysis showed a positive effect size of the food (*C. ovata*) cultured under warming conditions on the growth rate of *D. magna* (Fig. 7B). Conversely, the effect size of BPA and BPA × Warming on growth rate of *D. magna* was negative, although without significant differences between them (p-value = 0.18, post hoc Fisher's LSD; Fig. 7B).

4. Discussion

The assessment of the toxicity of emerging contaminants on organisms located at the base of food webs in a global change context is necessary to characterize the risk to these populations as well as their potential transfer throughout the food web. This study provides new information on how the effect of a chemical stressor (BPA) can be enhanced under future conditions of higher temperature, altering the performance of phytoplankton and zooplankton. For the first time, we

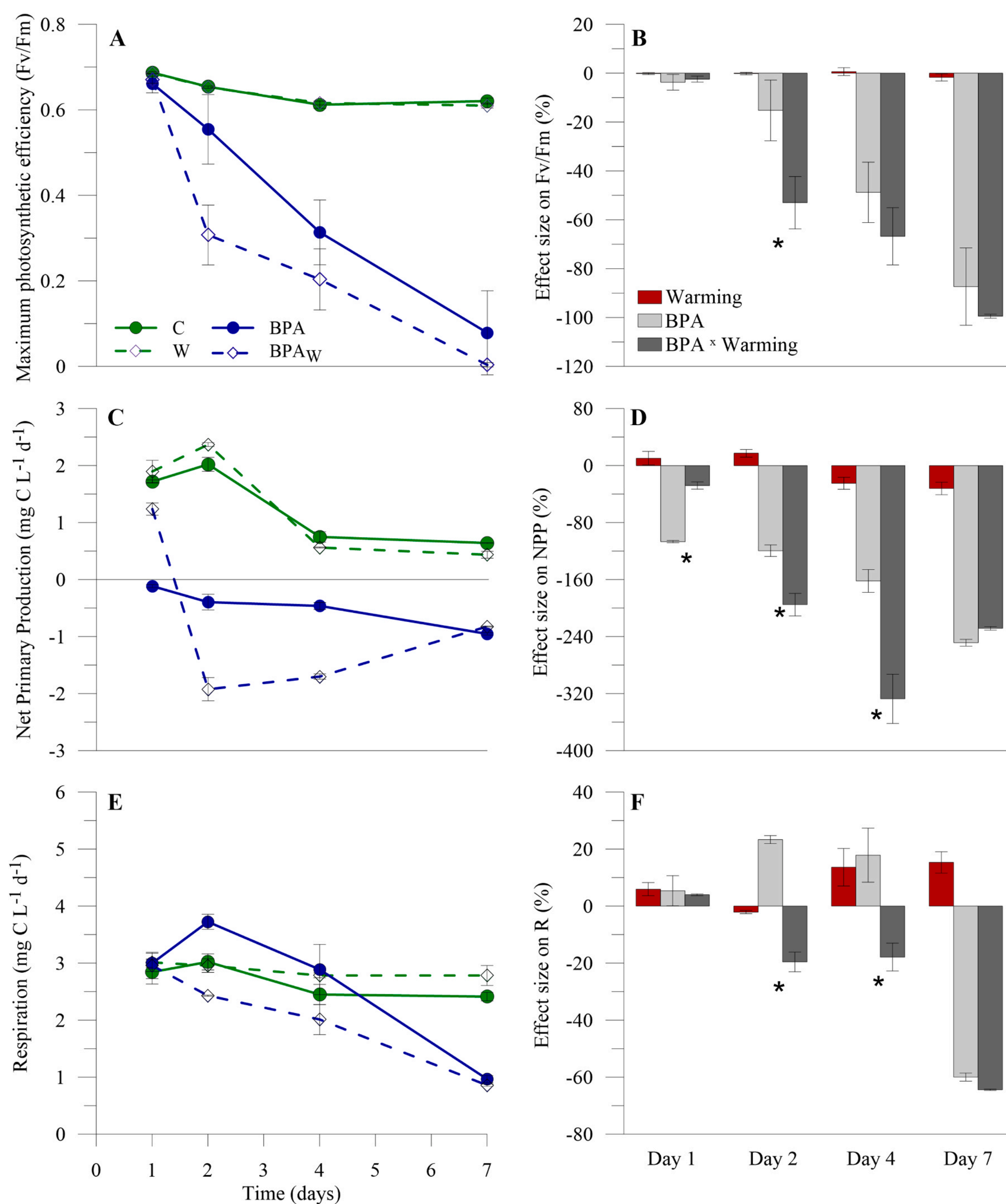


Fig. 4. Maximum photosynthetic efficiency (*Fv/Fm*), Net primary production (*NPP*) and Respiration (*R*), and for the different treatments and their corresponding effect sizes. *Fv/Fm* (A), *NPP* (C), and *R* (E) values are represented under the different treatments (Control treatment, (19ºC) [C]; Warming treatment, (24ºC) [W]; bisphenol A (BPA) treatment (5 mg BPA L^{-1}), [BPA]; and BPA×Warming interaction (24ºC and 5 mg BPA L^{-1}), [BPA_W]). Effect size of warming, BPA and BPA×Warming interaction on *Fv/Fm* (B), *NPP* (D), and *R* (F) of *C. ovata*, over 7 days of experiment. The asterisks indicate significant differences ($p<0.05$) between the effect size of BPA and BPA×Warming. Data are expressed as mean values ± SD (n = 3).

determine the physiological and metabolic response that allows us to understand the mechanisms underlying the population response of a ubiquitous phytoplankton species (*C. ovata*, (Choi et al., 2013)) exposed to the interaction of BPA and warming. Although environmental reality is more complex and the concentrations present in surface waters are lower than those used in this study, the results discussed here provide insights on how predicted increases in temperature modify the toxicity of BPA on phytoplankton, and alter the transfer of their effect to higher levels in the food web.

4.1. Sensitivity of *C. ovata* to BPA

Our initial assay using a gradient of BPA showed that concentrations of BPA <1 mg BPA L^{-1} did not exert a toxic effect on the abundance and growth rate of *C. ovata* after 7 days of incubation. Indeed, the observed positive effect of BPA at a low concentration (0.5 mg BPA L^{-1}) in our experiment could be related to a phenomenon known as hormesis, which has been previously documented in the literature (Calabrese and Baldwin, 2003), and that reflects the stimulatory effect of toxics substances on organisms at low doses. This could be attributed to the ability

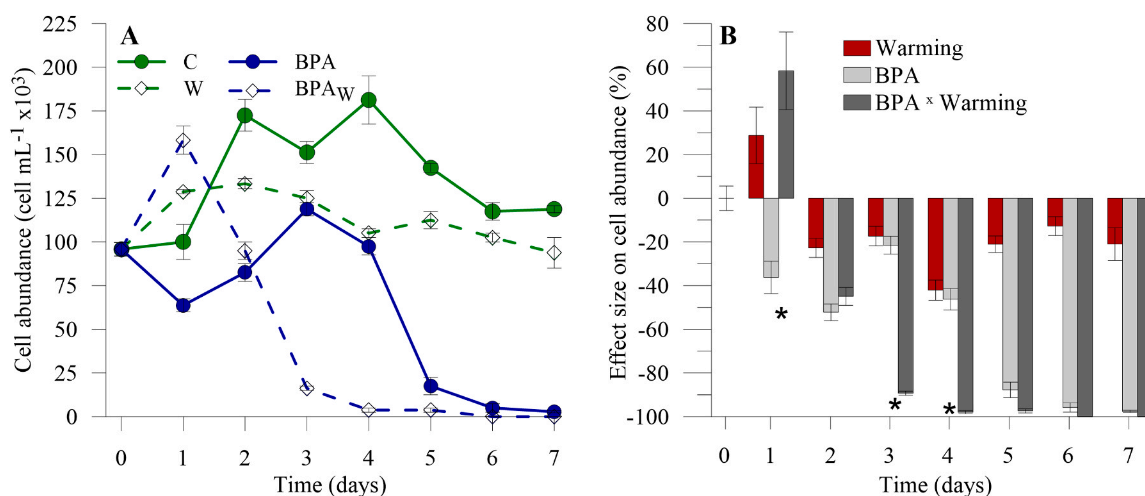


Fig. 5. Cell abundance (A) under different treatments and the corresponding effect size (B). Data are represented for the different treatments (Control treatment, (19ºC) [C]; Warming treatment, (24ºC) [W]; bisphenol A (BPA) treatment (5 mg BPA L^{-1}), [BPA]; and BPA×Warming interaction (24ºC and 5 mg BPA L^{-1}). Effect size of warming, BPA and BPA×Warming interaction on cell abundance of *C. ovata*, over 7 days of experiment. The asterisks indicate significant differences ($p<0.05$) between the effect size of BPA and BPA×Warming. Data are expressed as mean values ± SD (n = 3).

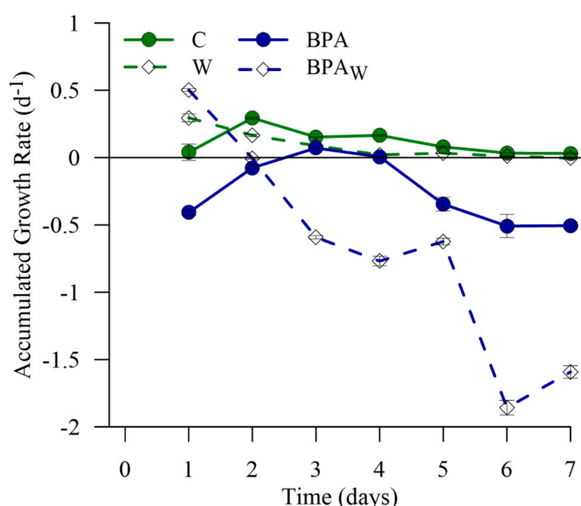


Fig. 6. Accumulated growth rate (GR) of *C. ovata* under different treatments. Data are represented for Control treatment, (19ºC) [C]; Warming treatment, (24ºC) [W]; bisphenol A (BPA) treatment (5 mg BPA L^{-1}), [BPA]; and BPA×Warming interaction (24ºC and 5 mg BPA L^{-1}) over 7 days of experiment. Data are expressed as mean values ± SD (n = 3).

of certain phytoplankton groups known as mixotrophs, among which are cryptophytes (Calderini et al., 2022), to use dissolved organic compounds as a source of carbon. In this regard, several microalgae have been reported to utilize phenol (subproduct derived from BPA) as a carbon source, such as *Chlorella pyrenoidosa* (Das et al., 2015) or *Isochrysis galbana* (Wang et al., 2019).

Thus, a concentration of 5 mg BPA L^{-1}, mimicking extreme pollution conditions after a BPA spill, was necessary to find significant negative effects of BPA on phytoplankton, over a short time scale (up to 7 days). On the other hand, a concentration of 10 mg BPA L^{-1} led to a total inhibition of phytoplankton growth. Our results show toxicity of BPA on *C. ovata* ($EC50_{72\,h}$ = 4.11 mg L^{-1}) similar to that reported for other phytoplankton species such as *C. vulgaris* ($EC50_{72\,h}$ = 3.32 mg L^{-1}) or *Desmodesmus subspicatus* ($EC50_{72\,h}$ = 6.48 mg L^{-1}) (Turan and Çakal Arslan, 2023). The toxicity of the phenolic compounds could arise from their ability to penetrate cells and the hydrophobic interaction with the cell membrane and membrane-bound organelles, as well as the formation of free radicals and reactive oxygen species (Michałowicz and Duda, 2007). All together can lead to disruption of the cell structure and function (Scragg, 2006). On the contrary, other eight phytoplankton species showed lower sensitivity to BPA, displaying no negative effects of BPA on their growth under conditions of 5 mg BPA L^{-1} (Nakajima et al., 2007).

4.2. Sensitivity of *C. ovata* to BPA × Warming

Since our objective was to determine how warming alters the toxic effect of BPA, it was necessary to work with a minimal BPA concentration that exerts a significant negative effect (i.e., 5 mg L^{-1} in our previous assay). However, it is important to underscore that while concentrations below 1 mg BPA L^{-1} did not manifest a significant impact on *C. ovata*, the presence of non-monotonic responses associated with exposure to endocrine disruptors, including BPA (Hill et al., 2018), does not exclude the toxicity of this compound at concentrations lower than those detected in our study and others. Also, additional investigations into the long-term toxicity of low BPA concentrations are necessary, as they could potentially have delayed effects both on phytoplankton and other organisms (Chen et al., 2015). On the other hand, it is possible that the growth of *C. ovata* at concentrations <1 mg BPA L^{-1} is due to a certain capacity to biodegrade and/or bioaccumulate BPA without being negatively affected, as has been reported for other phytoplankton species (e.g., (Ji et al., 2014)).

Our results demonstrate that exposure of *C. ovata* to 5 mg BPA L^{-1} had an inhibitory effect on its photosynthetic efficiency and showed negative values of net primary production. A previous study on *Monoraphidium braunii* showed that BPA was able of disrupting photosynthesis, thereby reducing the net efficiency of Chl-*a* and photosystem II performance (Gattullo et al., 2012). Other authors have also found that BPA inhibits cell division, promotes cell wall decomposition and disintegration of chlorophyll molecules (Li et al., 2009), and suppresses CO_2 assimilation and over-reduces the electron transport chain in plants (Li et al., 2018). Prolonged exposure to contaminants such as BPA induces an imbalance between reactive oxygen species (ROS) and antioxidants, culminating in the accumulation of ROS within chlorophyll-containing cells, thereby disrupting normal cellular functions and potentially leading to cell death (Yang et al., 2020). This could explain why BPA-induced stress stimulated the respiration of *C. ovata* at the beginning of the experiment ($p<0.05$ for day 2 and day 4), as a mechanism to compensate for the stress experienced (Noctor, 2006). All of this

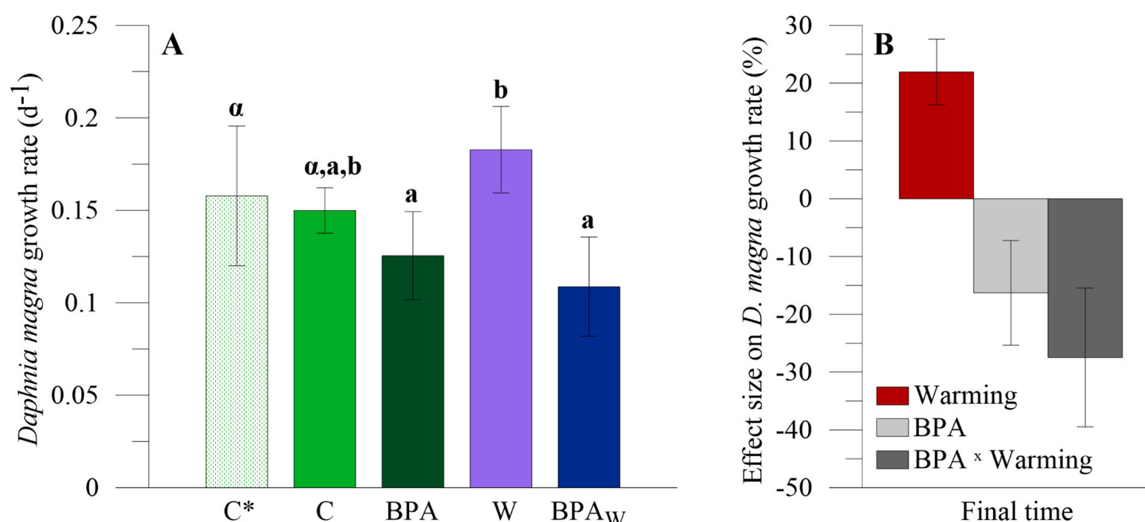


Fig. 7. Growth rate (A) of *Daphnia magna* under different treatments and the corresponding effect size (B). Data are represented for Control without dimethyl sulfoxide treatment [C*]; Control treatment, (19ºC) [C]; Warming treatment, (24ºC) [W]; bisphenol A (BPA) treatment (5 mg BPA L^{-1}), [BPA]; and BPA×Warming interaction (24ºC and 5 mg BPA L^{-1}). Effect size of warming, BPA and BPA×Warming interaction on growth rate of *Daphnia magna* was calculated at final time. Data are expressed as mean values ± SD (n = 3). Greek letters indicate the differences between the two control treatments (*t*-test analysis), while Latin alphabet letters show the differences from the *post-hoc* Fisher's LSD analysis between treatments.

translates into an inhibitory effect of BPA on the abundance of *C. ovata* and negative growth rates.

The 5°C increase in our experiment falls within the growth ranges of *C. ovata* (Weisse et al., 2016), as evidenced by the positive values of *NPP* and growth rate registered in the W treatment. However, the warming treatment showed lower values of *NPP* and growth rate compared to the control treatment (i.e., a negative effect), accompanied by an increase of respiration rate at the end of the experiment. This could be attributed to the fact that the warming exceeded the optimal growth temperature of our species under these conditions. Indeed, recent studies have shown that *C. ovata* exhibited a peak carbon production around 20°C (Montagnes et al., 2008), while Bergkemper and Weisse (Bergkemper and Weisse, 2017) observed a decline in cryptophytes abundance in an oligo-mesotrophic Austrian lake when water temperature reached 22°C–27°C. In this sense, our study contributes to improving the understanding of how the increasingly frequent and intense heatwaves, which expose organisms to temperatures above their optimal growth range, could impact the metabolism and growth of phytoplankton when exposed to chemical contaminants, such as BPA. Our study also highlights the need for additional, more realistic studies that assess the toxicity of BPA across a range of temperature variations.

Strikingly, our results revealed that the detrimental impact of *C. ovata* exposure to 5 mg BPA L^{-1} was accelerated under projected higher temperature conditions. Thus, the BPA×W interaction was negative synergistic, leading to the lowest values of photosynthetic efficiency, metabolic activity (*NPP* and Respiration), and accumulated growth rate. Limited research has explored how future temperature conditions may alter the ultimate impact of BPA on aquatic organisms. A thorough review has revealed that only a few articles have examined this interactive effect, and only one of them analyzed its impact on the composition of the phytoplankton community. Contrary to our findings, Theus et al (Theus et al., 2023). reported an antagonistic effect between both factors on the growth of three species of chlorophytes. Other studies in animals have shown that spermatogenesis and heart rate in two species of fish (*Astyanax bimaculatus* and Zebrafish) were more sensitive to the effect of BPA at lower temperatures (Ribeiro et al., 2021; Little and Seebacher, 2015). Nevertheless, synergisms seem to be common in nature and aquatic ecosystems (Villar-Argaiz et al., 2018), and in organisms like zebrafish it was clear that the increase in temperature can exacerbate the negative effects caused by endocrine disruptors (e.g., (Cardoso et al., 2017)). It is possible that the temperature increase accelerates many processes, such as uptake rate (Brown et al., 2004), which expose phytoplankton to higher internal doses of toxic compounds. Hence, under a global-change context, the impact of the interaction between contaminants (like BPA) and warming on organisms and ecosystems is a current hot-spot of research which warrants the need of more future studies.

4.3. *Transfer of the effects of BPA to zooplankton via herbivory*

Aquatic organisms can bioaccumulate contaminants via direct uptake from the water column or through dietary intake. As far as our knowledge goes, only a recent study has investigated the impact of Bisphenol A (BPA) on *Daphnia* through its diet (Álvarez et al., 2024). This investigation revealed that the main route of BPA assimilation in *D. magna* was through food with *Scenedesmus* sp. that had previously ingested BPA, exerting a negative effect on *D. magna* growth rate from concentrations higher than 0.65 mg BPA L^{-1}. However, our findings have shown that the adverse effects of BPA on physiological, metabolic, and growth variables measured in the primary producer only transferred to a toxic effect on the growth rate of *D. magna* (consumer) under jointly BPA and warming conditions. This result highlights the pressing need to assess how the different species composition of the phytoplankton community under warming conditions can influence the transfer of contaminants effects to higher trophic levels. Additionally, it is still unclear how temperature can increase the toxic effect of a contaminant through diet or even how contaminants can alter sensitivity to temperature. Thus, an increased vulnerability to temperature changes induced after a previous exposure to chemical stressors has recently been reported for zooplankton *D. magna (*López-Valcárcel et al., 2024*)*. On the other hand, some authors have mentioned that temperature increase leads to homeostatic, physiological, and clearance rate changes which could have led to higher contaminant ingestion and production of toxic metabolites in *Daphnia* sp. (Noyes et al., 2009; Kim et al., 2010)., and that these could also be transferred to higher trophic levels through feeding.

5. Conclusion

In our study, we show how environmental conditions (i.e., warming)

greatly influence the fitness of a phytoplankton species exposed to BPA, from the subcellular to cellular level, and how the effects on phytoplankton are transferred to primary consumers. Our results have shown that in a high-temperature scenario, the toxic effects of high concentrations of BPA were accelerated, having negative consequences on the physiological activity, metabolism, and survival of some phytoplankton species and their predators. Under projected scenarios of global change, the constant exposure to higher temperature and BPA concentrations in aquatic ecosystems can have negative consequences on the physiological activity, metabolism, and survival of *C. ovata* and growth rate of *D. magna*. In this regard, it is important to note that the ecotoxicological impact of BPA on phytoplankton is species-dependent, and therefore, the transfer of the effects to higher trophic levels will depend on the specific composition of the phytoplankton community.

Although the bioaccumulation capacity of BPA by *C. ovata* was not determined in our study, its ability to grow at concentrations higher than 1 mg BPA L^{-1} opens the door to future research on its potential bioremediation capabilities.

Finally, our results can be useful to support policy-making and decision-making in a rapidly changing global context, where the information available to environmental lawmakers regarding industrial chemicals and their effects on health and the environment is still limited.

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CRediT authorship contribution statement

Juan Manuel González Olalla: Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **María Vila Duplá:** Writing – review & editing, Methodology, Investigation, Data curation, Conceptualization. **Marco J Cabrerizo:** Writing – review & editing, Validation, Conceptualization. **Irene González Egea:** Writing – review & editing, Investigation, Formal analysis. **Gema Parra:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Juan Manuel Medina Sánchez:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **Presentación Carrillo:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Juan Manuel Medina Sanchez reports financial support was provided by Spain Ministry of Science and Innovation. Presentacion Carrillo reports financial support was provided by Government of Andalusia Ministry of Economic Transformation Industry Knowledge and Universities. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2024.117090.

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