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Long-term adverse effects of microplastics on *Daphnia magna* reproduction and population growth rate at increased water temperature and light intensity: Combined effects of stressors and interactions

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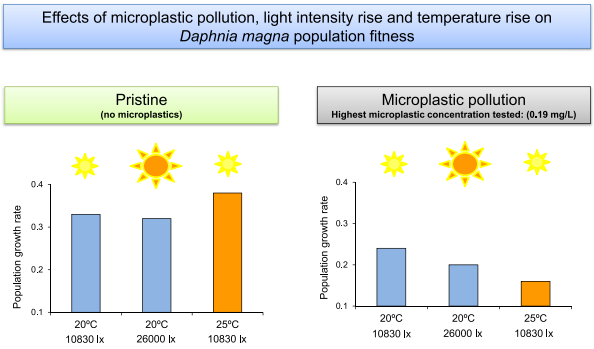
HIGHLIGHTS

- 21-D exposure to microplastics (MP) ≥0.09 mg/L reduced *D. magna* population fitness.
- At 20 °C, MP reduced the population fitness up to 27% (10,830 lx) or 38% (26,000 lx).
- At 25 °C/10830 lx, MP decreased the population fitness up to 59%.
- MP effects increased with light intensity (1.4 x) and temperature (2.2 x) rise.

GRAPHICAL ABSTRACT



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ABSTRACT

In many ecosystems, the zooplankton community has been pressured simultaneously by microplastic pollution and alterations resulting from global climate changes. The potential influence of light intensity rise (from 10,830 lx to 26,000 lx) and water temperature rise (from 20 °C to 25 °C) on the long term-toxicity of microplastics (MPs) to *Daphnia magna* were investigated. Three 21-day laboratory bioassays with model MPs (1–5 μm diameter) were carried out at (i) 20 °C/10830 lx, (ii) 20 °C/26000 lx, and (iii) 25 °C/10830 lx. In each bioassay, one control (no MPs) and three MP concentrations (0.04, 0.09, 0.19 mg/L) were tested. In all the bioassays, MPs caused parental and juvenile mortality, and reduced the somatic growth, reproduction and population growth rate. The MP EC_{50}s on living offspring (95% confidence interval within brackets) were 0.146 mg/L (0.142–0.151 mg/L) at 20 °C/10830 lx, 0.102 mg/L (0.099–0.105 mg/L) at 20 °C/26000 lx, and 0.101 mg/L (0.098–0.104 mg/L) at 25 °C/10830 lx. Relatively to the respective control group, 0.19 mg/L of MPs decreased the mean of the population growth rate by 27% at 20 °C/10830 lx, 38% at 20 °C/26000 lx and 59% at 25 °C/10830 lx. Based on the population growth rate and in relation to 20 °C/10830 lx (control, no MPs), the interaction between increased light intensity (26,000 lx) and MPs was synergism (at all the MP concentrations tested). The interaction between water temperature rise (25 °C) and MPs was antagonism at 0.04 mg/L of MPs and synergism at 0.09 and 0.19 mg/L of MPs. In the present scenario of climate changes and global MP pollution such findings raise high concern because

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zooplankton communities are crucial for aquatic biodiversity conservation, ecosystem functioning and services provided to humans. Further studies on the combined effects of MPs, other common pollutants, and alterations due to climate changes are needed.

1. Introduction

The global plastic pollution has been increasing over decades and such trend will be likely enhanced by consequences of the SARS-CoV-2 pandemic (Canning-Clode et al., 2020), mainly due to the widespread and intensive use of plastic materials, inadequate disposal after use, and limited capability of dealing with increased volumes of waste.

The pollution of aquatic ecosystems by microplastics (MPs), plastic particles with size lower than 5 mm, is a growing threat to aquatic biodiversity, ecosystem function, and services provided. MPs are widespread in aquatic ecosystems, have considerable environmental persistence, and their pollution may have ecological consequences (Du et al., 2020; Ma et al., 2020). MPs are incorporated by organisms (Jemec et al., 2016; Barboza et al., 2020) entering into food chains together with the other chemicals that microplastic particles transport adhered to their surfaces (Hahladakis et al., 2018; Hanslink et al., 2020), where they can be accumulated and transferred from prey to predators (Carbery et al., 2018). MPs can cause adverse effects in animals at different levels of biological organization (Sadler et al., 2019) and over generations (Martins and Guilhermino, 2018; Schür et al., 2020). As many aquatic organisms are used as food source to humans, their contamination by MPs is also a threat to human food safety and health (Miranda et al., 2019; Barboza et al., 2020).

The paradigm of MP pollution has been studied mainly in relation to the marine environment but the number of studies in freshwater organisms and ecosystems has been increasing considerably (Yao et al., 2020). In general, the levels of MPs in freshwaters are low (Koelmans et al., 2019; Picó and Barcelò, 2019; Xu et al., 2020). Nevertheless, considerable abundance of MPs, such as mean density of 20,264 particles per Km^{-2} in a large remote mountain lake (Free et al., 2014) and mean concentrations of 1.56 ± 1.64 mg/L in some urban lakes receiving treated wastewater effluents (Lasee et al., 2016) have been also documented. Moreover, plastic additives have been also detected in freshwater ecosystems, in some cases with environmental risk (Bolívar-Subirats et al., 2021).

In addition to MPs and other types of pollution, many freshwater ecosystems have been also impacted by global climate changes. Increase of water temperature, light intensity, and frequency of extreme events are examples of main driving pressures acting together with MPs on the biota, including on the zooplankton community that is crucial for aquatic ecosystem functioning. Cladoceran populations are important components of freshwater zooplankton communities. Along their evolution, they developed several types of responses and strategies to deal with environmental changes (Hoefnagel et al., 2018; Gust et al., 2019; Adamczuk, 2020). Nevertheless, dealing with the effects of multiple stressors is challenging and energy demanding, especially under strong and relatively rapid pressure.

In many freshwater ecosystems across the world, the cladoceran *Daphnia magna* is a keystone species. Shallow water ecosystems that are preferential habitats for this species due to reduced predation pressure (Giebelhausen and Lampert, 2001) are particularly vulnerable to the effects of climate changes and MP pollution. *D. magna* ingests a wide range of MPs (Aljaibachi and Callaghan, 2018; Frydkjær et al., 2017; Colomer et al., 2019; Elizalde-Veázquez et al., 2020), including relatively large fibres (Jemec et al., 2016). Some MPs are egested (Frydkjær et al., 2017) but others are internalized and cause toxic effects, such as mortality (Na et al., 2021), decreased reproduction (Besseling et al., 2014; Ogonowski et al., 2016; Pacheco et al., 2018), reduced population fitness and other effects over generations (Martins and Guilhermino, 2018; Schür et al., 2020). MPs are able to influence the bioaccumulation and toxicity of other environmental contaminants of concern to several organisms (Luis et al., 2015; Barboza et al., 2018; Guilhermino et al., 2018) and such interactions have been also documented in *D. magna* (Ma et al., 2016; Kim et al., 2017; Pacheco et al., 2018; Zocchi and Sommaruga, 2019).

Temperature rise influences the effects of MPs on *D. magna* (Sadler et al., 2019; Serra et al., 2020) and other organisms (Fonte et al., 2016) but the knowledge on this topic is still limited. This is a very relevant issue in relation to *D. magna* and other zooplankton species. Indeed, in the wild, temperature and light intensity that change with latitude are important drivers of their populations. Moreover, the rise of water temperature and light intensity that has been occurring in many ecosystems due to global climate changes may also modulate zooplankton populations, especially in shallow water ecosystems where the alterations have been generally greater that in deeper ones.

The objectives of the present study were to investigate the effects of light intensity and water temperature rise on the long-term toxicity of MPs to *D. magna*. To the best of our knowledge, the combined effects of light intensity rise and long-term exposure to MPs in *D. magna* are not known. The combined effects of temperature and MPs were previously investigated in *D. magna* (Sadler et al., 2019; Serra et al., 2020) but the knowledge is still very limited especially considering the great diversity of MPs, and the wide range of temperature variation within the vast area of *D. magna* geographical distribution. The following null hypotheses were tested: H_{01} - The rise of light intensity from 10,830 lx to 26,000 lx does not influence the effects of MPs on *D. magna* population fitness; H_{02} - The rise of water temperature from 20 °C to 25 °C does not influence the effects of MPs on *D. magna* population fitness.

2. Material and methods

2.1. Chemicals

The MPs tested were red fluorescent polymer microspheres from Cospheric Innovations in Microtechnology (U.S.A.), provided as dry powder (company reference: FMR-1.3 1–5 μm). According to the manufacturer, MPs have 1–5 μm of diameter, density of 1.3 g/cm^3, excitation wavelength of 575 nm, emission wavelength of 607 nm, their complete chemical profile is not available, and 1 mg of the product contains about 1.836E+8 spheres (estimate based on 2 μm average particle diameter). These MPs were selected because their size is in the low micro-scale, their fluorescence allows the quantification of MP concentrations in test medium using simple methods, their basic characterization was previously done, their behaviour over 48 h in the test medium used in the bioassays is known, and they caused chronic toxicity and transgenerational effects in *D. magna* (Martins and Guilhermino, 2018; Pacheco et al., 2018).

2.2. Abiotic factors tested

The abiotic factors tested were water temperature and light intensity. They were selected mainly due to their relevance in aquatic ecosystems, including in relation to global climate changes, and their ability to influence *D. magna* fitness (Giebelhausen and Lampert, 2001; Martins et al., 2013; Serra et al., 2019). Moreover, water temperature rise increases the toxicity of some MPs to *D. magna* (Sadler et al., 2019) and other species (Fonte et al., 2016).

The light intensities of 10,830 lx and 26,000 lx, hereafter indicated as moderate light (M-light) and high light (H-light), respectively, were tested. Both light intensities are in the range of natural variation, as well as the photoperiod of 16 h light (L): 8 h dark (D) that was used (Sellers, 1965; Forsythe et al., 1995).

The water temperatures tested were 20 °C and 25 °C which are common water temperatures in many aquatic systems, including in European ecosystems inhabited by *D. magna* and other daphnids in late spring and summer (Mitchell and Lampert, 2000; Castro et al., 2004).

As previously indicated, the null hypotheses tested were: H_{01} - The rise of light intensity from 10,830 lx to 26,000 lx does not influence the effects of MPs on *D. magna* population fitness; H_{02} - The rise of water temperature from 20 °C to 25 °C does not influence the effects of MPs on *D. magna* population fitness. The alternative hypotheses were: H_{A1} - The rise of light intensity from 10,830 lx to 26,000 lx influences the effects of MPs on *D. magna* population fitness; H_{A2} - The rise of water temperature from 20 °C to 25 °C influences the effects of MPs on *D. magna* population fitness.

2.3. Organisms, microplastic ingestion and acclimation conditions

The tested species was *Daphnia magna* Straus (clone A *sensus* Baird et al., 1989a) from laboratory cultures (ECOTOX - Laboratory of Ecotoxicology and Ecology of the Institute of Biomedical Sciences of Abel Salazar of the University of Porto) maintained in parthenogenetic reproduction as described in Martins and Guilhermino (2018). Briefly, females were maintained at water temperature of 20 ± 1 °C, photoperiod of 16 h light: 8 h dark, 10,830 lx. The culture medium was the American Society for Testing and Materials hard water (ASTM, 1980), enriched with 4 mL/L of *Ascophyllum nodosum* extract (Baird et al., 1989b) and vitamins (Bradley et al., 1993), hereafter indicated as test medium because it was also used in the bioassays. Food was *Chlorella vulgaris* provided every day from Monday to Friday, 3×10^5 cells/mL/female.

A first trial to investigate if the tested MPs were ingested by *D. magna* was conducted. Briefly, adult females were exposed individually to test medium containing a concentration of 160 mg/L of MPs. After 30 min, each female was observed in a stereomicroscope (Leica S9i) with an integrated camera (IC80 HD) and pictures were taken.

D. magna acclimation to the test conditions was carried out in chambers (Bronson PGC 1400, Netherlands) with control of temperature, light intensity and photoperiod (16 h light: 8 h dark). The light was from compact fluorescent lamps (Sylvania Lightning, Lynx CF-LE 55W/840), cool white, frosted/coated, luminous flux 4700 and 4000 K that emit low UV radiation. From parental cultures, 3rd brood juvenile females (> 6 h, < 24 h old) were isolated, maintained in glass beakers with 100 mL of test medium (1 female per beaker), and feed as indicated for parental cultures. When they produced the 3rd brood, juvenile females (> 6 h, < 24 h old) were isolated and divided into three groups for acclimation: one group (G1) was maintained at water temperature of 20 ± 1 °C and 10,830 lx; another group (G2) was maintained at water temperature of 20 ± 1 °C and 26,000 lx; the third group (G3) was maintained at water temperature of 25 ± 1 °C and 10,830 lx.

G1, G2 and G3 females were maintained in these conditions of light and water temperature for 3 generations, individually (i.e. one female per beaker) in 100 mL glass beakers containing 50 mL of test medium. They were feed daily (3×10^5 cells/mL/female of *C. vulgaris*). The other abiotic conditions were as previously indicated. Third brood juvenile females produced by the 3rd generation of females were used in the bioassays according their acclimation conditions.

2.4. Bioassays

Bioassays were carried out according the OECD guideline 211 (OECD, 2012) with punctual changes. They were carried out in a Bronson PGC 1400 chamber (Netherlands) with control of photoperiod (16 h light: 8 h dark; light provided by the compact fluorescent lamps previously indicated) and temperature. Three bioassays were performed: one carried out at water temperature of 20 ± 1 °C and 10,830 lx with juveniles produced by G1 descendent females, hereafter indicated as 20 °C/M-light; another bioassay carried out at water temperature of 20 °C ± 1 °C and 26,000 lx with juveniles produced by G2 descendent females (20 °C/H-light); and the third bioassay that was carried out at water temperature of 25 °C ± 1 °C and 10,830 lx with juveniles produced by G3 descendent females (25 °C/M-light). The bioassays were started with juvenile females (3rd brood, >6 h and <24 h old).

Females were exposed individually in 100 mL glass beakers with 50 mL of test medium. Beakers were covered but allowing air changes. The test medium was renewed at each 24 h, the exposure period was 21 days, and *C. vulgaris* (3×10^5 cells/mL/female, 0.322 mg carbon/female/day, Guilhermino et al., 1999) was used as food. Treatments were: control (test medium only), 0.05 mg/L of MPs, 0.1 mg/L of MPs and 0.2 mg/L of MPs (nominal concentrations). Treatments containing MPs were prepared by serial dilution of a stock solution (400 mg/L of MPs in test medium) into test medium.

At the beginning of the bioassay, at the time of test medium renewal in both fresh and old test medium, and at the end of the exposure period, the actual concentrations of MPs in test medium of treatments containing such particles were determined (Section 2.5). At the same time periods, light intensity (Roline RO-1332 Digital Luxmeter, Germany) and water temperature, pH, dissolved oxygen and conductivity (HACH HQ40d multi, U.S.A.) were measured.

The effect criteria were: the mean size at first brood release per parental female; the mean of the somatic growth per parental female (somatic growth); the mean of the number of days until the first brood release per parental female (first brood day); the mean of the total number of broods produced per parental female (brood number); the mean of the total number of offspring produced per parental female (total offspring); the mean of the number of living offspring per parental female (living offspring); the mean of the number of dead offspring (dead offspring); the mean of the number of aborted eggs per parental female (aborted eggs); and the intrinsic rate of population increase (population growth rate) that in *D. magna* can be used as an indicative of population fitness (Giebelhausen and Lampert, 2001).

The length of each female was determined from the length of the first exopodite of the second right antennae (Soares, 1989) of the released moults, which was measured using a Nikon SMZ800 stereomicroscope, U.S.A. It was used to express the size at the first brood release, and to calculate the somatic growth of each parental female during the bioassay as:

length of the exopodite of the last moult released - length of the exopodite of the first moult released.

Population growth rate was calculated using the Lotka (1913) equation as indicated in Martins et al. (2013). Mortality was recognised by the immobilization for 15 s under a brilliant light. The offspring and other data from females that died before the end of the bioassay were not included in data analyses. Females were observed at least twice a day. Moults, offspring produced and dead females were removed as soon as they were observed.

2.5. Determination of microplastic actual concentrations in test medium

The actual concentrations of MPs in test medium were determined by spectrofluorimetry (excitation wavelength: 575 nm; emission wavelength: 607 nm) as in Martins and Guilhermino (2018). A solution with a MP concentration of 8 mg/L was prepared in test medium. This solution was serial diluted in test medium (1:2 *v*/v) to obtain a series of solutions with MP concentrations ranging from 4 mg/L to 0.125 mg/L. The process was repeated three times with different test medium in distinct days. The fluorescence of all solutions was measured (Jasco FP-6200

spectrofluorimeter, Japan), plotted against the corresponding MP nominal concentrations, and the following linear regression model was fitted to the data ($N = 49$, $R = 99.9\%$):

$$\text{concentration of MPs (mg/L)} = -0.033 + 0.026 \times \text{fluorescence (F units)}$$

At the beginning of each bioassay, at its end, and at the time of each test medium renewal, the fluorescence of freshly prepared (0 h) and/or old (24 h) test medium of each beaker of treatments with nominal MP concentrations of 0.1 mg/L and 0.2 mg/L was measured. MP concentrations were determined from the previously indicated linear regression model. The fluorescence of test media with MP nominal concentration of 0.05 mg/L could not be measured due to lack of sensitivity of the method.

For each bioassay, the mean of fluorescence and of MP concentrations per treatment (0.1 or 0.2 mg/L) were determined (Table 1). For each beaker, the deviation of MP actual concentrations at 0 h relatively to the corresponding nominal concentration was calculated as in Guilhermino et al. (2018):

$$\text{Deviation (\%)} = \text{module of } 100 - [\text{actual concentration (mg/L)} \times 100/\text{nominal concentration (mg/L)}]$$

The decrease of MP concentration in test medium of each beaker over the test medium renewal period (24 h), hereafter indicated as MP decay, was determined as:

$$\text{MP decay (\%)} = \text{module of } 100 - (\text{actual concentration at 24 h in mg/L} \times 100/\text{actual concentration at 0 h in mg/L})$$

The deviation of MP actual concentrations in freshly prepared test medium (0 h) relatively to the nominal concentration was always lower than 20% (Table 1). The MP decay over 24 h in some beakers was higher than 20% (Table 1). For this reason, the time-weighted mean of MP concentration in test media of each replicate along the bioassay was determined according to OECD (2012). The total mean per treatment, namely 0.09 mg/L or 0.19 mg/L (Table 1), were considered the estimated exposure concentrations (EEC) of MPs over the bioassay in the treatments containing the two highest MP concentrations. Regarding the lowest MP concentration tested for which the MP actual concentration could not be determined, the EEC was calculated as:

$$\text{EEC (mg/L)} = \text{nominal concentration (mg/L)} - \text{mean decay in the treatment} \times \text{nominal concentration (mg/L)}$$

The obtained EECs of MPs in treatments with the lowest nominal concentration were 0.039 mg/L at 20 °C/M-light, 0.037 mg/L at 20°/H-light and 0.038 mg/L at 25 °C/M-light. Their mean (0.04 mg/L) was used as the EEC of MPs in treatments containing the lowest MP concentration.

The biological results were expressed in relation to the EECs of MPs, namely 0.04 mg/L, 0.09 mg/L or 0.19 mg/L.

2.6. Data analyses

Data sets with normal distribution and homogeneity of variances were analysed by one-way Analyses of Variance (ANOVA). When significant differences were found, the Tukey's multi-comparison test was used to discriminate significant different treatments, and to determine the no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC) of MPs. When normal distribution and/or homogeneity of variances were not achieved, the Kruskal-Wallis test was used. When significant differences were found, the nonparametric Tukey-type test of Miller for equal sample sizes or of Dunn for unequal sample sizes were used (Zar, 1999). The NOEC was the highest MP concentration that did not induce effects significantly different from the control group, and the LOEC was the lowest MP concentration that induced effects significantly different from those observed in the control group (OECD, 2012).

To estimate the concentrations of MPs that caused 10%, 20% and 50% of effect (EC_{10}, EC_{20} and EC_{50}, respectively), a logistic model (lower limit = 0) was fitted to living offspring data set of each bioassay, as in Martins et al. (2013):

$$y = c + ((d-c)/(1 + \exp.\{b\,[\ln(x) - \ln(e)]\}))$$

with: d = upper limit; c = lower limit; e = EC_{50}, the concentration that causes 50% of reduction on living offspring production, and b = proportional to the slope around the EC_{50}.

To investigate the type of interaction between increased light and MPs, and between increased temperature and MPs, the conceptual approach to interpret the type of interaction from population or community response data in studies with factorial experimental design described in Crain et al. (2008) with some modifications was used. Briefly, the conceptual approach is based on the comparison of the effects caused by the simultaneous exposure to two or more stressors (combined effect) and the sum of the effects caused by each of the stressors alone (independent effects), and uses the individual and interactive effect sizes measured with Hedge's *d* before across studies combination by meta-analysis (Crain et al., 2008).

In our study, the population growth rate was used as population response to stressors and the main objectives were to investigate the interactions between the rise of light intensity (from 10,830 lx to 26,000 lx) and MPs, and increased water temperature (from 20 °C to 25 °C) and MPs. Because under combined exposures, the type of interaction may be different at low, medium and high concentrations of the stressors, especially in the case particles such as MPs (e.g. Pacheco et al., 2018), the type of interaction for each MP concentration tested, namely 0.04 mg/L, 0.09 mg/L and 0.19 mg/L was analysed.

The interaction between increased light intensity and MPs was investigated considering three scenarios: increased light intensity

Table 1
Mean (± SD) of fluorescence (Fluo) and actual concentrations (conc) of microplastics (MP) in freshly prepared (0 h) and old (24 h) test media, and deviation (dev) of MP actual concentration at 0 h from nominal ones, decay of MP actual concentrations in test media over 24 h (decay), and estimated exposure concentration (EEC) per treatment along the bioassay. Temp – Temperature; Light – light intensity. Nom – nominal. N1 – number of samples analysed. N2 – number of replicates per treatment. In the decay column distinct letters indicate significant differences among treatments ($p < 0.05$).

Temp (°C)	Light (lux)	MP Nom conc (mg/L)	N1	Fluo 0 h	Fluo 24 h	MP actual conc 0 h (mg/L)	MP actual conc 24 h (mg/L)	Dev (%)	Decay (%)	N2	EEC (mg/L)
20	10,830	0.1	210	5.28 ± 0.09	4.36 ± 0.08	0.104 ± 0.002	0.080 ± 0.006	4 ± 2	23 ± 5 a,b	10	0.0918 ± 0.0002
20	26,000	0.1	210	5.25 ± 0.06	4.23 ± 0.05	0.104 ± 0.002	0.077 ± 0.001	4 ± 2	26 ± 2 b	10	0.0896 ± 0.0002
25	10,830	0.1	189	5.26 ± 0.08	4.3 ± 0.1	0.103 ± 0.002	0.079 ± 0.003	3 ± 2	24 ± 3 a	9	0.0904 ± 0.0002
20	10,830	0.2	189	9.3 ± 0.1	8.2 ± 0.1	0.209 ± 0.004	0.180 ± 0.004	5 ± 2	14 ± 1 c	9	0.1946 ± 0.0003
20	26,000	0.2	168	9.24 ± 0.06	8.20 ± 0.06	0.207 ± 0.001	0.180 ± 0.002	3.6 ± 0.7	13.1 ± 0.9 c	8	0.1934 ± 0.0003
25	10,830	0.2	147	9.23 ± 0.06	8.18 ± 0.06	0.207 ± 0.002	0.180 ± 0.001	3.5 ± 0.8	13 ± 1 c	7	0.1931 ± 0.0002

(26,000 lx) and low MP concentration (0.04 mg/L); increased light intensity (26,000 lx) and medium MP concentration (0.09 mg/L); increased light intensity (26,000 lx) and high MP concentration (0.19 mg/L).

The interaction between increased water temperature and MPs was investigated considering three scenarios: increased water temperature (25 °C) and low MP concentration (0.04 mg/L); increased water temperature (25 °C) and medium MP concentration (0.09 mg/L); increased water temperature (25 °C) and high MP concentration (0.19 mg/L). In all the scenarios, the control treatment (no MPs) was water temperature of 20 °C and light intensity of 10,830 lx.

Following Crain et al. (2008) and considering each scenario, the individual effect of stressor A (d_a) alone and of stressor B (d_b) alone were calculated (Gurevitch et al., 2000):

$$\text{Stressor A}, d_a = [(X_A - X_{ct})/s]\, J_{(m)}$$

$$\text{Stressor B}, d_b = [(X_B - X_{ct})/s]\, J_{(m)}$$

where X is the mean of the population growth rate under exposure to stressor A (X_A), control (X_{ct}) or stressor B (X_B), s is the pooled standard deviation of the two groups, and $J(m)$ is a constant to small sample bias correction (Hedges and Olkin, 1985).

Then, the main effects of the stressor A (d_A), stressor B (d_B) and their interaction (d_I) were calculated as in Gurevitch et al. (2000):

$$\text{Stressor A}, d_A = [((X_A + X_{AB}) - (X_B + X_{ct}))/2s]\, J_{(m)}$$

$$\text{Stressor B}, d_B = [((X_B + X_{AB}) - (X_A + X_{ct}))/2s]\, J_{(m)}$$

$$\text{Interaction}, d_I = [((X_{AB} + X_B) - (X_A + X_{ct}))/2s]\, J_{(m)}$$

where X are the means of the population growth rates in the control (X_{ct}), under exposure to the stressor A (X_A), to the stressor B (X_B) or both stressors (X_{AB}), s is the pooled standard deviation, and $J(m)$ is as before indicated. The sampling variance for each Hedge's d calculated as indicated in Gurevitch et al. (2000), and the appropriate two-tailed critical value of the normal distribution were used to calculate the lower and upper 95% confidence interval (95% CI) limits (Gurevitch et al., 1992).

For each scenario and working with no weighted Hedge's, the broad category of the interaction type was identified based on the direction (positive or negative) of individual effect sizes, namely d_a of stressor A alone and d_b of stressor B alone, was identified as in Crain et al. (2008): (a) both individual stressors negative; (b) one negative and the other positive; (c) both positive. Considering the broad category identified, and the interaction effect size with the corresponding 95% confidence interval (95% CI), the interaction type was classified as in Crain et al. (2008): if the 95% CI overlapped zero, the interaction was addition; if the 95% CI did not overlap zero and the broad category was (a) or (b), the type of interaction was synergism when the interaction effect was negative, and antagonism when the interaction effect was positive; if the 95% CI did not overlap zero and the broad category was (c), the type of interaction was synergism when the interaction effect was positive, and antagonism when the interaction effect was negative.

In all the analyses, the significance level was 0.05. The SPSS statistical package, version 26.0 was used for descriptive statistics, ANOVAs, Tukey test and the Kruskal-Wallis test. Miller's and Dunn's nonparametric Tukey-type tests were carried out in Microsoft Excel following Zar (1999). The fitting of the logistic model to offspring living data and the estimate of EC_{10}, EC_{20} and EC_{50} values was done using the extension package *drc* for dose-response analysis using R (Ritz et al., 2015). The calculations to determine the type of interaction were done in Microsoft excel.

3. Results

In the preliminary trial, *D. magna* ingested the MPs tested, as shown by its gut full of particles after 30 min of exposure to a high concentration of MPs (Fig. 1). MPs were also found inside the body, in the brood chamber, stuck in gills and adsorbed to appendices and to the body surface (Fig. 1).

3.1. General conditions of the bioassays

The mean (± SD) of water temperature, dissolved oxygen and pH recorded in fresh and old test media of treatments of the bioassays are shown in Table S1. In treatments of the bioassays carried out at 20 °C, the water temperature mean (± SD) per treatment was 20.2 ± 0.2 °C at 20 °C/M-light, and 20.7 °C ± 0.1 or ± 20.7 °C 0.2 SD at 20 °C/H-light. At 25 °C/M-light, the water temperature mean (± SD) per treatment was 25.1 ± 0.2 or 25.0 ± 0.2 °C. In individual treatments of all the bioassays, the mean (± SD) of dissolved oxygen per treatment ranged from 8.12 ± 0.06 mg/L to 8.13 ± 0.06 mg/L, and the mean (± SD) of pH per treatment ranged from 8.3 ± 0.1 to 8.5 ± 0.1 pH units.

No parental mortality was recorded in any of the control treatments. In all the bioassays, the mean of living offspring number in the control was higher than 60 (Table 2). The coefficient of variation in control treatments was 2.5% in the bioassay at 20 °C/M-light, 2.6% in the bioassay at 20 °C/M-light, and 3.5% in the bioassay at 20 °C/M-light.

Significant differences in MP decay among treatments were found ($H_5 = 831.718$, $p < 0.001$). The MP decay was higher in treatments containing 0.09 mg/L of MPs than in those with 0.19 mg/L of MPs (Table 1).

3.2. Effects of light intensity and water temperature in the absence of microplastics

In the controls of the three bioassays, all the parental females survived until the end of the exposure period, all the offspring produced was alive and no aborted eggs were observed.

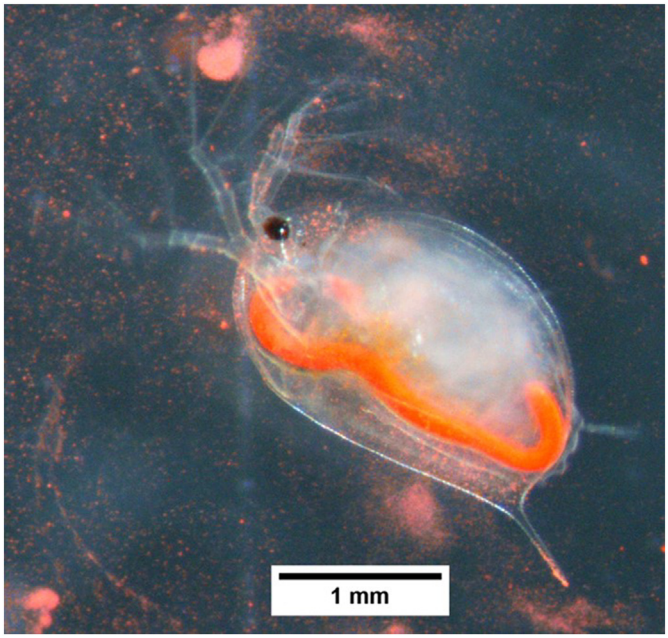

Fig. 1. *Daphnia magna* after 30 min of exposure to a high concentration of microplastics. The gut full of microplastic particles is visible (orange). Microplastic particles appearing as pink-orange adherent to the appendices and body surface, and inside the brood chamber and other body parts are also visible.

Table 2
Mean (± SD) of size at the first brood release, total somatic growth, first brood day number, brood number, living offspring number and population growth rate per parental female in control treatments of bioassays carried out at water temperature of 20 °C and light intensity of 10,830 lx (20 °C/M-light), water temperature of 20 °C and light intensity of 26,000 lx (20 °C/H-light), and water temperature of 25 °C and light intensity of 10,830 lx (25 °C/M-light). The results of one-way ANOVA (F), Kruskal-Wallis (H), and the Miller's multi-comparison test per effect criterion are also indicated. N – number of parental females that survived until the end of the bioassay; different letters after the mean ± SD indicate significant ($p \leq 0.05$) differences among control groups for each effect criterion.

	N	Size at 1st brood (mm)	Total growth (mm)	1st brood day (day number)	Brood number	Living offspring number	Population growth rate
20 °C/M-light	10	0.205 ± 0.003 a	0.206 ± 0.005	8.8 ± 0.4 a	5 ± 0 a	91 ± 2	0.33 ± 0.01 a
20 °C/H-light	10	0.198 ± 0.007 a	0.203 ± 0.006	8.9 ± 0.3 a	5 ± 0 a	92 ± 2	0.32 ± 0.01 a
25 °C/M-light	10	0.175 ± 0.008 b	0.204 ± 0.005	6 ± 0 b	8 ± 0 b	93 ± 3	0.38 ± 0.01 b
F or H		$H_2 = 22.274$ $p < 0.001$	$H_2 = 1.374$ $p = 0.503$	$H_2 = 24.869$ $p < 0.001$	$H_2 = 29.000$ $p < 0.001$	$F_{2, 27} = 1.578$ $p = 0.225$	$H_2 = 20.395$ $p < 0.001$

The means of different effect criteria in females of the control groups are shown in Table 2. Significant differences in the size at the first brood release, first day brood number, brood number and population growth rate among the three control groups were found (Table 2). There were no significant differences in any of the effect criteria between females of the bioassay carried out at 20 °C/M-light and those of the bioassay at 20 °C/H-light. Significant differences in several effect criteria between females of the bioassays carried out at 20 °C (20 °C/M-light and 20 °C/H-light) and those exposed at 25 °C/M-light were found (Table 2). Relative to females exposed at 20 °C, those exposed at 25 °C showed lower size at the first brood release (15% of reduction), decreased first brood day number (32% of reduction), higher brood number (60% of increase) and greater population growth rate (15% increase). No significant differences in the living offspring number or somatic growth among the three control groups were found (Table 2).

3.3. Long-term effects of microplastics at water temperature of 20 °C and moderate light intensity

At 20 °C/M-light, the mortality of parental females per treatment with MPs was 0% at 0.04 mg/L and 0.09 mg/L, and 10% at 0.19 mg/L that occurred after 12 days of exposure (Table 3).

Significant differences in the size at first brood release, somatic growth, total offspring, living offspring, dead offspring and population growth rate among treatments were found (Table 3). In the range of concentrations tested and in relation to the control group at 20 °C/M-light, MPs caused significant reduction of the size at the first brood release (up to 7% of decrease), somatic growth (up to 13% of decrease), total offspring (up to 44% of decrease), living offspring (up to 68% of decrease) and population growth rate (up to 27% of decrease). MPs also caused the release of dead juveniles up to 43% of the total number of offspring released by females exposed to 0.19 mg/L. The NOEC of MPs for different effect criteria ranged from 0.04 mg/L to 0.19 mg/L, and the LOEC of MPs ranged from 0.09 mg/L to >0.19 mg/L, depending of the effect criteria (Table 3). No aborted eggs occurred in any treatment, and there were no significant differences in the first brood day number and in the brood number among treatments (Table 3).

The model fitted to the living offspring produced at 20 °C/M-light under different MP concentrations is shown in Fig. 2. The 21-day EC_{10}, EC_{20} and EC_{50} of MPs to *D. magna* reproduction based on this effect criterion are shown in Table 4.

3.4. Long-term effects of microplastics at water temperature of 20 °C and high light intensity

At 20 °C/H-light, the mortality of parental females in treatments with MPs was 0% at 0.04 mg/L and 0.09 mg/L, and 20% at 0.19 mg/L (Table 3). One of the parental females died after 8 days of exposure and the other after 18 days.

Significant differences in the size at the first brood release, somatic growth, total offspring number, living offspring number, dead offspring number and population growth rate among treatments were found (Table 3). In relation to the control group at 20 °C/H-light, MPs caused significant reduction of the size at the first brood release (up to 12% of decrease), somatic growth (up to 22% of decrease), total offspring number (up to 57% of decrease), living offspring number (up to 83% of decrease) and population growth rate (up to 38% of decrease). MPs also induced the release of dead juveniles, which represented 60% of the total offspring released by females exposed to 0.19 mg/L of MPs. The NOEC of MPs ranged from 0.04 to 0.19 mg/L, and the LOEC from 0.09 to >0.19 mg/L depending of the effect criterion considered (Table 3). No significant differences in the first brood day number and in the number of broods among treatments were found (Table 3). However, there was a slight delay of the first brood release (from 8.9 to 10 days, 12%) and a slight decrease of the total number of broods (4%) at 0.19 mg/L of MPs. No aborted eggs were observed in any treatment.

The toxicity curve based on living offspring data produced under exposure to distinct concentrations of MPs at 20 °C/H-light is shown in Fig. 2, and the 21-day EC_{10}, EC_{20} and EC_{50} of MPs based on living offspring are indicated in Table 4.

3.5. Long-term effects of microplastics at water temperature of 25 °C and moderate light intensity

At 25 °C/M-light, the mortality of parental females in treatments with MPs was 0% at 0.04 mg/L, 10% at 0.09 mg/L, and 30% at 0.19 mg/L (Table 3). At 0.09 mg/L of MPs, the mortality occurred after 16 days of exposure, whereas at 0.19 mg/L of MPs the females died after 5, 8 and 13 days of exposure.

At 25 °C/M-light, significant differences in all the effect criteria among treatments were found (Table 3). In relation to the control group at 25 °C/M-light, MPs caused significant reduction of the size at the first brood release (up to 8% of reduction), somatic growth (up to 20% of reduction), number of broods released (up to 43% of reduction), total offspring number (up to 78% of reduction), living offspring number (up to 91% of reduction) and population growth rate (up to 59% of reduction). MPs also caused the release of dead juveniles, which accounted for 55% of the total number of offspring released by females exposed to 0.19 mg/L of MPs, and increased the time until the release of the first brood up to 73% (from 6 to 10.4 days, Table 3). Depending of the effect criterion, the NOEC of MPs ranged from 0.04 mg/L to 0.09 mg/L, and the LOEC from 0.09 mg/L to 0.19 mg/L (Table 3).

The toxicity curve of MPs on *D. magna* living offspring at 25 °C/M-light is shown in Fig. 2, and the 21-day EC_{10}, EC_{20} and EC_{50} of MPs estimated from it are indicated in Table 4.

3.6. Comparison of microplastic long-term effects at different light intensity and water temperature and interactions between the stressors

Fig. 2 shows that MPs reduced more the number of living offspring produced at 20 °C/H-light and 25 °C/M-light than at 20 °C/M-light. In relation to the EC_{50} of MPs at 20 °C/M-light, the EC_{50} was reduced by 30% (1.4 folds) at 20 °C/H-light, and by 31% (1.4 folds) at 25 °C/M-light (Table 4). For all the MP concentrations, there were significant differences among the three bioassays (Table 4). At all the MP concentrations, the number of offspring produced by females exposed at 20 °C/M-light

Table 3
Percentage of parental mortality, and mean (± SD) of the size at first brood release, somatic growth, first brood day number, brood number, total offspring number, living offspring number, dead offspring number and population growth rate per parental female in each treatment of the bioassays (bioassay 1: water temperature of 20 °C and light intensity of 10,830 lx; bioassay 2: water temperature of 20 °C and light intensity of 26,000 lx; bioassay 3: water temperature of 25 °C and light intensity of 10,830 lx). The results of Kruskal-Wallis and the Dunn's tests, and NOEC and LOEC values of MP per effect criterion are also indicated. PM – percentage of parental mortality. N – number of parental females that survived until the end of the bioassay; different letters after the mean indicate statistical significant differences ($p \leq 0.05$) among treatments of the same bioassay per effect criterion.

	PM (%)	N	Size (mm)	Growth (mm)	1st brood (days)	Brood number (n)	Total offspring (n)	Living offspring (n)	Dead offspring (n)	Population growth rate
20 °C/M-light										
Control	0	10	0.205 a ± 0.003	0.206 a ± 0.005	8.8 ± 0.4	5 ± 0	91 a ± 2	91 a ± 2	0 a	0.33 a ± 0.01
0.04 mg/L MP	0	10	0.206 a ± 0.000	0.198 a,b ± 0.007	9 ± 0	5 ± 0	88 a,b ± 2	88 a,b ± 2	0 a	0.317 a,b ± 0.005
0.09 mg/L MP	0	10	0.202 a,b ± 0.004	0.189 b,c ± 0.008	9 ± 0	5 ± 0	78 b,c ± 3	67 b,c ± 3	12 b ± 3	0.298 b,c ± 0.006
0.19 mg/L MP	10	9	0.19 b ± 0.01	0.18 c ± 0.01	9 ± 0	5 ± 0	51 c ± 3	29 c ± 2	22 b ± 4	0.24 c ± 0.01
H			$H_3 = 23.455$ p < 0.001	$H_3 = 24.665$ p < 0.001	$H_3 = 5.957$ $p = 0.114$	–	$H_3 = 33.651$ $p < 0.000$	$H_3 = 33.634$ p < 0.001	$H_3 = 36.776$ p < 0.001	$H_3 = 33.184$ p < 0.001
NOEC (mg/L)			0.09	0.04	0.19	0.19	0.04	0.04	0.04	0.04
LOEC (mg/L)			0.19	0.09	> 0.19	> 0.19	0.09	0.09	0.09	0.09
20 °C/H-light										
Control	0	10	0.198 a ± 0.007	0.203 a ± 0.006	8.9 ± 0.3	5 ± 0	92 a ± 2	92 a ± 2	0 a ± 0	0.32 a ± 0.01
0.04 mg/L MP	0	10	0.200 a ± 0.006	0.198 a ± 0.006	9 ± 0	5 ± 0	83 a,b ± 3	83 a,b ± 3	0 a ± 0	0.304 a,b ± 0.004
0.09 mg/L MP	0	10	0.196 a ± 0.007	0.17 b ± 0.01	9 ± 0	5 ± 0	60 b,c ± 2	46 b,c ± 2	14 b ± 1	0.265 b,c ± 0.005
0.19 mg/L MP	20	8	0.174 b ± 0.123	0.158 b ± 0.007	10 ± 1	4.8 ± 0.5	40 c ± 2	16 c ± 2	24 b ± 2	0.20 c ± 0.02
H			$H_3 = 16.040$ $p = 0.001$	$H_3 = 30.641$ p < 0.001	$H_3 = 7.186$ $p = 0.066$	$H_3 = 7.708$ $p = 0.052$	$H_3 = 34.812$ p < 0.001	$H_3 = 34.785$ p < 0.001	$H_3 = 35.885$ p < 0.001	$H_3 = 34.656$ p < 0.001
NOEC (mg/L)			0.09	0.04	0.19	0.19	0.04	0.04	0.04	0.04
LOEC (mg/L)			0.19	0.09	> 0.19	> 0.19	0.09	0.09	0.09	0.09
25 °C/M-light										
Control	0	10	0.175 a ± 0.008	0.204 a ± 0.005	6 a ± 0	8 a ± 0	93 a ± 3	93 a ± 3	0 a	0.38 a ± 0.01
0.04 mg/L MP	0	10	0.171 a ± 0.004	0.201 a,b ± 0.008	6 a ± 0	8 a ± 0	83 a,b ± 1	83 a,b ± 1	0 a	0.380 a,b ± 0.008
0.09 mg/L MP	10	9	0.165 a,b ± 0.007	0.184 b,c ± 0.007	7 a ± 0	8 a ± 0	59 b,c ± 5	47 b,c ± 4	12 b ± 2	0.315 b,c ± 0.008
0.19 mg/L MP	30	7	0.161 b ± 0.006	0.163 c ± 0.004	10.4 b ± 0.8	4.6 b ± 0.5	20 c ± 2	8 d ± 2	11 b ± 1	0.16 c ± 0.02
H			$H_3 = 15.697$ p = 0.001	$H_3 = 28.415$ p < 0.001	$H_3 = 34.800$ p < 0.001	$H_3 = 34.602$ p < 0.001	$H_3 = 32.780$ p < 0.001	$H_3 = 32.771$ p < 0.001	$H_3 = 31.540$ p < 0.001	$H_3 = 28.521$ p < 0.001
NOEC (mg/L)			0.09	0.04	0.09	0.09	0.04	0.04	0.04	0.04
LOEC (mg/L)			0.19	0.09	0.19	0.19	0.09	0.09	0.09	0.09

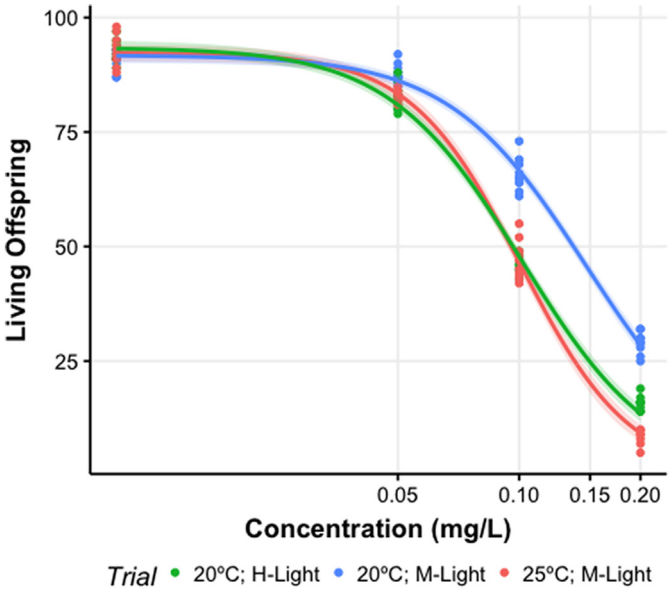

Fig. 2. Effects of 21-day exposure to microplastics on *Daphnia magna* living offspring at water temperature of 20 °C and light intensity of 10,830 lx (blue), water temperature of 20 °C and light intensity of 26,000 lx (green), and water temperature of 25 °C and light intensity of 10,830 lx (red).

was higher than the number of offspring produced by females exposed to the corresponding treatments of the other two bioassays, which had no significant differences between them (Table 4).

For all the MP concentrations, there were also significant differences in the somatic growth of females exposed to corresponding treatments of distinct bioassays (0.04 mg/L: $H_2 = 0.934$, $p = 0.627$; 0.09 mg/L: $H_2 = 13.634$, $p = 0.001$; 0.19 mg/L: $H_2 = 18.679$, $p < 0.001$). At MP concentrations of 0.09 and 0.19 mg/L, the somatic growth was higher in females exposed at 20 °C/M-light than in females exposed at 20 °C/H-light or at 25 °C/M-light, which have no significant differences between them (Fig. 3a). There were also significant differences in the population growth rate of *D. magna* among bioassays (0.04 mg/L: $H_2 = 25.806$, $p < 0.001$; 0.09 mg/L: $H_2 = 25.806$, $p < 0.001$; 0.19 mg/L: $H_2 = 25.806$, $p < 0.001$). At 0.04 and 0.09 mg/L of MPs the population growth rate was higher at 20 °C/M-light and 25 °C/M-light than at 20 °C/H-light; among females exposed to 0.19 mg/L of MPs the population growth rate was higher at 20 °C/M-light than at 20 °C/H-light or 20 °C/M-light (Fig. 3b). The highest reduction of *D. magna* population growth rate caused by 0.19 mg/L of MPs was observed at 25 °C (Fig. 3b).

The values of the individual and interactive Hedge's *d* with the respective 95% CI are represented in Fig. 4 (the values are indicated in Table S3). Regarding increased light intensity (26,000 lx) and MPs (Fig. 4a), in all the scenarios considered (a-1, a-2 and a-3), the 95% CI did not overlap zero and the individual effects of stressors were both negative. Under exposure to increased water temperature (25 °C) and the lowest concentration of MP tested (0.04 mg/L, Fig. 4b-1), the 95% CI did not overlap zero, temperature had a positive individual effect, MPs had an individual negative effect, and the interactive effect was positive. At higher concentrations of MPs, namely 0.09 mg/L (Fig. 4, b-2) or 0.09 mg/L (Fig. 4, b-3), the 95% CI did not overlap zero, temperature had a positive individual effect, MPs had a negative individual effect and the interactive effect was negative.

4. Discussion

All the bioassays complain with the validity criteria of the OCED guideline (OECD, 2012), namely mortality lower than 20% and mean of total offspring number per female that survived until the bioassay ≥60. Moreover, the recommendations (OECD, 2012) regarding water temperature and pH variation per beaker (temperature variation: < 2 °C; pH: range 6–9 and variation <1.5 pH units), oxygen levels above 3 mg/L, and the coefficient of variation around the mean of living offspring produced per animal in the control groups was always below 25% (OECD, 2012) were also accomplished. Therefore, all the bioassays can be considered valid.

During the interval of test medium renewal the concentrations of MPs in test medium decreased. At water temperature of 20 °C and 10,830 lx, the MP decay found (14% - 24%) compare with the range of values documented in previous studies carried out with the same type of MPs under comparable conditions (Martins and Guilhermino, 2018; Pacheco et al., 2018).

Several factors may have contributed to MP decay in test medium over the interval of test medium renewal (24 h). Likely, the tested females have ingested some MPs, as occurred in the preliminary trial carried out before the bioassays, and in other studies that documented the ingestion of different types of MPs by *D. magna* (Jemec et al., 2016;

Table 4
Microplastic (MP) concentrations estimated to cause 10% (EC_{10}), 20% (EC_{20}) and 50% (EC_{50}) of the number of living offspring produced per female after 21 days of exposure at distinct water temperatures and light intensities, and comparison of living offspring number per MP concentration among bioassays. Temp – water temperature; Light – light intensity; SE – standard error of the EC_x estimate. 95% CL – confidence limits at 95%. In the down part of the table, different letters after water temperature and light intensity indicate significant differences ($p \leq 0.05$) among the bioassays for each MP concentration.

Temp (°C)	Light (lx)	Parameter	EC_{10} (mg/L)	EC_{20} (mg/L)	EC_{50} (mg/L)
20	10,830	Estimate	0.062	0.085	0.146
		SE	0.0023	0.0023	0.0024
		95% CL	(0.057–0.067)	(0.080–0.090)	(0.142–0.151)
20	26,000	Estimate	0.044	0.060	0.102
		SE	0.0016	0.0016	0.0016
		95% CL	(0.041–0.047)	(0.057–0.063)	(0.099–0.105)
25	10,830	Estimate	0.051	0.065	0.101
		SE	0.0019	0.0018	0.0015
		95% CL	(0.047–0.054)	(0.061–0.069)	(0.098–0.104)

Criterion and conditions	MP concentration (mg/L)		
	0.04	0.09	0.19
Living offspring	$H_2 = 16.662$	$H_2 = 19.153$	$H_2 = 20.489$
	$p < 0.001$	$p < 0.001$	$p < 0.001$
20 °C/M-light	a	a	a
20 °C/H-light	b	b	b
25 °C/M-light	b	b	b

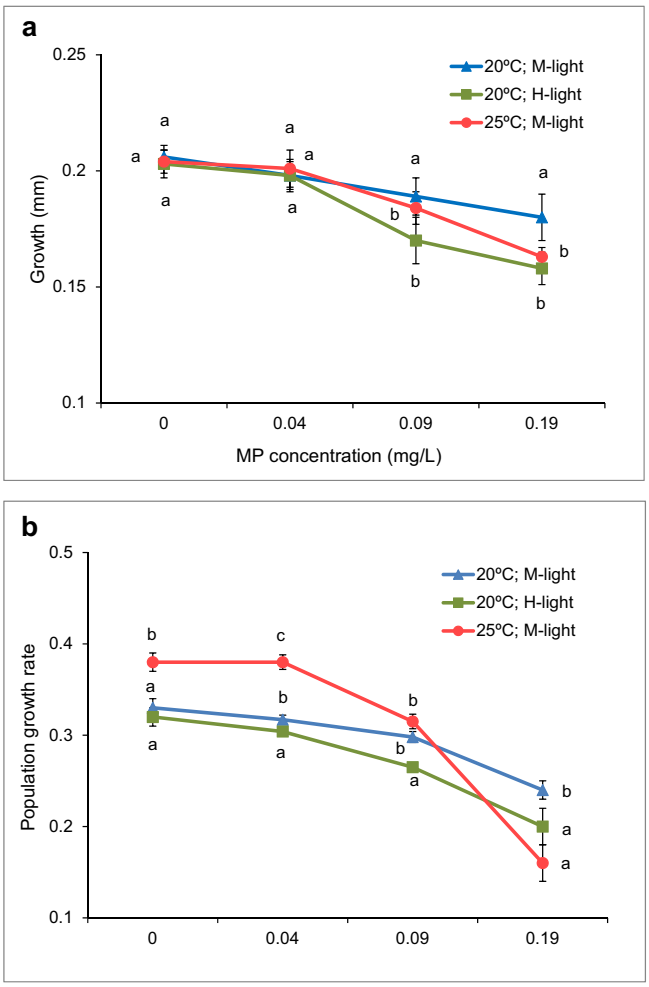

Fig. 3. Effects of 21-day exposure to microplastics on *Daphnia magna* somatic growth (a) and population growth rate (b) at water temperature of 20 °C and light intensity of 10,830 lx (blue), at water temperature of 20 °C and light intensity of 26,000 lx (green), and water temperature of 25 °C and light intensity of 10,830 lx (red). The values are the mean of the females that survived until the end of the bioassay with the corresponding standard error. Different letters near the mean indicate statistical significant differences per microplastic concentration among the three bioassays carried out.

Ma et al., 2016; Ogonowski et al., 2016; Frydkjær et al., 2017). MP uptake through gills and other routes may have also occurred. In test medium, some MPs may have also bind to *C. vulgaris* cells used to feed *D. magna*, as suggested in a previous study where other microalgae were exposed to the same type of MPs (Prata et al., 2018), being ingested together with food. The tested MPs were found to form hetero-aggregates with cells of the marine microalgae *Tetraselmis chuii* (Prata et al., 2018), and hetero-aggregates including cells of freshwater microalgae and other types of MPs were also documented (Lagarde et al., 2016), including in experiments with microalgae and *D. magna* where their sedimentation was also reported (Chen et al., 2020). Thus, the formation of hetero-aggregates including *C. vulgaris* cells and MPs with further sedimentation into the beakers bottom may have also contributed to MP decay in test medium. Other processes, such as MP adsorption to beaker walls may have also contributed to reduce the concentrations of MPs (Luis et al., 2015; Pacheco et al., 2018).

The lower (~13–14%) MP decay in treatments with 0.19 mg/L of MPs than in those with 0.09 mg/L of MPs (~23–26%) may have been due to lower food intake by females exposed to the highest concentration of MPs. Indeed, the presence of high number of MP particles in test medium together with food reduces *D. magna* filtration and microalgae intake (Ogonowski et al., 2016; Colomer et al., 2019). If feeding of females exposed to the highest MP concentration tested was reduced, lower ingestion of microalgae cells with MPs adsorbed may have contributed to lower MP decay at 0.19 mg/L than at 0.09 mg/L of MPs. The procedure used to determine the concentrations of MPs in test media may also have had lower sensitivity at 0.09 mg/L than at 0.19 mg/L of MPs. If so, this factor may have also contributed to the differences of MP decay in test media between the treatments with 0.09 mg/L and 0.19 mg/L of MPs.

The concentrations of MPs tested (0.04, 0.09 and 0.19 mg/L) are in the range of MP levels documented in the water of freshwater systems, being lower than some of the values reported in impacted ecosystems, such as mean concentrations of 1.56 ± 1.64 mg/L in lakes and 5.51 ± 9.09 mg/L in wetlands of Texas, U.S.A. (Lasee et al., 2016). In real scenarios, *D. magna* and other zooplankton species may actually be exposed to higher concentrations of MPs because in general very small MPs present in the water are not quantified (Andrady, 2017), they feed on microalgae that may have MPs bound (or inside the cells in the case of nano-sized plastics), MPs are retained in the gut and gills for some time, and accumulation of some small MPs in internal organs and tissues may occur.

4.1. In the absence of microplastics, light intensity rise had no significant effects on D. magna fitness, whereas water temperature rise increased it

The means of the effect criteria determined in the control group of the bioassay carried out at water temperature of 20 °C and 10,830 lx are in the range of corresponding values previously documented in *D. magna* exposed to comparable abiotic conditions and food regimes (e.g. Giebelhausen and Lampert, 2001; Guilhermino et al., 1999; Martins et al., 2013; Vandenbrouck et al., 2011).

The rise of light intensity from 10,830 lx to 26,000 lx at water temperature of 20 °C had no significant effects on *D. magna* individual and population fitness. In the absence of other stressors (e.g. environmental contaminants, fish kairmones), *D. magna* population fitness is a compromise among the effects of light intensity, photoperiod, temperature and food availability (Giebelhausen and Lampert, 2001; Kessler and Lampert, 2004; Gust et al., 2019). In our experimental conditions, females were exposed every day to 8 h of light that is within the most favourable range for *D. magna* filtration (Serra et al., 2019) and reproduction (Gust et al., 2019). Also, the water temperature was in the range most adequate for the species (Mitchell and Lampert, 2000; Giebelhausen and Lampert, 2001), there was availability of adequate food, and no other stressors were present. Moreover, females were previously acclimated to increased light intensity that is an important factor (Mitchell et al., 2004; Coggins et al., 2017; Serra et al., 2019), and the light was from lamps that emit low UV radiation minimizing the risk of adverse effects that high UV radiation levels induce on *D. magna* (Storz and Paul, 1998). Such favourable conditions may explain the comparable population fitness at 10830 lx and 26,000 lx in the control groups at water temperature of 20 °C. These results agree with studies from the literature carried out with other *D. magna* clones exposed to comparable environmental conditions, such as the study of Effertz and von Elert (2017) where no immediate effects of light intensity variation on *D. magna* somatic growth in the absence of fish kairomones were found.

Under light intensity of 10,830 lx, water temperature rise from 20 °C to 25 °C reduced by 15% the size at the first brood release and accelerated juvenile development leading to an earlier release of the first brood (from 8 to 6 days), suggesting that energy and other resources were allocated from juvenile growth to development and reproduction. These results are in agreement with previous studies where *D. magna* was exposed to comparable water temperatures and with the temperature-size rule for ectotherms in general (Heugens et al., 2006; Vandenbrouck et al., 2011; Martins et al., 2013; Hoefnagel et al., 2018; Im et al., 2020).

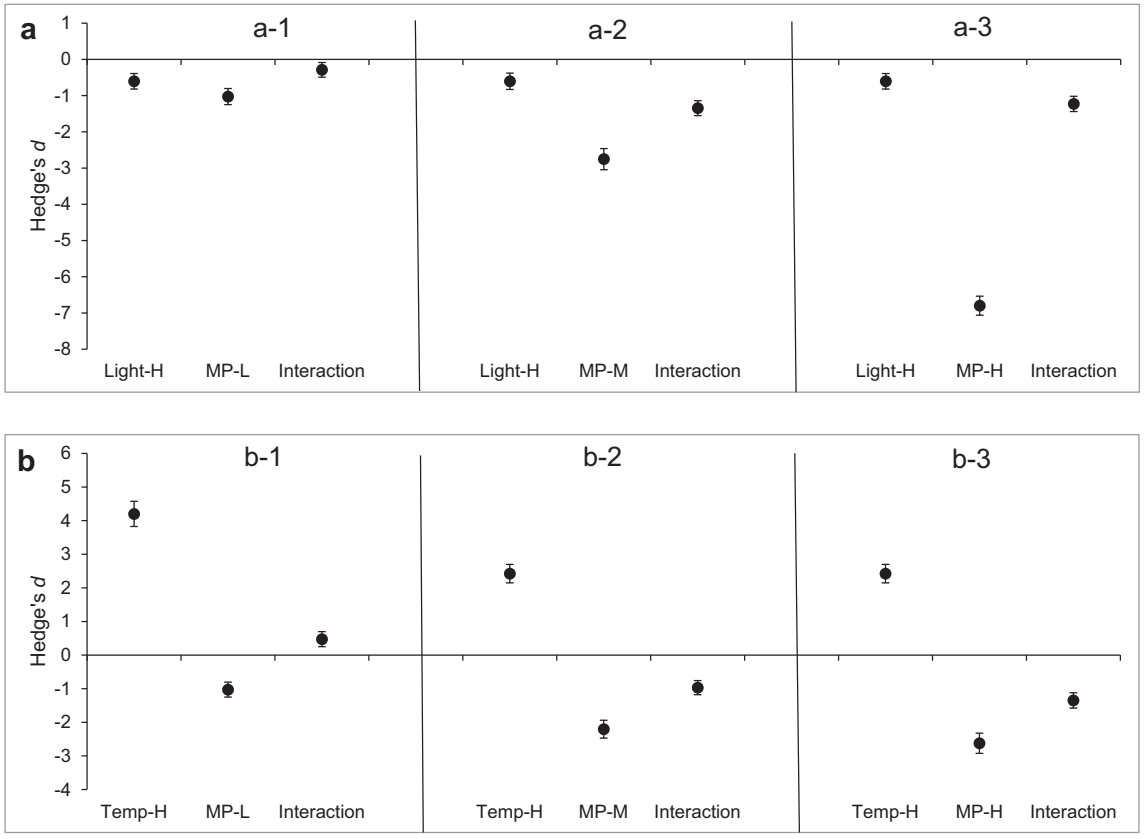

Fig. 4. Individual and combined effects of light intensity rise (from 10,830 lx to 26,000 lx) and microplastics (MP) (a), and water temperature rise (from 20 °C to 25 °C) and MP (b) on the population growth rate of *Daphnia magna*, compared with control (10,830 lx, 20 °C water temperature). The dots represent the individual and interactive effect sizes measured with Hedge's *d* and the corresponding 95% confidence interval (vertical bars). The following scenarios were considered: increased light intensity and 0.04 mg/L of MP (a-1), increased light intensity and 0.09 mg/L of MP (a-2), increased light intensity and 0.19 mg/L of MP (a-3); increased water temperature and 0.04 mg/L of MP (b-1), increased water temperature and 0.09 mg/L of MP (b-2), and increased water temperature and 0.19 mg/L of MP (b-3). Light-H: individual effect of increased light intensity alone. MP-L: individual effect of 0.04 mg/L of MP alone. MP-M: individual effect of 0.09 mg/L of MP alone. MP-H: individual effect of 0.19 mg/L of MP alone. Interaction: interactive effect of the two stressors. Temp-H: individual effect of increased water temperature alone.

The results of the present study also show that the earlier start of reproduction at water temperature of 25 °C and 10,830 lx resulted in higher number of broods (8 instead of 5) and greater (15%) population growth rate than at water temperature of 20 °C and 10,830 lx. Thus, the reproduction period was longer and likely more energy demanding at water temperature of 25 °C than at 20 °C. Previous studies showed that after long-term exposure to 25 °C, *D. magna* filtration rate is higher than at 20 °C (Burns, 1969). Also, in *D. magna* long-term acclimated to temperatures in the range from 16 °C to 29 °C, activity, respiration, hearth beat, and metabolism increased with temperature rise, whereas the body size and mass decreased likely due to higher energy demands at warmer temperatures (Khan and Khan, 2008). Moreover, in *D. magna* maintained for generations at 20 °C and then exposed for 96 h to distinct temperatures, higher energy reserves in juveniles exposed to temperatures in the 22–26 °C range than in those exposed to 20 °C were found, suggesting increased metabolism induced by rapid temperature rise (Filho et al., 2011).

In the literature, lower *D. magna* fitness at 25 °C than at 20 °C was also reported (e.g. Giebelhausen and Lampert, 2001; Im et al., 2020). The distinct findings regarding the effects of temperature on *D. magna* reported are due to differences in factors known to influence the response of *D. magna* to thermal variation. Such factors include genetic constitution and variability, phenotypic plasticity and density of tested populations (Mitchell and Lampert, 2000; Mitchell et al., 2004; Messiaen et al., 2010; Janssen et al., 2017; Bruijning et al., 2018), life stage (Hoefnagel et al., 2018) and sex (Mikulski et al., 2011). Environmental factors also contribute to distinct findings, namely water depth, stratification and light influencing diurnal vertical migration behaviour in the water column (Mitchell and Lampert, 2000), food quality and availability (Giebelhausen and Lampert, 2001; Im et al., 2020), previous acclimation and type of thermal stress (Paul et al., 2004; Seidl et al., 2005; Coggins et al., 2017; Janssen et al., 2017; Müller et al., 2018; Adamczuk, 2020), oxygen levels (Paul et al., 2004; Zeis et al., 2004), presence of other stressors (Messiaen et al., 2010; Martins et al., 2013), among other factors.

4.2. At water temperature of 20 °C and moderate light, microplastics reduced D. magna fitness

The preliminary trial with a high concentration of MPs confirmed that *D. magna* ingested the MPs tested. It also showed that the MPs can be retained in *D. magna* gills and in the gut, enter into the brood chamber and inside the body, and attach to body surface including to appendices. MPs in gills may reduce the efficiency of respiration and other gill functions, inside the gut may cause local lesions and other reactions, inside the body may cause toxicity by several ways, in the brood chamber leads to exposure of eggs, embryos and juveniles to MPs and their chemicals during early developmental phases, and at the body surface and appendices may compromise swimming and other behaviour, as previously discussed (Martins and Guilhermino, 2018; Pacheco et al., 2018), as well as the sensorial perception of food, predators and other stimulus.

In the bioassay carried out at water temperature of 20 °C and 10,830 lx, there was mortality of one parental female after 12 days of exposure to 0.19 mg/L confirming that MPs can cause cumulative mortality in *D. magna*. The decrease of the size at first brood release, somatic growth,

total offspring, living offspring and population growth rate in females exposed to MPs (≥ 0.09 mg/L) indicates significant reduction of *D. magna* individual and population fitness caused by MPs. This is in good agreement with previous findings in *D. magna* exposed to the same type of MPs (Martins and Guilhermino, 2018; Pacheco et al., 2018) and to other types of MPs, including nanoplastics (e.g. Besseling et al., 2014; Eltemsah and Bøhn, 2019; Schür et al., 2020). However, no significant effects of MPs on *D. magna* survival, morphology, growth and reproduction were also documented (Imholf et al., 2017; Coady et al., 2020). Such differences among studies may be due to several factors known or suspected of influencing the toxicity of MPs, such as MP proprieties, MP concentrations tested, food levels and type, abiotic conditions, and differences of the clones tested, among others (Ogonowski et al., 2016; Imholf et al., 2017; Sadler et al., 2019). Independently of their causes, such differences highlight the need of more research.

The reduction of *D. magna* population fitness by the MPs tested may have been induced by several processes likely acting together. Such processes may include reduction of food intake in the presence of MPs, physical effects of the particles, and chemical toxicity of MPs and/or of their components, among others, as previously discussed (e.g. Martins and Guilhermino, 2018; Pacheco et al., 2018). Briefly, regarding physical effects, the presence of MPs in test media could have decrease microalgae ingestion by *D. magna*, as suggested by the results of MP decay in test media. In studies with *D. magna* exposed to other MPs, high number of MP particles in test medium reduced filtration and microalgae ingestion (Ogonowski et al., 2016; Colomer et al., 2019; Serra et al., 2020). Decreased food ingestion may lead to less energy available and need of energy allocation from growth and reproduction to face MP-induced stress, increased need of tissue repair, and maintenance of basic functions (Martins and Guilhermino, 2018; Pacheco et al., 2018). Indeed, in *D. magna* exposed to environmental contaminants, changes in energy allocation and in energy reserves may occur (Vandenbrouck et al., 2011; Sengupta et al., 2016). Potential retention of MPs in gills in both parental females and juveniles may have decreased the efficiency of gill functions, including respiration, contributing to fitness decrease and ultimately leading to dead of parental females and juveniles developing into the brood chamber (Pacheco et al., 2018). Other physical effects, such as lesions in gut walls caused by MPs, may have also occurred.

Plastics and MPs generally contain several chemicals, including additives (Hahladakis et al., 2018; Campanale et al., 2020). Such chemicals can be released from plastics into the water and cause toxicity as shown in studies with *D. magna* exposed to plastic leachates (Jemec et al., 2016; Belzagui et al., 2021) and plastic components (Jang and Ji, 2015), and reared in plastic objects (Cuhra et al., 2017). Release of plastic components and additives from MPs may also occur inside *D. magna* body after MP uptake (Martins and Guilhermino, 2018; Pacheco et al., 2018). Several of these chemicals, such as bisphenol A (BPA), can cause mortality, endocrine disruption with potential effects on growth, reproduction, and adverse effects over generations in *D. magna* (Mansilha et al., 2013; Jang and Ji, 2015).

BPA and BPAF were detected in the MPs tested (Table S2) at 1 ± 0.05 μg/kg (mean ± SD) and below the limit of quantification (1 μg/kg), respectively (Table S2). However, the concentrations of BPA (0.00009 and 0.00019 ng/L) to which females were likely exposed in the treatments containing 0.09 and 0.19 mg/L of MPs seem to be very low to cause significant effects on *D. magna* reproduction. For example, Mansilha et al. (2013) did not found significant effects on the total number of living offspring per female in *D. magna* exposed for 21 days to 3000 μg/L of BPA. Also, multigenerational exposure to BPA migrated from plastic products induces xenoestrogenic effects in *D. magna* (Mansilha et al., 2013), whereas in the present study MPs reduced reproduction. Thus, the effects were likely caused by other MP components with different modes of action and/or by the particles them self. MPs (and/or their associated chemicals) can induce neurotoxicity through acetylcholinesterase activity inhibition, oxidative stress and damage (Barboza et al., 2018; Guilhermino et al., 2018), among other effects in aquatic species, including in *D. magna* (e.g. Sadler et al., 2019). Such effects may have also occurred in *D. magna* exposed to the highest concentrations of MPs tested but they were not investigated. Chemical toxicity may have also contributed to mortality among parental females and juveniles developing in the brood chamber.

The NOEC and LOEC of the MPs obtained at 20 °C/M-light for the number of living juvenile production, compare with the corresponding values previously documented (Martins and Guilhermino, 2018; Pacheco et al., 2018), and together with 21-day EC_{10}, EC_{20} and EC_{50} at of MPs at different light intensities and water temperatures contribute to increase baseline values that may be used in modelling and risk assessment of MPs.

4.3. Light intensity rise increased the adverse effects of microplastics on *D. magna* fitness, and the type of interaction was synergism

Although in the control groups *D. magna* coped well with the rise of light intensity from 10,830 lx to 26,000 lx, the results were different in the presence of MPs. Indeed, the increase of mortality in parental females exposed to the highest MP concentration (from 10% to 20%), the significant differences between the toxicity curves of MPs on living offspring at distinct light intensity, the lower EC_{10}, EC_{20} and EC_{50} of MPs on reproduction at 26000 lx than at 10830 lx, and the significant lower somatic growth and population growth rate at 0.09 and 0.19 mg/L of MPs indicate that light intensity rise increased the adverse effects of MPs on *D. magna* individual and population fitness, leading to H_{01} rejection and acceptance of H_{A1}.

During the exposure period, parental females were in accelerated growth or in reproduction that are highly energy demanding processes. Adapting and responding to chemically induced stress is also energy expensive (Sengupta et al., 2016). Thus, under MP exposure, females likely needed additional energy but the presence of MPs in test media may have decreased food ingestion, as found for other MPs (Ogonowski et al., 2016). The increase of light intensity may have also influenced the swimming activity leading to alterations in both MP and microalgae uptake. Thus, at 26000 lx females may have needed to allocate more energy from growth and reproduction to deal with MP-induced stress and increased light intensity than at 10830 lx, as suggested by the comparison of MP toxicity curves at distinct light intensity. Indeed, based on the living offspring EC_{50} ratio (20 °C/M-light EC_{50} / 20 °C/H-light EC_{50}), the reproductive toxicity of MPs was 1.4 fold increased by light intensity rise.

Considering the criteria to identify the type of interaction in factorial studies with stressors (Crain et al., 2008) and the values of the individual and interactive Hedge's *d* with the respective 95% CI obtained, the type of interaction between increased light (26,000 lx) and MP stress on *D. magna* population growth rate in relation to the control (10,830 lx and water temperature of 20 °C, no MPs) was synergism at all the concentrations of MPs tested. The approach used (Crain et al., 2008) based on Gurevitch et al. (2000) and previous studies (e.g. Hedges and Olkin, 1985; Gurevitch et al., 1992) was very important to detect the type of interaction even at low concentrations of MPs.

4.4. Water temperature rise increased the adverse effects of microplastics on *D. magna* fitness and the type of interaction changed with microplastic concentrations

Water temperature rise from 20 °C to 25 °C increased the long-term toxicity and adverse effect of MPs on *D. magna* fitness, as indicated by higher parental mortality (reaching 30% at 0.19 mg/L of MPs), lower EC_{50} of MPs on living offspring, lower somatic growth and lower population growth rate at the higher MP concentrations tested in females exposed at water temperature of 25 °C than at 20 °C (10,830 lx in both cases). Thus, H_{02} was rejected and H_{A2} was accepted. Also, under exposure to 0.19 mg/L of MPs, the release of the first brood was delayed and

the total number of broods was lower. Moreover, females exposed to 0.09 and 0.19 mg/L of MPs, produced significantly lower number of total offspring and living offspring.

Overall, these findings suggest that metabolic and other costs, such as the activation of antioxidant defences to respond to increased production of reactive oxygen species and avoid lipid peroxidation, among other mechanisms to cope with thermal stress effects that *D. magna* has at warmer temperatures (Khan and Khan, 2008; Im et al., 2020) may have contributed to the increase of MP toxicity at water temperature of 25 °C. Higher filtration and respiration rates at water temperature of 25 °C than at 20 °C (Burns, 1969; Khan and Khan, 2008) and potential modification of swimming behaviour at increased water temperature may have also increased the uptake of MPs and/or of the chemicals that they contain. Moreover, water temperature rise may have also changed the distribution, biotransformation, interaction with molecular targets and elimination of microplastics (and/or of their chemicals), contributing to higher MP toxicity at water temperature of 25 °C than at 20 °C.

Independently of the mechanisms involved, the comparison of bioassays at 20 °C/M-light and 25 °C/M-light indicate that water temperature modulated the long-term effects of MPs on *D. magna* fitness. Based on the ratio of the MP EC_{50}s on living offspring, the rise of temperature from 20 °C to 25 °C increased the toxicity of MPs by 1.4 folds. Based on the maximal decrease of the population growth rate in relation to the respective control group (27% at 20 °C, 59% at 25 °C), under 10,830 lx, the rise of water temperature from 20C to 25 °C increased the adverse effects of MPs by 2.2 fold. Moreover, MPs completely inversed the positive effects of water temperature rise on *D. magna* population fitness in their absence (control groups).

Using the approach described in Crain et al. (2008), based on the individual and interactive effects of increased temperature and each of the MP concentrations on *D. magna* population growth rate in relation to the control (water temperature of 20 °C, 10830 lx), the type of interaction was antagonism at 0.04 mg/L of MPs, and synergism at the higher concentrations of MPs tested (0.09 and 0.19 mg/L). Differences in the type of interaction at low and high levels of stressors have been reported, such as in *D. magna* exposed to the same type of MPs and gold nanoparticles (Pacheco et al., 2018), and in *D. magna* exposed to ammonium and temperature variation (Serra et al., 2020). Synergism between temperature rise and another type of MPs on *D. magna* filtration capacity was also found (Serra et al., 2020), and synergism between temperature and other environmental contaminants (e.g. cadmium) in *D. magna* population growth rate has been also documented (Heugens et al., 2006). Interaction of temperature with several other stressors (e.g. nutrients, toxins, toxicants, salinity, UV), among others, have been widely documented in population and community studies with different organisms as highlighted in meta-analysis studies (e.g. Crain et al., 2008). However, the interactions between two or more stressors are complex, can be influenced by several other factors, and more knowledge is needed. Regarding MPs, the challenges are increased by the very high diversity of the plastic particles found in the environment.

4.5. Implications to natural ecosystems

The results of the present work and studies from the literature carried out with diverse types of MPs in the laboratory (Martins and Guilhermino, 2018; Pacheco et al., 2018; Schür et al., 2020; Zimmermann et al., 2020) and in more realistic scenarios using mesocosms deployed in the field (Aljaibachi et al., 2020) showed reduced *D. magna* fitness under long-term exposure to MPs. Such results suggest that in natural ecosystems, the fitness of wild *D. magna* populations is likely reduced by MP pollution.

The results of the present study also show that the rise of light intensity or water temperature within ecologically relevant values increase the adverse long-term effects of MPs on *D. magna* population fitness. According to the results obtained, in shallow freshwater ecosystems particularly small ones, under comparable levels of other stressors, the reduction of *D. magna* population fitness caused by MPs is expected to be higher in regions with high light intensity than in those with low light intensity, and at higher water temperatures than at lower ones. In the same ecosystem, the effects of MPs on *D. magna* population fitness may be different along the year depending of the light intensity and temperature variation patterns, among other factors (e.g. food availability), making more difficult to assess risks. In real scenarios, the combined adverse effects of MPs, water temperature and light intensity on *D. magna* may be higher than those found here due to the negative effects of UV radiation, food limitation, and the presence of other stressors, such as predators, parasites and other pollutants acting together (e.g. Heugens et al., 2006; Coors and De Meester, 2008; Serra et al., 2020). Indeed, the cumulative effects of multiple stressors frequently result in increased adverse effects (Crain et al., 2008).

D. magna and other zooplankton species have adaptation responses to face light intensity and water temperature alterations, such as changes in the diurnal vertical migration (DVM) pattern, among several others (Storz and Paul, 1998; Kessler and Lampert, 2004; Bruijning et al., 2018; Serra et al., 2019). Regarding DVM, under increased light and/or temperature, *D. magna* and other daphnids tend to increase the time spent in deeper layers of the water to decrease the exposure to intense light, UV radiation and high temperature of superficial water (Storz and Paul, 1998; Kessler and Lampert, 2004). However, the DVM depends also of other factors, such as food availability, oxygen levels and temperature in different layers of the water (Kessler and Lampert, 2004). Spending more time in deeper layers of the water may decrease the uptake of low density MPs that are mainly in superficial water. However, in thermally stratified lakes, spending more time in the hypolimnion generally reduces phytoplankton intake leading to fitness reduction if the availability of other adequate food resources in deeper layers of the water is limited. Moreover, in the hypolimnion, the water temperature is generally low and oxygen levels may be also reduced requiring additional energy to maintain basic functions. In general, such conditions are not adequate for growth and reproduction of daphnids. Also, spending more time in lower layers of the water column may increase the exposure to high density MPs that tend to be more abundant in bottom water, as well as to other environmental contaminants present in this layer of the water and in superficial sediment.

In shallow water systems (e.g. ponds, rock pools), changes in DVM may have a limited efficacy in preventing exposure to intense light and UV radiation and to increased water temperature. Therefore, in such ecosystems, the combined effects of MP pollution, and increased light intensity, UV radiation and water temperature may be particularly challenging to *D. magna* populations, as well as to populations of other species. Moreover, increased water temperature may reduce the oxygen concentration in the water, which can act as an additional stress factor (Storz and Paul, 1998). Increased light intensity, UV radiation and water temperature may also promote changes in MPs (Andrady, 2017), such as greater fragmentation originating smaller particles that in general are more toxic, and facilitating the release of plastic components, including additives (Mansilha et al., 2013) increasing their bioavailabilty in the water. MP fragmentation may also occur in *D. magna* gut after MP ingestion as found in other species (Dawson et al., 2018). The formation of smaller MPs facilitates their passage through biological barriers (Miranda et al., 2019), as well as their distribution inside the body and accumulation in internal organs and tissues, increasing the probability of adverse effects caused by MPs and the chemicals that they contain in sensitive biological targets. Moreover, MP fragments are more toxic to *D. magna* than regular shaped MP particles (An et al., 2021).

In the present study, *D. magna* was previously acclimated to the light intensity and water temperature tested. In *D. magna* and several other organisms, previous or gradual acclimation to changes in light intensity and temperature is very important because allows adaptation through responses at molecular, biochemical and physiological levels (Paul et al., 2004; Coggins et al., 2017; Gust et al., 2019), as well as at individual and population levels (Giebelhausen and Lampert, 2001;

Khan and Khan, 2008; Bruijning et al., 2018; Hoefnagel et al., 2018). However, in the wild, changes may occur rapidly. Moreover, the frequency of extreme events, such as heatwaves, is increasing as result of global climate changes. Sudden changes in environmental conditions may influence considerably the individual traits, genetic composition and population dynamics of *D. magna* (Adamczuk, 2020). The outcome may be reduced population fitness, especially if other stressfully conditions exist, such as food limitation, pollution and predators. In such conditions, the combined effects of MP pollution, high light intensity and increased water temperature, and other stressors on zooplankton species may be difficult to overcome.

In the wild, *D. magna* populations have genetic diversity and plasticity. Because some clones of *D. magna* are more susceptible to MPs, other environmental contaminants, and physical factors variation than others (Mitchell et al., 2004; Messiaen et al., 2010; Sadler et al., 2019), MP pollution may have also adverse impacts on the diversity of *D. magna* populations, especially under simultaneous exposure to other stressors. Negative effects of MPs on other zooplankton species, both freshwater and marine, and interspecific differences of sensitivity to MPs were also found (Botterell et al., 2019; Jaikumar et al., 2019). Moreover, MPs can influence interspecific relationships (van Colen et al., 2020). Therefore, changes at community level with potential implications to ecosystem functioning may also occur.

In aquatic ecosystems, zooplankton plays a major role in controlling phytoplankton populations and is an important food resource to higher trophic levels, among other ecological functions. As aquatic ecosystems around the world are polluted with MPs (Picó and Barcelò, 2019; Du et al., 2020; Xu et al., 2020), in some cases with considerable MP pollution levels (Moore et al., 2011; Free et al., 2014; Lasee et al., 2016), the reduction of *D. magna* fitness under MP exposure highlights the potential consequences of MP pollution to aquatic biodiversity, ecosystem functioning, and ecosystem services.

5. Conclusions

The influence of increased light intensity (26,000 lx) and water temperature (25 °C) on the long-term toxicity of MPs (microspheres, 1–5 μm diameter, concentrations between 0.04 and 0.19 mg/L) to *D. magna* were investigated in relation to water temperature of 20 °C and light intensity of 10,830 lx. At all the environmental conditions tested, MPs caused parental mortality and the release of immobile juveniles, and reduced the size at the first brood release, the somatic growth, the number of total and living offspring released, and the population growth rate. At increased water temperature (25 °C), MPs also delayed the release of the first brood and decreased the total number of broods produced. The NOEC values ranged from 0.04 to 0.09 mg/L of MPs, and the LOEC values from 0.09 to 0.19 mg/L of MPs, depending of the effect criterion, water temperature and light intensity considered.

At water temperature of 20 °C and light intensity of 10,830 lx, the EC_{50} of MPs on living offspring was 0.146 mg/L (95 CL: 0.142–0.151). The rise of light intensity to 26,000 lx increased the effects of MPs on living offspring by about 1.4 fold (EC_{50} = 0.102 mg/L, 95% CI: 0.099–0.105 mg/L of MPs), and water temperature rise to 25 °C had a comparable effect in this effect criterion (EC_{50} = 0.101 mg/L, 95% CL: 0.098–0.104 mg/L of MPs). Based on the population growth rate maximal decrease (at 0.19 mg/L of MPs), the long-term (21-day) adverse effects of MPs on *D. magna* population fitness increased (1.4 fold) with the rise of light intensity from 10,830 lx to 26,000 lx, and with the rise of water temperature from 20 °C to 25 °C (2.2 fold).

Using the conceptual approach to identify the type of interaction between stressors in factorial population or community studies described in Crain et al. (2008) applied to the effects of increased light intensity (26,000 lx) and MPs (0.04, 0.09 or 0.19 mg/L) on *D. magna* population growth rate measured through the Hedge's *d* in relation to the control (no MPs) at light intensity of 10,830 lx and water temperature of 20 °C, the type of interaction between the two stressors was found to be synergism at all the MP concentrations tested. Using the same approach, the type of interaction between increased water temperature (25 °C) and microplastics was antagonism at the lowest concentration of MPs tested (0.04 mg/L), and synergism at higher MP concentrations (0.09 and 0.19 mg/L).

The previously indicated findings raise concern because *D. magna* and other zooplankton species play a fundamental function in aquatic ecosystem functioning, MP pollution is a worldwide paradigm, and temperature and light intensity variate with latitude and have been increasing in many aquatic ecosystems across the globe due to global climate changes. Further studies, especially on the combined long-term effects of MPs, light intensity and temperature increase, and other alterations resulting from climate changes are needed.

CRediT authorship contribution statement

L. Guilhermino: Conceptualization, Methodology, Investigation, Validation, Formal analysis, Resources, Data Curation, Writing - Original Draft, Writing - Review & Editing, Visualization, Supervision, Project administration, Funding acquisition.

A. Martins: Methodology, Investigation, Writing - Review & Editing.

S. Cunha: Methodology, Investigation, Validation, Resources, Data Curation, Writing - Original Draft (bisphenol analyses), Writing - Review & Editing, Visualization, Funding acquisition.

J.O. Fernandes: Methodology, Investigation, Validation, Resources, Data Curation, Writing - Review & Editing, Visualization, Funding acquisition.

Declaration of competing interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.147082.

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