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Warmer water, high light intensity, lithium and microplastics: Dangerous environmental combinations to zooplankton and Global Health?

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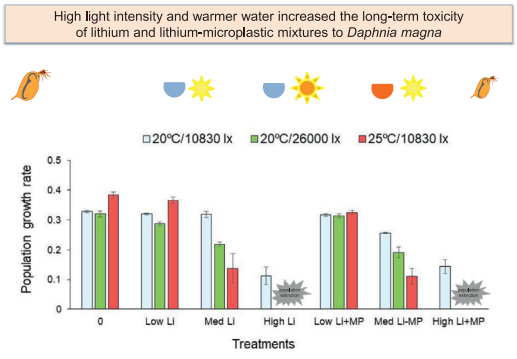
HIGHLIGHTS

- Warmer water increased the long-term toxicity of Li and Li-MPs mixtures to *D. magna*.
- High light intensity (low UV) also augmented the toxicity of Li and Li-MPs mixtures.
- Temperature rise and chemical stress interact synergistically in all the scenarios.
- Light intensity rise and chemical stress interact mainly synergistically.
- 0.08 and 0.1 mg/L of Li, alone and in Li-MPs mixtures caused population extinction.

GRAPHICAL ABSTRACT



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ABSTRACT

Nowadays there is a high concern about the combined effects of global warming and emerging environmental contaminants with significant increasing trends of use, such as lithium (Li) and microplastics (MPs), both on wildlife and human health. Therefore, the effects of high light intensity (26,000 lx) or warmer water temperature (25 °C) on the long-term toxicity of Li and mixtures of Li and MPs (Li-MPs mixtures) were investigated using model populations of the freshwater zooplankton species *Daphnia magna*. Three 21-day bioassays were done in the laboratory at the following water temperatures and light intensities: (i) 20 °C/10830 lx; (ii) 20 °C/26000 lx (high light intensity); (iii) 25 °C/10830 lx (warmer temperature). Based on the 21-day EC_{50}s on reproduction, high light intensity increased the reproductive toxicity of Li and Li-MPs mixtures by ~1.3 fold; warmer temperature increased the toxicity of Li by ~1.2 fold, and the toxicity of Li-MPs mixtures by ~1.4 fold based on the concentration of Li, and by ~2 fold based on the concentrations of MPs. At high light intensity, Li (0.04 mg/L) and Li-MPs mixtures (0.04 Li + 0.09 MPs mg/L) reduced the population fitness by 32 % and 41 %, respectively. Warmer temperature, Li (0.05 mg/L) and Li-MPs mixtures (0.05 Li + 0.09 MPs mg/L) reduced it by 63 % and 71 %, respectively. At warmer temperature or high light intensity, higher concentrations of Li and Li-MPs mixtures lead to population extinction. Based on the population growth rate and using data of bioassays with MPs alone done simultaneously, Li and MPs interactions were antagonistic or

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synergistic depending on the scenario. High light intensity and chemical stress generally acted synergistically. Warmer temperature and chemical stress always acted synergistically. These findings highlight the threats of long-term exposure to Li and Li-MPs mixtures to freshwater zooplankton and Global Health in a warmer world.

1. Introduction

Global warming is progressing faster than previously predicted (IPCC, 2019, 2021). Acting together with other consequences of global climate changes and human population growth (e.g., pollution, habitat degradation and loss, and overexploitation of natural resources, among others), it is decreasing the biodiversity, changing the species distribution and the dynamics of communities, and increasing the risks posed by exotic invasive species (Dudgeon, 2019; Martínez-Megías and Andreu Rico, 2022; Talukder et al., 2022). Such environmental changes are also altering the patterns of human diseases (Maharjan et al., 2021; Santos-Guzman et al, 2021; Yang et al., 2021; Grobusch and Grobusch, 2022), promoting the emergence of new ones and facilitating pandemics, rising the sea water levels, and shaping landscapes determining their adequacy to human settlement and activities. They are also having economic and social consequences, and increasing the risk of conflicts for limited resources, such as freshwater (Dudgeon, 2019; Jerez et al., 2021). Therefore, the ongoing environmental alterations pose major challenges to wildlife and human society.

Among the effects of global warming, the increase of temperature and light intensity in many regions across the world, raises high concern as it can affect the behaviour, health and wellbeing of humans and other animals *per see*, and influence the effects of other stressors (Martins et al., 2013; Ferreira et al., 2016; Nieto et al., 2016; Serra et al., 2020; Guilhermino et al., 2021a; Lyu et al., 2021). Adaptation to a warmer world with more light requires further knowledge on the long-term combined effects of temperature elevation, light intensity rise, and pollution. Natural substances intensively explored and environmental contaminants of high concern with global distribution, significant increasing trends of presence in the environment, and able to cause neurotoxicity and reproductive toxicity, such as lithium (Li) and plastic particles with size lower than 5 mm known as microplastics (MPs), require special attention because they have the potential to reduce the fitness of populations rapidly.

Li is a metal naturally present on earth that occurs in all the environmental compartments (Bolan et al., 2021; Chaves et al., 2021). It is used in several types of industries (Kszos and Stewart, 2003) and health care (Haupt et al., 2021). Li has having an extraordinary trend of demand mostly due to its use in energy storage devices (Marín et al., 2021) resulting in growing environmental contamination in many areas (Choi et al., 2019; Bolan et al., 2021; Melchor-Martínez et al., 2021). It is widely spread in food webs, is bioconcentrated and bioaccumulated, and has high biological activity (Aral and Vecchio-Sadus, 2008; García-Seoane et al., 2016; Bolan et al., 2021; Thibon et al., 2021). In addition to neurotoxicity (Oliveira et al., 2011; Viana et al., 2020; Costa et al., 2021) and reproductive toxicity (Kszos et al., 2003; Martins et al., 2022), long-term exposure to Li can cause a variety of adverse effects in animals (Davis et al., 2018; Tkatcheva et al., 2015; Kim et al., 2017; Liu et al., 2018; Jing et al., 2021), including humans (Harari et al., 2016; Diserens et al., 2021; Verdoux et al., 2021). Li acts through several mechanisms that are not yet completely elucidated, despite the high number of studies on the topic, the great therapeutic use of this metal, and the possibility of new applications in health care (Haupt et al., 2021; Krull et al., 2022), energy production and storage, among others.

Our society has a high dependency of plastics that resulted in a huge and very challenging global pollution problem. Despite all the efforts, the worldwide growing trend of plastic pollution continues and has been accelerated by the SARS-CoV-2 pandemics (Canning-Clode et al., 2020; Guilhermino et al., 2021a, 2021b; Ray et al., 2022). Among plastics, MPs are of special concern due to their global occurrence in the environment (Amelia et al., 2021; Kumar et al., 2021; Torres-Agullo et al., 2021; Talbot and Chang, 2022), wild animals and food webs (Guilhermino et al., 2021b; Vital et al., 2021; Bertoli et al., 2022; Bhutton and You, 2022), high environmental persistence, long-range circulation in the environment, diversity of properties (Andrady, 2017), and bioactivity. Some MPs are accumulated by living organisms, as well as many of the additives and other environmental contaminants that they generally contain (Campanale et al., 2020; Eder et al., 2021). In addition to neurotoxicity (Barboza et al., 2018b; Sulukan et al., 2021) and reproductive toxicity (Pacheco et al., 2018; Liu et al., 2022; Schür et al., 2020; Schwarzer et al., 2022), MPs can cause many other toxic effects (Castro-Castellon et al., 2021; Gonçalves and Bebianno, 2021; Guimarães et al., 2021; Kukkola et al., 2021), and reduce the fitness of populations (Martins and Guilhermino, 2018; Amorim and Scott-Fordsmand, 2021; Trotter et al., 2021). They also interact with the toxicity of other contaminants in animals (Pacheco et al., 2018; Thi et al., 2021; Eder et al., 2021), and may modify large-scale environmental processes (Agathokleous et al., 2021). Exposure to MPs and associated chemicals likely has also adverse effects on the human health (Barboza et al., 2018a; Campanale et al., 2020; Prata et al., 2020; Ferrante et al., 2022), and a recent study documenting the presence of MPs in human blood (Leslie et al., 2022) augmented the concerns. More research, as well as methodological and technological improvement, is needed (Eder et al., 2021; Kukkola et al., 2021; Ockenden et al., 2021) to increase our understanding on the MPs paradigm and its impacts.

As Li and MPs occur globally (Aral and Vecchio-Sadus, 2008; Bolan et al., 2021; Wang et al., 2021b; Talbot and Chang, 2022), are used together in many industries (e.g., batteries, electric vehicles, electronics, plastics, Li-extraction and transformation) and products of daily use (e.g., mobile phones, computers, electric devices), humans and other animals likely are simultaneously exposed to them along their life-time. Changes of temperature and/or light can modify the gene expression, physiology, behaviour and life traits of animals (e.g., Mitchell and Lampert, 2000; Huegens et al., 2006; Bae et al., 2016; Gust et al., 2019; Ulbing et al., 2019; Serra et al., 2020; Cremer et al., 2022), as exposure to Li (e.g., Kszos and Stewart, 2003; Nagato et al., 2013; Kim et al., 2017; Viana et al., 2020; Martins et al., 2022) and MPs (Sadler et al., 2019; Pacheco et al., 2018; Lyu et al., 2021; Liu et al., 2022) can also do. Light influences the effects of neuroactive environmental contaminants (Simão et al., 2019) and both Li (e.g., Oliveira et al., 2011; Viana et al., 2020) and MPs (e.g., Barboza et al., 2018a; Sarasamma et al., 2020) act in the nervous system. Moreover, in exposed animals, interactions between MPs and Li (Costa et al., 2021; Martins et al., 2022), temperature and MPs (e.g., Ferreira et al., 2016; Sulukan et al., 2021), temperature and Li (Rodríguez et al., 2021), and light and MPs (Guilhermino et al., 2021a) were found. Therefore, it is possible that warmer temperature and/or high light intensity influence the combined effect of Li and MPs.

Water quality is crucial to achieve the United Nations Development Sustainable Goals. Poor water quality compromises biodiversity, ecosystem services, and favours the spread of animal and human diseases, including zoonotic and infectious ones (Dudgeon, 2019; Santos-Guzman et al, 2021; Zamir et al., 2022). Many freshwater ecosystems are particularly vulnerable to global warming, chemical contamination by Li and MPs, among other impacts, due to their characteristics (e.g., low water volume, shallow waters) and localization (e.g., Li naturally enriched regions, urbanized and industrial areas, proximity to e-trash dumping sites). Freshwater scarcity is already a huge problem in many regions, and getting water of good quality is a growing global paradigm.

Daphnia magna is a small freshwater crustacean widely used in environmental research and safety assessment that has several characteristics favouring its use as a model to investigate the long-term effects of environmental changes in relation to 'Global Health'. It is a zooplankton organism

and zooplankton populations significantly contribute to water of good quality, as well as to several other ecosystem goods and services. *D. magna* has an advanced nervous system (Kim et al., 2017; Bedrossiantz et al., 2021), a relatively short life cycle, a wide range of distribution and high ecological relevance in diverse types of freshwater ecosystems (Effertz and von Elert, 2017; Serra et al., 2019). Moreover, *D. magna* behaviour, life traits, individual and population fitness, and responses to stressors are influenced by temperature (Mitchell and Lampert, 2000; Khan and Khan, 2008; Martins et al., 2013; Im et al., 2020) and light (Storz and Paul, 1998; Effertz and von Elert, 2017; Gust et al., 2019; Guilhermino et al., 2021a). Several types of MPs induce long-term toxicity in this species (e.g., Pacheco et al., 2018; Schür et al., 2020; An et al., 2021; Liu et al., 2022), whereas others do not (e.g., Hiltunen et al., 2021). In *D. magna*, Li disrupts several signalling pathways (Kim et al., 2017), causes several metabolomic alterations (Nagato et al., 2013), and reduces survival, reproduction and the population fitness after long-term exposure (Bozich et al., 2017; Martins et al., 2022).

The goals of the present study were to investigate the effects of high light intensity or warmer temperature on the long-term toxicity of Li, alone and combined with MPs (Li-MPs mixtures), to *D. magna*, including the potential toxicological interactions between stressors on the population fitness.

This study is important because temperature and light intensity have been rising in many regions of the planet changing the patterns of environmental, animal and human threats and often increasing their adverse effects (e.g., Dudgeon, 2019; Maharjan et al., 2021; Watts et al., 2021). Moreover, urbanization and human activity are increasing artificial light at night, disrupting circadian cycle patterns and interfering with gene expression and physiology of animals, leading to life-trait changes (Maszczyk et al., 2021; Cremer et al., 2022). Furthermore, the growing global trends of Li and MPs environmental contamination are very concerning, and one needs to have a better understanding of global warming effects on their long-term toxicity.

2. Material and methods

2.1. Null and alternative hypotheses

Four null hypotheses were tested: H_{01} – High light intensity does not increase the long-term toxicity of Li to *D. magna*; H_{02} – High light intensity does not increase the long-term toxicity of Li-MPs mixtures to *D. magna*; H_{03}: Warmer temperature does not increase the long-term toxicity of Li to *D. magna*; H_{04} – Warmer temperature does not increase the long-term toxicity of Li-MPs mixtures to *D. magna*. The alternative hypotheses to H_{01}, H_{02}, H_{03} and H_{04} were, respectively: H_{A1} – High light intensity increases the long-term toxicity of Li to *D. magna*; H_{A2} – High light intensity increases the long-term toxicity of Li-MPs mixtures to *D. magna*; H_{A3} – Warmer temperature increases the long-term toxicity of Li; H_{A4} – Warmer temperature increases the long-term toxicity of Li-MPs mixtures to *D. magna*.

2.2. Water temperature, light intensity and chemicals

Water temperatures of 20 °C and 25 °C and light intensity of 10,830 lux (lx) and 26,000 lx were chosen for this study mainly because they are ecologically relevant, the variation of water temperature or light intensity between the indicated values or in ranges covering them influence the performance of *D. magna* (Mitchell and Lampert, 2000; Hoefnagel et al., 2018; Serra et al., 2019), and the long-term toxicity of some contaminants to this species (Vandenbrouck et al., 2011; Martins et al., 2013; Serra et al., 2020; Guilhermino et al., 2021a).

In the experiments, Li was used as lithium chloride (LiCl), p.a. (Merck, Germany). The MPs tested were fluorescent microspheres (dry powder, Cospheric Innovations in Microtechnology, U.S.A., reference of the product: FMR-1.3), with the following properties according to the manufacturer: 1–5 μm diameter, 1.3 g/cm^3 density, ~1.836E^{+8} polymer microspheres per mg of the product, excitation wavelength of 575 nm and emission wavelength of 607 nm. These MPs were chosen for this work because they are very small and fluorescent, induce long-term toxicity in *D. magna* (Martins and Guilhermino, 2018; Guilhermino et al., 2021a), their behaviour in the test medium was studied before (Pacheco et al., 2018), and they interacted with Li in *D. magna* long-term exposed simultaneously to both contaminants (Martins et al., 2022).

The other chemical substances used were obtained from Sigma-Aldrich (Germany), Merck (Germany), and other suppliers as indicated in the Supplementary material (Section 1).

2.3. Model populations, experimental design and exposure conditions

The model populations of *D. magna* tested (G1, G2 and G3) were from laboratorial individual cultures, and were previously acclimated for three generations to the light intensity and water temperature to be tested, as described in Guilhermino et al. (2021a). Briefly, the populations were acclimated in chambers (Bronson PGC 1400 chambers, Netherlands; light from Sylvania Lightning, Linx CF-LE 55W/840 lamps, cool white fluorescent, low UV radiation), with controlled temperature, light intensity and photoperiod (16 h light and 8 h dark – 16 h L: 8 h D). G1 was acclimated to light intensity of 10,830 lx and water temperature of 20 ± 1 °C (20 °C/10830 lx), G2 to water temperature of 20 ± 1 °C and light intensity of 26,000 lx (20 °C/26000 lx), and G3 to water temperature of 25 ± 1 °C and light intensity of 10,830 lx (25 °C/10830 lx). All females were maintained in parthenogenetic reproduction, in hard water of the American Society for Testing and Materials - ASTM (ASTM, 1980), with vitamins and an extract of *Ascophyllum nodosum* (Martins and Guilhermino, 2018), hereafter indicated as test medium. Each female was maintained in a 100 mL glass beaker with 50 mL of test medium, and feed with 3 × 10^5 cells/mL/daphnia of *Chlorella vulgaris* cultured in the laboratory (Guilhermino et al., 2021a).

Three 21-day bioassays testing the effects of Li, alone and in mixture with MPs (Li-MPs mixtures), were carried out according to the OECD guideline 211 (OECD, 2012), with punctual alterations, at the following water temperature and light intensity: 20 °C/10830 lx with G1 (moderate conditions), 20 °C/26000 lx with G2 (high light intensity), and 25 °C/10830 lx with G3 (warmer temperature). This design was selected to investigate the effects of high light intensity or warmer water temperature, separately, on the long-term toxicity of Li and Li-MPs mixtures, allowing testing each of the hypotheses and diagnosing the potential interactions between each pair of stressors on *D. magna* population growth rate in different exposure scenarios (Table 1). The results of the bioassay at moderate conditions were described and discussed in detail elsewhere (Martins et al., 2022), and some of them were used in the present study for comparative purposes. Selected data from three 21-day bioassays testing the effects of MPs alone at moderate conditions, high light intensity and warmer temperature with G1, G2 and G3, respectively (Guilhermino et al., 2021a), were used to investigate some of the combined effects of stressors. All the bioassays testing the effects of MPs or the effects of Li and Li-MPs mixtures carried out at the same temperature and light intensity were performed simultaneously in the same test chamber, their treatments were prepared from the same Li and MPs stock solutions, the parental females were from the same model population, and the control was the same. The other experimental conditions (e.g., food, test medium, exposure conditions and time, among others) were similar for all the bioassays.

The bioassays were conducted in the above-indicated test chambers with the same lamps, under a photoperiod of 16 h L: 8 h D. The test medium was the same of the acclimation period, and it had at least ~52.5 mg/L of Na, among and other ions (Ca, K, Mg), from ASTM. In each bioassay, the treatments were: control (test medium), 0.02, 0.04 and 0.08 mg/L of Li alone, and 0.02 Li + 0.05 MPs mg/L, 0.04 Li + 0.1 MPs mg/L and 0.08 Li + 0.2 MPs mg/L of Li-MPs mixtures. These concentrations were selected based on the findings of studies investigating the toxicity of Li in *D. magna* (Nagato et al., 2013; Bozich et al., 2017; Kim et al., 2017) and *C. dubia* (Kszos et al., 2003), and the long-term effects of the same type of MPs in *D. magna* (Martins and Guilhermino, 2018; Pacheco et al., 2018).

Table 1
Scenarios used to determine the type of interaction between pairs of stressors. The concentrations indicated are the estimated exposure concentrations. Sc – scenarios (Sc1–Sc18). Temperature – water temperature. Li – lithium. MP – microplastics. Li-MP – lithium-microplastic mixtures. L – low concentration. M – medium concentration. H – high concentration. Light-H – high light intensity (26,000 lx). Tem-H – warmer water temperature (25 °C).

Interaction	Sc	Stressor A	Stressor B	Combined (interaction)	Temperature Light intensity Exposure	Control
Li × MP	Sc1	Li - L	MP - L	Li-MP - L	20 °C	Control group at 20 °C,
	Sc2	Li - M	MP - M	Li-MP - M	26,000 lx	26,000 lx
	Sc3	Li - H	MP - H	Li-MP - H		
Li × MP	Sc4	Li - L	MP - L	Li-MP - L	25 °C	Control group at 25 °C,
	Sc5	Li - M	MP - M	Li-MP - M	10,830 lx	10,830 lx
	Sc6	Li - H	MP - H	Li-MP - H		
Li × Light-H	Sc7	Li - L	Light-H	Li - L, Light-H	20 °C	Control group at 20 °C,
	Sc8	Li - M	Light-H	Li - M, Light-H	26,000 lx	10,830 lx
	Sc9	Li - H	Light-H	Li - H, Light-H		
Li-MP × Light-H	Sc10	Li-MP - L	Light-H	Li-MP - L, Light-H	20 °C	Control group at 20 °C,
	Sc11	Li-MP - M	Light-H	Li-MP - M, Light-H	26,000 lx	10,830 lx
	Sc12	Li-MP - H	Light-H	Li-MP - H, Light-H		
Li × Tem-H	Sc13	Li - L	Tem-H	Li - L, Tem-H	20 °C	Control group at 20 °C,
	Sc14	Li - M	Tem-H	Li - M, Tem-H	26,000 lx	10,830 lx
	Sc15	Li - H	Tem-H	Li - H, Tem-H		
Li-MP × Tem-H	Sc16	Li-MP - L	Tem-H	Li-MP - L, Tem-H	25 °C	Control group at 20 °C,
	Sc17	Li-MP - M	Tem-H	Li-MP - M, Tem-H	10,830 lx	10,830 lx
	Sc18	Li-MP - H	Tem-H	Li-MP - H, Tem-H		

Their environmental realism is discussed in Section 4. The treatments were prepared immediately before the starting of each bioassay and each test medium renewal, by diluting stock solutions of LiCl (200 mg/L), and MPs (400 mg/L) in the case of Li-MPs mixtures, into test medium.

Each bioassay was initiated with juvenile females (>6 h and <24 old) from the 3rd brood of the model populations previous acclimatized to the water temperature and light intensity to be tested. Each female was put in a 100 mL glass beaker with 50 mL of test medium, which was renewed at each 24 h, and fed daily with *C. vulgaris* (3×10^5 cells/mL/daphnia, ~0.322 mg of carbon/daphnia/day, Guilhermino et al., 1999). In all the bioassays, the beakers were not agitated during the day, and ten individually exposed females were used per treatment.

The effect criteria were the mortality of parental females, the total somatic growth (somatic growth), the day of the first brood release, the total number of broods released (brood number), the number of total offspring (total offspring), the number of living offspring (living offspring), the number of dead offspring (dead juveniles), the number of aborted eggs, and the intrinsic rate of population increase (population growth rate), as indicative of population fitness (OECD, 2012; Martins and Guilhermino, 2018). Females were observed more than once per day, offspring and moults were immediately removed, and the data from parental females that died before the end of the exposure period were not analysed, except for parental mortality.

Test medium temperature, dissolved oxygen and pH were measured (HACH HQ40d multi probe, U.S.A.) in all the beakers at the beginning and at the end of the bioassays, and at each time of test medium renewal, in freshly prepared and 24 h old test medium, hereafter indicated as fresh and old, respectively. At these times, samples of fresh and/or old test medium were collected to determine the actual concentrations of Li and MPs. Light intensity was also measured (Roline RO-1332 Digital Luxmetter, Germany).

2.4. Determination of the actual concentrations of Li and MPs in test medium

The procedures used to determine the total concentrations of Li (hereafter indicated as actual concentrations of Li) in randomly selected samples of fresh and old test medium were previously described (Martins et al., 2022), and a short description is in the Supplementary material (Section 1). The detection (LOD) and quantification limits (LOQ) of the instrumental method were 2.20 and 6.70 μg/L, respectively (Martins et al., 2022). To each sample of test medium, the deviation of Li actual concentration relatively to the nominal one was determined as in Guilhermino et al. (2021a). To compare the exposure conditions, the results of the actual concentrations of Li determined in fresh and old test medium from the three bioassays, in a total of 333 samples, were analysed together.

Since in previous studies with the same type of MPs and test medium, the concentrations of the particles decreased within 24 h (Guilhermino et al., 2021a), all the test medium samples collected from beakers where the females survived until the end of the bioassay were analysed, namely 21 samples of fresh test medium and 21 samples of old test medium per beaker (each beaker with a parental female alive until the end of the exposure period) of Li-MPs mixtures. The total number of samples per bioassay was: 2646 at moderate conditions, 2520 at high light intensity, and 2268 at warmer temperature. The actual concentrations of MPs in test medium samples with nominal concentrations of 0.1 and 0.2 mg/L of MPs were determined by spectrofluorimetry as described in previous studies (Pacheco et al., 2018; Martins and Guilhermino, 2018). The actual concentrations of MPs in samples with nominal concentration of 0.05 mg/L were determined as described in Guilhermino et al. (2021a) due to low sensitivity of the method in this range. A brief description of the procedures is provided in the Supplementary material (Section 2). The deviation of the actual concentrations of MPs relatively to the nominal ones, and the reduction of the concentration of MPs during the interval of test medium renewal (MPs decay) were calculated as in previous studies (e.g., Guilhermino et al., 2021a). The time weighted means (TMW) were also estimated (OECD, 2012) because in some replicates the MPs decay was higher than 20 %, and were used to determine the estimated exposure concentrations (EECs) of MPs along the bioassays. The EECs in the Li-MPs mixtures with MPs nominal concentrations of 0.1 and 0.2 mg/L determined in the three bioassays were analysed together to compare the exposure conditions of MPs in mixture treatments of the same and distinct bioassays.

2.5. Data analyses

Data were analysed using the Mann-Whitney test (U) to compare two data sets or the Kruskal-Wallis test (H) to compare multiple data sets. When the Kruskal-Wallis test indicated significant differences among treatments, pairwise comparisons (with Bonferroni correction for multiple tests) were carried out to discriminate different treatments and determine the no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC) when adequate.

The NOEC and LOEC for each effect criterion, and the concentrations of Li or Li-MPs mixtures that caused 10 %, 20 % and 50 % of inhibition on *D. magna* reproduction (living offspring) after 21 days of exposure (21-day

EC_{10}, EC_{20} and EC_{50}, respectively) were determined in relation to the EECs along the 21-day exposure period. The 21-day EC_xs was calculated from a logistic model, with lower limit of zero, fitted to the appropriate data of each bioassay (Guilhermino et al., 2021a).

The potential interactions between pairs of stressors on *D. magna* population growth rate were investigated as described in Guilhermino et al. (2021a), with the effect sizes measured with no weighted Hedge's *d*, following Crain et al. (2008) and previous studies (Gurevitch et al., 1992, 2000). Briefly, the independent and combined effects of stressors, and the corresponding 95 % confidence intervals (95 % CI) were calculated (Gurevitch et al., 1992, 2000). Considering each scenario (2 stressors), if the 95 % CI of the combined effect (interaction) overlapped zero, the interaction was addition; otherwise, individual effects of both stressors negative or one negative and the other positive, the interaction was synergism when the combined effect was negative, and antagonism when the combined effect was positive (Crain et al., 2008). The pairs of stressors and the 18 exposure scenarios considered are indicated in Table 1. To calculate the combined effects (interaction) between each pair of stressors, data from bioassays carried out simultaneously with females from the same model populations testing MPs alone (Guilhermino et al., 2021a), and Li and Li-MPs mixtures at moderate water temperature (20 ± 1 °C) and light intensity of 10,830 lx (Martins et al., 2022) were used.

The *drc* extension package for dose-response analysis in R (Ritz et al., 2015) was used to logistic model fitting and parameter estimates. Hedges' *d* and their 95 % CI were determined in Microsoft Excel. The IBM SPSS statistical package (version 26) was used for the other data analyses. The selected significance level was 0.05.

3. Results

In the control groups, all the parental females survived until the end of the bioassays, the mean of the living offspring number per female was higher than 60, and the coefficients of variation ranged from 2.5 % to 3.5 % (Guilhermino et al., 2021a), therefore in accordance with OECD (2012). The variation of test medium temperature, pH and dissolved oxygen in the bioassays at high light intensity and warmer temperature (Table S1) are also in accordance with OECD (2012), as well as those measured during the bioassay at moderate conditions (Martins et al., 2022).

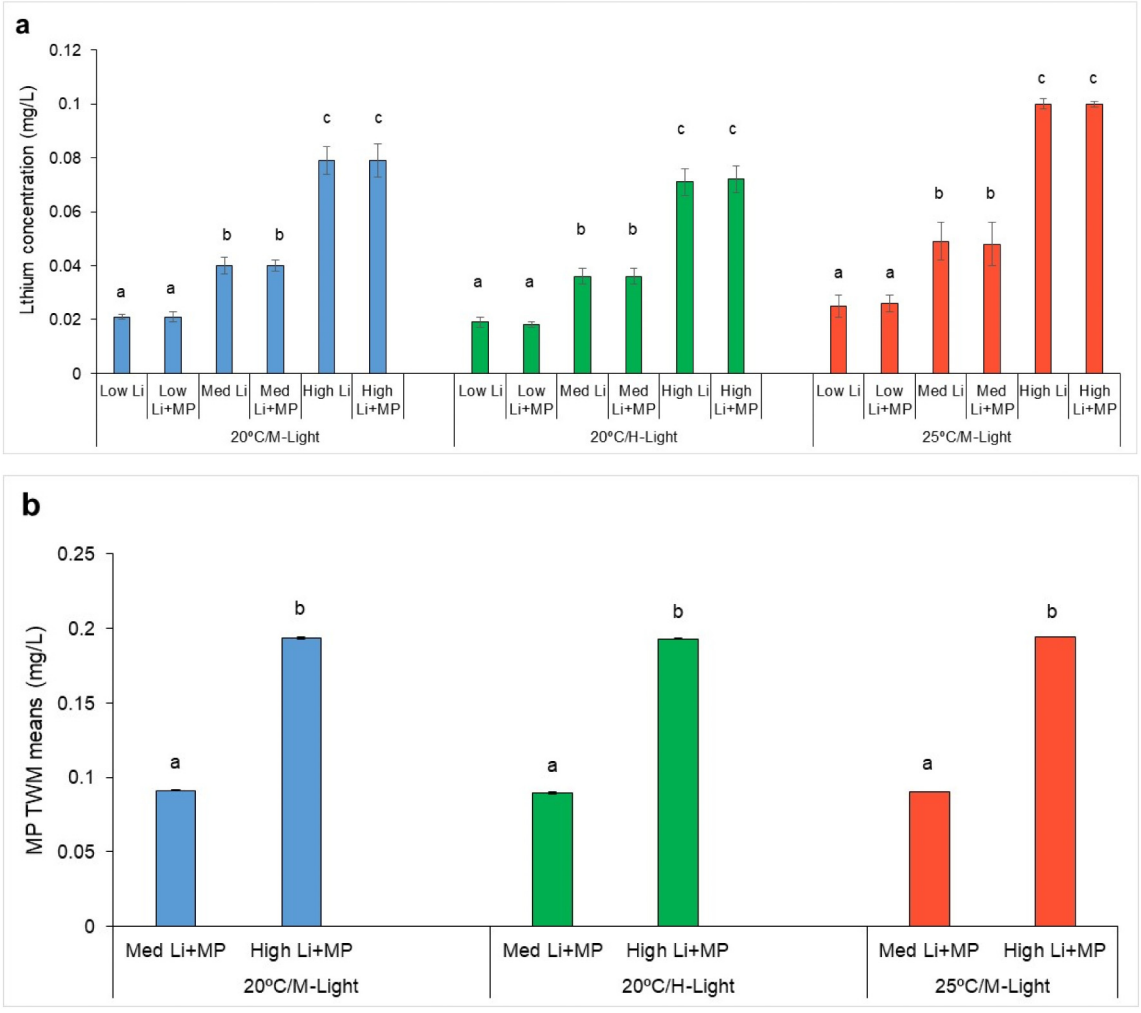

Fig. 1. Mean and standard deviation (bars above the means) of the actual concentrations of lithium (Li) per treatment indicated in the top (a) and of time weight means (TWM) of microplastics (MPs) per treatment indicated in the second part of the figure (b) determined along the bioassays carried out at moderate water temperature of 20 °C and moderate light intensity of 10,830 lx (20 °C/M-Light, blue bars), moderate water temperature and high light intensity of 26,000 lx (20 °C/H-light, green bars), and warmer water temperature of 25 °C and moderate light intensity (25 °C/M-light, red bars). Low Li – lowest concentration of Li. Med Li – medium concentration of Li. High Li – highest concentration of Li. Low Li + MP – lowest concentration of Li-MPs mixtures. Med Li + MP – medium concentration of Li-MPs mixtures. High Li + MP – highest concentration of Li-MPs mixtures. In Li analyses, the number of samples per treatment ranged from 12 to 20. In MP analyses, for each female alive until the end of the bioassay, a TWM was calculated from the actual concentrations of MPs determined in fresh and old test medium along the 21 days of exposure (42 samples), and the means of the TMW per treatment (4–10) are shown in the Figure. The data of the bioassay at 20 °C/M-Light was from Martins et al. (2022).

3.1. Exposure concentrations of Li and MPs along the bioassays

In the control groups of all the bioassays, the concentrations of Li were lower than the LOQ.

Regarding the actual concentrations of Li in test medium samples of the other treatments, no significant differences in the actual concentrations of Li between fresh and old test medium were found ($U = 0.261$, $p = 0.609$, $N = 333$). The means per treatment are shown in Fig. 1a. There were significant differences among treatments ($H_{17} = 318.818$, $p < 0.001$, $N = 333$), but there were no significant differences ($p > 0.05$) among treatments with the same nominal concentration of Li (with or without MPs), either of the same bioassay or distinct bioassays (Fig. 1a). At moderate conditions or high light intensity, the deviation of Li actual concentration relatively to the nominal one in each replicate was always lower than 20 %, thus in agreement with OECD (2012). Because the Li actual concentrations were above 80 % of the nominal ones and remained relatively stable during the interval of test renewal, the EECs of Li along these bioassays were indicated as the nominal ones (OECD, 2012), namely 0.02, 0.04 and 0.08 mg/L. In some replicates of the bioassay at warmer temperature, the deviation of Li actual concentration relatively to the nominal one was higher than the 20 % recommended (OECD, 2012). Therefore, despite the no significant differences in relation to the corresponding treatments of the other bioassays, the EECs at warmer temperature were indicated as the total mean of the actual concentrations of Li in treatments with the same concentration of the metal, namely 0.03, 0.05 and 0.1 mg/L, and the 21-days ECx were determined in relation to these concentrations.

In fresh test medium samples from all the replicates and bioassays, the deviations of the MPs actual concentrations relatively to the nominal ones were lower than 20 %, therefore in agreement with OECD (2012). The means (±SD) of MPs decay within the interval of test medium renewal (24 h) per treatment ranged from 22.7 ± 0.5 % to 26.1 ± 0.2 % in treatments with the nominal concentration of 0.1 mg/L, and from 12.8 ± 0.1 % to 13.2 ± 0.2 % in treatments with the nominal concentration of 0.2 mg/L. Regarding the TWM, there were significant differences among treatments ($H_5 = 39.617$, $p < 0.001$, $N = 44$) but the TWM of treatments with the same nominal concentration of MP were not significantly ($p > 0.05$) different (Fig. 1b). Therefore, their total means were used as the EECs of MPs along the bioassays, namely 0.04 mg/L and 0.09 mg/L of MPs. In Li-MPs mixtures with the lowest nominal concentration of MPs (0.05 mg/L), the means of MPs concentrations ranged from 0.037 mg/L to 0.039 mg/L, with a total mean (±SD) of 0.038 ± 0.001 mg/L. Thus, 0.04 mg/L was used as the EEC in mixture treatments with the lowest concentration of MPs.

To simplify the text, the EECs will be indicated as: lowest (0.02 or 0.03 mg/L), medium (0.04 or 0.05 mg/L) and highest (0.08 or 0.1 mg/L) concentrations of Li; lowest (0.02 Li + 0.04 MPs mg/L or 0.03 Li + 0.04 MPs mg/L), medium (0.04 Li + 0.09 MPs mg/L or 0.05 Li + 0.09 MPs mg/L) and highest (0.08 Li + 0.19 MPs mg/L or 0.1 Li + 0.19 MPs mg/L) concentrations of Li-MPs mixtures. Aggregates of MPs and aggregates of MPs and microalgae cells and cell debris were observed in the bottom of the beakers.

3.2. Long-term toxicity of Li and Li-MPs mixtures at high light intensity

At high light intensity, the medium and the highest concentrations of Li and Li-MPs mixtures induced parental mortality (Fig. 2), which occurred between the days 8 to 21 (Table 2).

Compared to the control group exposed at the same conditions of water temperature and light intensity (Table 2), exposure to the medium concentrations of Li or Li-MPs mixtures delayed by 1.2 fold the first brood release, caused juvenile mortality (43 % and 40 % for Li and Li-MPs mixtures, respectively), and reduced the brood number by 20 %, the total offspring (by 41 % and 62 %, respectively), the living offspring (by 66 % and 77 %, respectively) and the population growth rate (by 32 % and 41 %, respectively). At the highest concentration of Li and Li-MPs mixtures, the somatic growth was reduced (by 49 % and 46 %, respectively) and reproduction did not occur. The NOEC and LOEC values per effect criterion are indicated in Table 2.

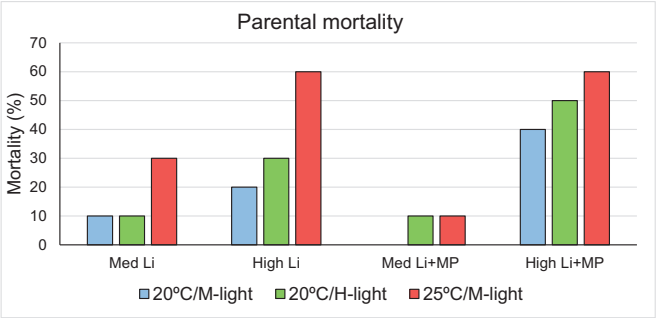

Fig. 2. Percentage of cumulative mortality of parental females (*Daphnia magna*) after 21 days of exposure to lithium alone (Li) or in mixture with microplastics (Li-MPs mixtures) at water temperature of 20 °C and light intensity of 10,830 lx (20 °C/M-light, blue bars), water temperature of 20 °C and light intensity of 26,000 lx (20 °C/H-light, green bars) and water temperature of 25 °C and light intensity of 10,830 lx (25 °C/M-light, red bars). Med Li – medium concentration of Li. High Li – highest concentration of Li. Med Li + MP – medium concentration of Li-MPs mixtures. High Li + MP – highest concentration of Li-MPs mixtures. At the beginning of each bioassay, there were 10 females per treatment. The data at 20 °C/M-light was from Martins et al. (2022).

The toxicity curves of Li and Li-MPs mixtures (based on the concentrations of Li or MPs) on *D. magna* living offspring are shown in Fig. 3, and the 21-day EC_{10}, EC_{20}, and EC_{50} are indicated in Table 3.

The individual and combined effects of Li and MPs on *D. magna* population growth rate at high light intensity, measured through Hedge's *d*, are shown in Fig. 4a. Under exposure to the lowest or medium concentrations of Li-MPs mixtures, the interaction was antagonism. At the highest concentration of Li-MPs mixtures, the type of interaction could not be determined because the population growth rate was not calculated (females did not reproduce).

3.3. Long-term toxicity of Li and Li-MPs mixtures at warmer water temperature

At warmer temperature, the parental mortality of Li and Li-MPs mixtures reached 60 % (Fig. 2), and occurred after 2 to 19 days of exposure depending on the treatments (Table 2).

Compared to the control group at the same water temperature and light intensity (Table 2), exposure to 0.04 mg/L of Li or to the medium concentration of the Li-MPs mixtures decreased the somatic growth (by 20 % and 41 %, respectively), increased the time until the first brood release (by 1.5 and 1.7 fold, respectively), caused juvenile mortality (58 % and 78 %, respectively), and reduced the brood number (by 38 % and 50 %, respectively), the total offspring (by 87 % and 86 %, respectively), the living offspring (by 95 % and 97 %, respectively) and the population growth rate (by 63 % and 71 %, respectively). At 0.08 mg/L of Li alone, the somatic growth was reduced by 59 %, the time of the first brood release was 2.4 fold increased and all the juveniles released were dead. Under exposure to the highest concentration of Li-MPs mixtures, the somatic growth was reduced by 75 % and reproduction did not occur. The NOEC and LOEC values are indicated in Table 2.

The toxicity curves of Li and Li-MPs mixtures on reproduction are shown in Fig. 3, and the 21-d EC_{10}, EC_{20} and EC_{50} are depicted in Table 3.

The individual and combined effects of Li and MPs on *D. magna* population growth rate at warmer temperature measured through Hedge's *d* are shown in Fig. 4b, except at the highest concentration of Li-MPs mixtures because living offspring was not produced. The interaction was synergism at the lowest concentration of Li-MPs mixtures and antagonism at the medium concentration.

3.4. Effects of high light intensity or warmer water temperature

As shown in Fig. 2, the parental mortality caused by the medium concentration Li, and by the highest concentration of Li or Li-MPs mixtures

Table 2
Mean (± standard deviation) of the 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 carried at water temperature of 20 °C and light intensity of 26,000 lx, and water temperature of 25 °C and light intensity of 10,830 lx. Li – lithium. MP – microplastics. All the concentrations are in mg/L. Light – light intensity; Temp – water temperature; N – number of parental females that survived until the end of the bioassay; M – Percentage of mortality. PM – days or interval of days where parental mortality occurred; n – number; # - females did not reproduce. (-) – not determined as the females did not reproduce. Different letters after the mean indicate statistical significant differences (Kruwkal-Wallis test and pairwise comparisons, $p \leq 0.05$) among treatments of the same bioassay per effect criterion. The data of the control treatments were first published in Guilhermino et al. (2021a).

Concentrations (mg/L), light and temperature, statistical test	N	PM (days)	Growth (mm)	1st brood (day number)	Brood Number (n)	Total offspring (n)	Living offspring (n)	Dead offspring (n)	Population growth rate
20 °C/26000 lx									
Control	10	–	0.203 ± 0.006 a	8.9 ± 0.3 a	5 ± 0 a	92 ± 2 a	92 ± 2 a	0 ± 0 a	0.32 ± 0.01 a
0.02 Li	10	–	0.184 ± 0.007 a,b	9 ± 0 a	5 ± 0 a	66 ± 3 a,b	65 ± 3 a,b	1.4 ± 0.8 a,b	0.287 ± 0.006 a,b
0.04 Li	9	16	0.167 ± 0.007 a,b	11 ± 0 b	4 ± 0 b	54 ± 2 b,c	31 ± 2 b,c	23 ± 1 c	0.218 ± 0.008 b
0.08 Li	7	8–14	0.103 ± 0.008 c	(−) #	0 ± 0 b #	0 ± 0 c #	0 ± 0 c #	0 ± 0 #	(−) #
0.02 Li + 0.04 MP	10	–	0.14 ± 0.01 b,c	9 ± 0 a	5 ± 0 a	83 ± 3 a,b	82 ± 2 a,b	1 ± 1 a,b	0.313 ± 0.007 a
0.04 Li + 0.09 MP	9	17	0.13 ± 0.01 b,c	11 ± 0 b	4 ± 0 b	35 ± 4 c	21 ± 4 c	14 ± 2 b,c	0.19 ± 0.02 b
0.08 Li + 0.19 MP	5	8–21	0.11 ± 0.01 c	(−)	0 ± 0 b #	0 ± 0 c #	0 ± 0 c #	0 ± 0 #	(−)
Kruskal-Wallis			$H_4 = 56.117$ $p < 0.001$	$H_4 = 45.579$ $p < 0.001$	$H_6 = 59.000$ $p < 0.001$	$H_6 = 57.791$ $p < 0.001$	$H_6 = 57.806$ $p < 0.001$	$H_4 = 40.985$ $p < 0.001$	$H_4 = 42.514$ $p < 0.001$
NOEC Li			0.04	0.02	0.02	0.02	0.02	0.02	0.02
LOEC Li			0.08	0.04	0.04	0.04	0.04	0.04	0.04
NOEC Li + MP			<0.02 + 0.04	0.02 + 0.04	0.02 + 0.04	0.02 + 0.04	0.02 + 0.04	0.02 + 0.04	0.02 + 0.04
LOEC Li + MP			0.02 + 0.04	0.04 + 0.09	0.04 + 0.09	0.04 + 0.09	0.04 + 0.09	0.04 + 0.09	0.04 + 0.09
25 °C/10830 lx									
Control	10	–	0.204 ± 0.005 a	6 ± 0 a	8 ± 0 a	93 ± 3 a	93 ± 3 a	0 ± 0 a	0.38 ± 0.01 a
0.03 Li	10	–	0.184 ± 0.009 a,b	6.4 ± 0.5 a,b	8 ± 0 a	80 ± 4 a,b	74 ± 3 a,b	6 ± 1 a,b	0.37 ± 0.01 a,b
0.05 Li	7	7–14	0.163 ± 0.006 a,b,c	9 ± 0 b,c	5 ± 0 a,b	12 ± 2 c	5 ± 2 b	7 ± 1 b	0.14 ± 0.05 c
0.1 Li	4	2–17	0.083 ± 0.005 b,c	14.3 ± 0.5 c	2.8 ± 0.5 b	4 ± 1 c	0 ± 0 b	4 ± 1 a,b	(−)
0.03 Li + 0.04 MP	10	–	0.13 ± 0.01 c	7 ± 0 a,b,c	8 ± 0 a	64 ± 3 a,b,c	56 ± 3 a,b	8 ± 1 b	0.324 ± 0.008 b,c
0.05 Li + 0.09 MP	9	12	0.12 ± 0.01 c	10 ± 0 c	4 ± 1 b	13 ± 5 b,c	3 ± 2 b	10 ± 4 b	0.11 ± 0.03 c
0.1 Li + 0.19 MP	4	2–19	0.052 ± 0.008 c	(−)	0 ± 0 b #	0 ± 0 c #	0 ± 0 b #	0 ± 0 #	(−)
Kruskal-Wallis			$H_6 = 50.886$ $p < 0.001$	$H_5 = 45.155$ $p < 0.001$	$H_6 = 52.504$ $p < 0.001$	$H_6 = 50.389$ $p < 0.001$	$H_6 = 50.572$ $p < 0.001$	$H_5 = 35.578$ $p < 0.001$	$H_4 = 40.751$ $p < 0.001$
NOEC Li			0.05	0.03	0.05	0.03	0.03	0.03	0.02
LOEC Li			0.1	0.05	0.1	0.05	0.05	0.05	0.05
NOEC Li + MP			<0.03 + 0.04	0.03 + 0.04	0.03 + 0.04	0.03 + 0.04	0.03 + 0.04	<0.03 + 0.04	<0.03 + 0.04
LOEC Li + MP			0.03 + 0.04	0.05 + 0.09	0.05 + 0.09	0.05 + 0.09	0.05 + 0.09	0.03 + 0.04	0.03 + 0.04

was greater at high light intensity or warmer temperature than at moderate conditions.

Compared to the long-term effects of Li on *D. magna* reproduction (living offspring) at moderate conditions (Fig. 3), the exposure at high light intensity or warmer temperature moved the toxicity curves towards lower concentrations. The 21-day EC_{10} and EC_{20} of Li to reproduction were lower at high light intensity than at warmer temperature and the 95 % CI did not overlap, whereas the 21-day EC_{50}s were close (Table 3). Under exposure to Li-MPs mixtures and in relation to the curve at moderate conditions (Fig. 3), the curves at high light intensity or warmer temperature were also shifted towards lower concentrations. The differences were more pronounced at warmer temperature than at high light intensity, and are highlighted through the comparison of the Li-MPs mixture curves based on the concentrations of MPs. As shown in Table 3, the 21-day EC_{50} of Li-MPs mixtures were lower at warmer temperature than at high light intensity.

The long-term effects of Li and Li-MPs mixtures on *D. magna* somatic growth at different light intensities and water temperatures are shown in Fig. 5a. The comparison of distinct bioassays per treatment indicated significant differences under exposure to the highest concentration of Li and at all the concentrations of Li-MPs mixtures (Fig. 5a, Table 3). There were no significant differences in the somatic growth between females exposed at moderate conditions and those exposed to high light intensity in any treatment. Compared to 20 °C/10830 lx, at warmer temperature, the somatic growth was significantly reduced under exposure to the highest concentration of Li and at all the concentrations of Li-MPs mixtures. Compared to high light intensity, at warmer temperature, the somatic growth was significantly lower at the highest concentration of Li-MPs mixtures.

The long-term effects of Li and Li-MPs mixtures on *D. magna* population growth rate are shown in Fig. 5b and Table 3. Compared to 20 °C/10830 lx, at high light intensity, the population growth rate was lower in all the treatments, except in the control group and in the lowest concentration of Li-MPs mixtures, where there were no significant differences. Compared to 20 °C/10830 lx, at warmer temperature, the population growth rate was significantly higher in the control group and in the lowest concentration of Li, there were no significant differences at the lowest concentration of Li-MPs mixtures, and the population growth rate was significantly reduced at the medium concentrations of Li and Li-MPs mixtures. In relation to high light intensity, at warmer temperature, the population growth rate was higher in the control group and under exposure to the lowest concentration of Li, and there were no significant differences in the other treatments despite the lower population growth rate mean.

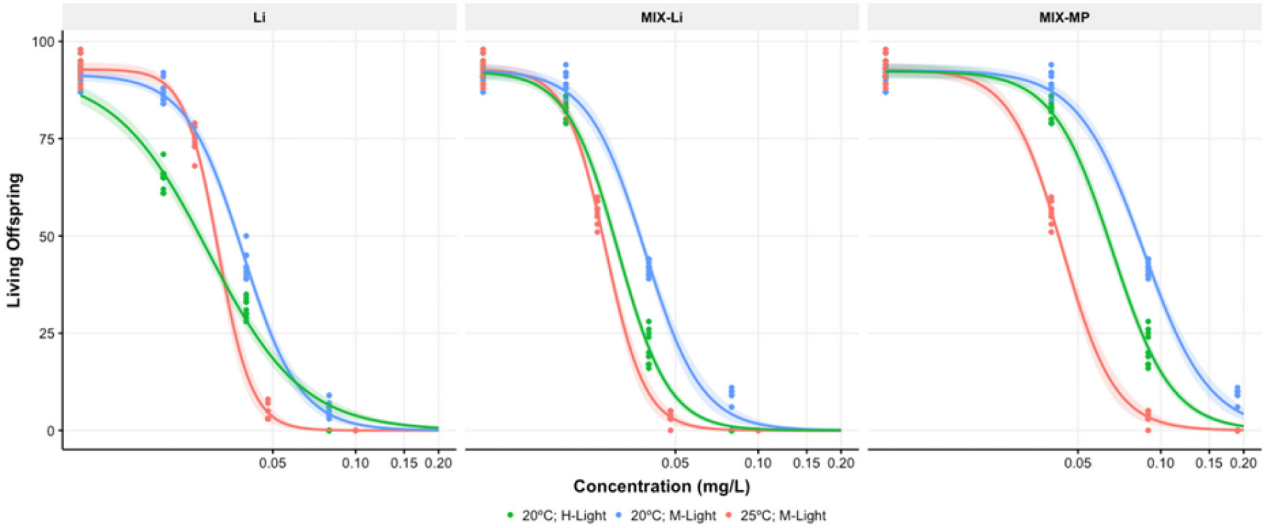

Fig. 3. Toxicity curves of lithium alone (Li), lithium-microplastic mixtures based on the concentration of lithium (MIX-Li), and lithium-microplastic mixtures based on the concentration of microplastics (MIX-MP) on *Daphnia magna* living offspring number per female after 21 days of exposure at water temperature of 20 °C and light intensity of 10,830 lx (20 °C/M-Light, blue), water temperature of 20 °C and light intensity of 26,000 lx (20 °C/H-Light, green), and water temperature of 20 °C and light intensity of 18,300 lx (25 °C/M-Light, red). Each dot represents the number of living offspring produced per females of each treatment that survived until the end of the exposure period. For the curves at 20 °C/H-Light and 25 °C/M-Light, the number of females per treatment is indicated in Table 2. For the curves at 20 °C/M-Light, the number of females per treatment ranged from 6 to 10, and the data were from Martins et al. (2022).

As the individual effects of Li and high light intensity on *D. magna* population growth rate were both negative, as well as their combined effects, the two stressors act synergistically in the two exposure scenarios (Fig. 6a). High light intensity also interacted synergistically with the medium concentration of Li-MPs mixtures but its interaction with the lowest concentration of Li-MPs mixtures was week antagonism (Fig. 6b).

Warmer temperature always acted synergistically with Li or Li-MP mixtures (Fig. 7).

4. Discussion

The concentrations of Li tested are within the values documented in environmental waters, which range from not detected (e.g., Kavanagh et al., 2017) until extremely high values in brine water, such as >4000 ppm (López Steinmetz and Salvi, 2021). They include concentrations up to 0.091 mg/L in surface water (Kavanagh et al., 2017), up to 1.7 mg/L in groundwater (Lindsey et al., 2021), up to 2.21 mg/L in natural mineral water commercially available (Neves et al., 2020), and up to 2.98 mg/L in drinking waters (Steinmetz et al., 2021). Moreover, in some areas, the concentration of Li in the water has been increasing along with human population density, including in drinking water (Choi et al., 2019). The occurrence of MPs and other plastic particles also variates greatly (Wang et al., 2021b), and includes values up to 3622 items/L in stream water (Simmerman and Coleman Wasik, 2020), up to 51.7 mg/m^3 (Rodrigues et al., 2018) and up to 1,146,418.36 items/m^3 (Moore et al., 2011) in river water, up to 23.98 ± 10.61 items 100/m^3 in estuarine water (Rodrigues et al., 2019), and means of 1.56 ± 1.64 mg/L and 5.51 ± 9.90 mg/L in lake and wetland waters, respectively (Lasee et al., 2017). Therefore, the tested concentrations of Li and MPs are environmentally realistic.

Table 3
Estimated effective concentrations of lithium (Li) and in lithium-microplastic mixtures (Li-MP), based on the concentration of Li and in the concentration of microplastics (MPs), causing 10 % (EC_{10}), 20 % (EC_{20}) and 50 % (EC_{50}) of reduction on *Daphnia magna* living offspring number per female after 21-d exposure with the respective standard error (SE) and 95 % confidence limits (95 % CL), and results of the comparison of the somatic growth and population growth rate among corresponding treatments of different bioassays (Kruskal-Wallis test, $p \leq 0.05$). Substance – substance to each the estimates were made. Low Li – low concentration of lithium. Medium Li – medium concentration of lithium. High Li – high concentration of lithium. Low Li-MP – low concentration of Li-MP mixtures. Medium Li-MP – medium concentration of Li-MP mixtures. High Li-MP – high concentration of the mixtures.

Temperature Light intensity	Condition	Substance	21-d EC_{10}		21-d EC_{20}		21-d EC_{50}	
			EC_{10} (mg/L)	95 % CL (mg/L)	EC_{20} (mg/L)	95 % CL (mg/L)	EC_{50} (mg/L)	95 % CL (mg/L)
20 °C, 26000 lx	Li alone	Li	0.012	0.011–0.014	0.017	0.015–0.018	0.029	0.028–0.031
	Li-MP	Li	0.019	0.018–0.021	0.023	0.022–0.024	0.031	0.030–0.032
	Li-MP	MP	0.039	0.036–0.041	0.047	0.045–0.050	0.067	0.064–0.069
25 °C, 10830 lx	Li alone	Li	0.023	0.023–0.024	0.026	0.025–0.027	0.032	0.031–0.032
	Li-MP	Li	0.019	0.018–0.020	0.022	0.022–0.023	0.028	0.027–0.028
	Li-MP	MP	0.027	0.025–0.029	0.032	0.031–0.034	0.044	0.043–0.045

Effect criterion	Treatments (mg/L) and pairwise comparisons					
	Low Li	Medium Li	High Li	Low Li-MP	Medium Li-MP	High Li-MP
Somatic growth	$H_2 = 5.556$ $p = 0.062$	$H_2 = 0.835$ $p = 0.659$	$H_2 = 14.887$ $p < 0.001$	$H_2 = 13.208$ $p < 0.001$	$H_2 = 18.879$ $p < 0.001$	$H_2 = 9.738$ $p = 0.008$
Population growth rate	$H_2 = 25.806$ $p < 0.001$	$H_2 = 20.432$ $p < 0.001$	–	$H_2 = 7.349$ $p = 0.025$	$H_2 = 23.093$ $p < 0.001$	–

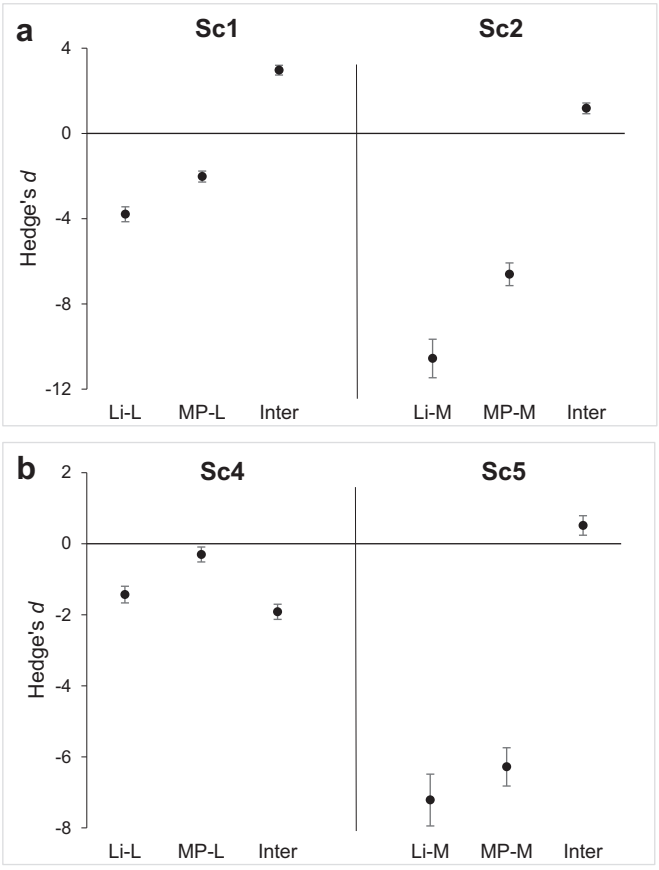

Fig. 4. Independent and combined effects of lithium (Li) and microplastics (MPs) on the population growth rate of *Daphnia magna* at water temperature of 20 °C and light intensity of 26,000 lx (a), and water temperature of 25 °C and light intensity of 10,830 lx (b). The dots represent the individual and interactive (inter, mixture) effect sizes measured with Hedge's *d* with the corresponding 95 % confidence interval (vertical bars). At each water temperature and light intensity, the following exposure scenarios were considered: lowest concentrations of Li, MPs and Li-MPs mixtures (Sc1, Sc4) and medium concentrations of Li, MPs and Li-MP mixtures (Sc2, Sc5). The number of females in treatments with Li alone, MPs alone and in the mixture by this order were: 10, 10, 10 in Sc1; 9, 10, 9 in Sc2; 10, 10, 10 in Sc3; and 7, 9, 9 in Sc4. The data of MPs alone used to calculate the Hedge's *d* were from Guilhermino et al. (2021a).

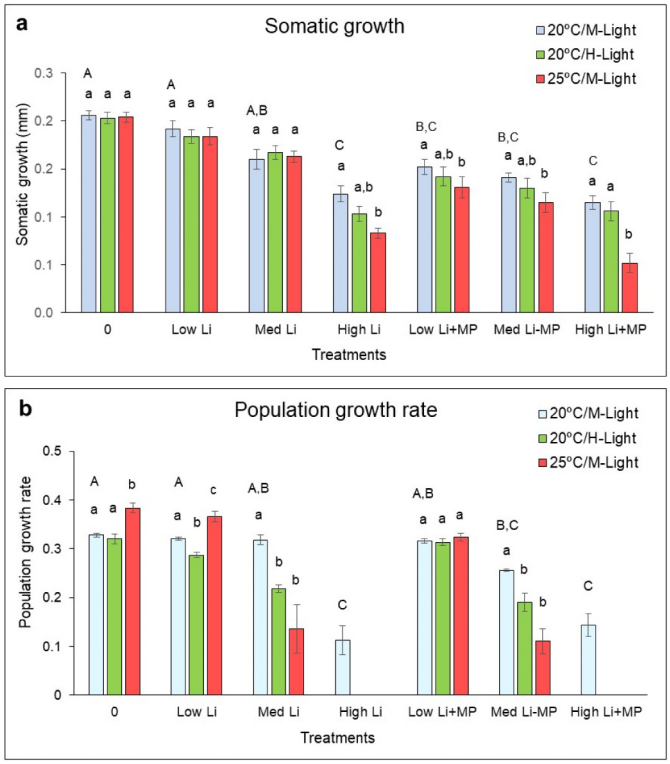

Fig. 5. Means and standard deviation of *Daphnia magna* somatic growth (a) and population growth rate (b) exposed for 21 days to lithium (Li) alone or to mixtures of Li and microplastics (MPs) at water temperature of 20 °C and light intensity of 10,830 lx (20 °C/M-light, blue), water temperature of 20 °C and light intensity of 26,000 lx (20 °C/H-light, green), and water temperature of 25 °C and light intensity of 18,300 lx (25 °C/M-light, red). Low Li – lowest concentration of Li. Med Li – medium concentration of Li. High Li – highest concentration of Li. Low Li + MP – lowest concentration of Li-MPs mixtures. Med Li + MP – medium concentration of Li-MPs mixtures. High Li + MP – highest concentration of Li-MPs mixtures. Different common letters indicate significant differences among treatments with the same concentration of Li or of the mixtures; capital letters indicated significant differences among treatments of the bioassay at 20 °C/M-Light (Kruskall Wallis test and pairwise comparisons, $p \leq 0.05$). The comparison of treatments and the number of females per treatment (N) in the bioassays at 20 °C/H-Light and 25 °C/M-Light are indicated in Table 2. In the bioassay at 20 °C/M-Light, the N was: 10 in the control, in the Low Li, in the Low Li-MP and in the Med Li-MP; 9 in the Med Li; 8 in the High Li; and 6 in the High Li-MP, and the data were from Martins et al. (2022).

4.1. *D. magna* population growth rate in the control groups

The comparison of *D. magna* population growth rate in the control groups of the three bioassays through the Kruskal-Wallis test and pairwise comparisons indicated no significant differences between distinct light intensities at 20 °C, suggesting that high light density did not induce stress or that stress adaptation occurred. The individual effect of high light intensity on the population growth rate, measured through Hedges' *d*, was negative, as well as its 95 % CI indicating that high light intensity reduced the population growth rate and supporting the second hypothesis. The individual effect of light was calculated from the difference between the means of the population growth rate in the control group at high light intensity and in the control group at moderate conditions, the pooled standard deviation of the two groups, and included a constant correcting for small sample bias (Gurevitch et al., 2000; Hedges and Olkin, 1985). Under comparable favourable conditions, *D. magna* water filtration increased with light intensity after prolonged exposure (Serra et al., 2019). High water filtration, food intake and activity, and growth and reproduction imply high metabolism, biotransformation, elimination of the resulting toxic metabolites and repair, requiring additional energy and other resources, such as basic molecules (Vandenbrouck et al., 2011; Guilhermino et al., 2021a). In the absence of other stressors, *D. magna* population fitness results from trade-off responses to light, temperature and food (Storz and Paul, 1998; Effertz and von Elert, 2017; Gust et al., 2019; Im et al., 2020; Stábile et al., 2021). Therefore, it is hypothesised that water filtration increased with light intensity, as documented in Serra et al. (2019), triggering slight energy allocation from the somatic growth to cope with the increased metabolic costs and maintain reproduction, overtime leading to a slightly negative effect on the population growth rate. At 20 °C and low UV radiation, *D. magna* exposure to light (0.48 μmol s^{-1} m^{-2}) or dark (<0.1 μmol s^{-1} m^{-2}) did not significantly affect the somatic growth nor the size until the first reproduction in the absence of predator cues, leading to the conclusion that light intensity does not affect resource allocation in this species (Effertz and von Elert, 2017). However, in a study carried at 23 ± 1 °C and low UV radiation, where *D. magna* was exposed to different light conditions (darkness, 300, 800 and 1500 lx, low UV radiation), at water temperature of 23 ± 1 °C, the somatic growth and reproduction increased with the augment of light intensity up to up 800 lx, compared to which a delay in the first brood release, lower growth and reproduction were observed at 1500 lx (Zeini and Akel, 2020), showing that increased light intensity can

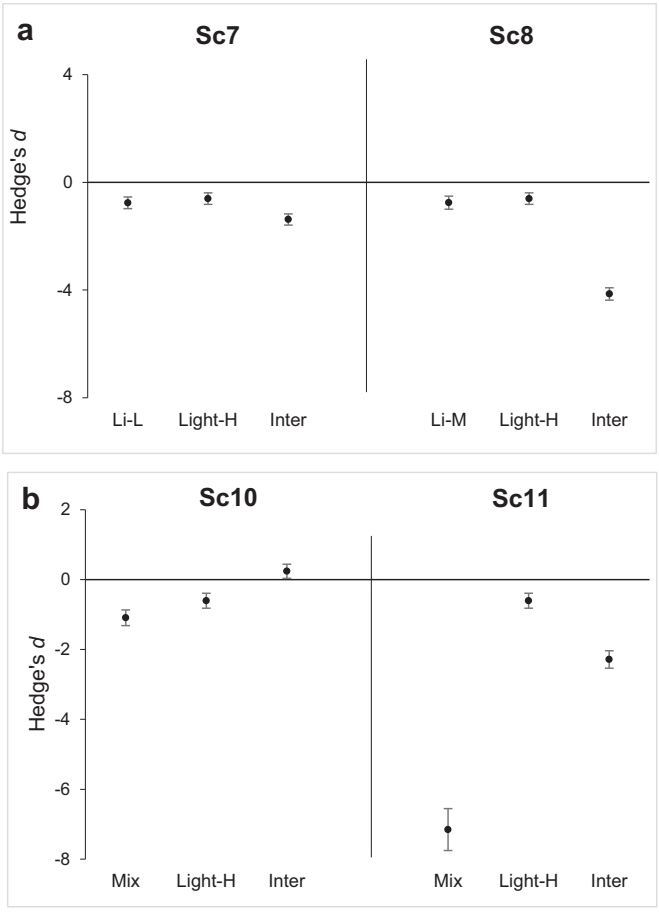

Fig. 6. Independent and combined effects high light intensity (26,000 lx) and lithium (Li) in the top (a), and between high light intensity and Li-microplastic mixtures (Mix) in the bottom (b). The dots represent the individual and interactive (inter, mixture) effect sizes measured with Hedge's *d* with the corresponding 95 % confidence interval (vertical bars). The exposure scenarios were: Sc7 - lowest concentration of Li (Li-L), high light intensity (Light-H) and lowest concentration of Li at high light intensity (Inter); Sc8 - medium concentration of Li (Li-M), high light intensity (Light-H) and medium concentration of Li at high light intensity (Inter); Sc10 - lowest concentration of Mix, high light intensity (Light-H) and lowest concentration of Mix at high light intensity (Inter); Sc11 – medium concentration of Mix, high light intensity (Light-H) and medium concentration of Mix at high light intensity (Inter). The number of females in treatments with Li alone, Light-H and Inter by this order were: 10, 10, 10 in Sc7; and 9, 10, 9 in Sc8. The number of females in treatments with the Mix, Light-H and Inter by this order were: 10, 10, 10 in Sc10; and 10, 10, 9 in Sc11. The data of Light-H (control) used to calculate the Hedge's *d* were from Guilhermino et al. (2021a), and those of Li and Mix at 20 °C/M-Light were from Martins et al. (2022).

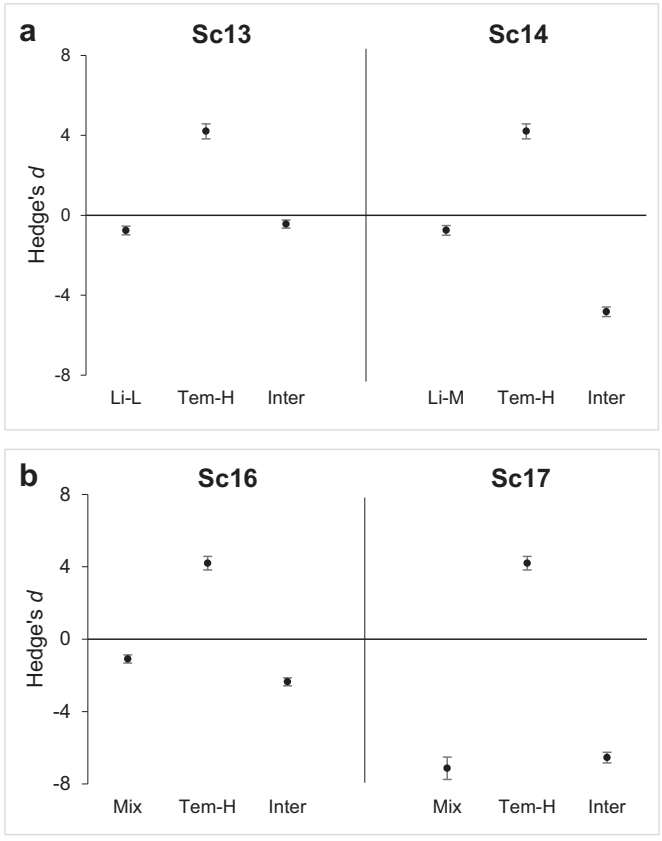

Fig. 7. Independent and combined effects warmer water temperature (25 °C) and lithium (Li) in the top (a), and between warmer water temperature and Li-microplastic mixtures (Mix) in the bottom (b). The dots represent the individual and interactive (inter, mixture) effect sizes measured with Hedge's *d* with the corresponding 95 % confidence interval (vertical bars). The exposure scenarios were: Sc13 - lowest concentration of Li (Li-L), warmer water temperature (Tem-H) and lowest concentration of Li at warmer water temperature (Inter); Sc14 - medium concentration of Li (Li-M), warmer water temperature (Tem-H) and medium concentration of Li at warmer water temperature (Inter); Sc16 - lowest concentration of Mix, warmer water temperature (Tem-H) and lowest concentration of Mix at increased water temperature (Inter); Sc17 - medium concentration of Mix, warmer water temperature (Tem-H) and medium concentration of Mix at warmer water temperature (Inter). The number of females in treatments with Li alone, Tem-H and Inter by this order were: 10, 10, 10 in Sc13; and 9, 10, 7 in Sc14. The number of females in treatments with the Mix, Tem-H and Inter by this order were: 10, 10, 10 in Sc16; and 10, 10, 9 in Sc17. The data of Tem-H used to calculate the Hedge's *d* were from Guilhermino et al. (2021a), and those of Li and Mix at 20 °C/M-Light were from Martins et al. (2022).

cause adverse effects in *D. magna*, thus supporting our hypothesis. Nevertheless, because the effects of light changes are influenced by several factors (e.g., Gust et al., 2019; Serra et al., 2019; Cremer et al., 2022) and some conditions were different, it will be interesting to test the hypothesis in further studies including the energy metabolism.

Warmer temperature increased the population growth rate of *D. magna* due to accelerated development, earlier release of the first brood and higher number of broods produced during the exposure period (Guilhermino et al., 2021a), in agreement with other studies (Heugens et al., 2006; Vandenbrouck et al., 2011; Hoefnagel et al., 2018; Im et al., 2020).

4.2. High light intensity increased Li toxicity, and the interaction was synergistic

The long-term toxicity of Li to *D. magna* was higher at high light intensity than at moderate conditions, as shown by the higher parental mortality at 0.08 mg/L, the increase of the reproductive toxicity by ~1.3 fold based on the ratio between the 21-day EC_{50}s on living offspring, which were 0.039 mg/L at 20 °C/10830 lx (Martins et al., 2022) and 0.029 mg/L at high light intensity, and the complete impairment of the reproductive success at 0.08 mg/L of Li leading to population extinction in one generation. Therefore, H_{01} was rejected and H_{A1} was accepted. These findings are in line with the ability of light changes to influence the effects of stressors, such as neuroactive pharmaceuticals (Simão et al., 2019), MPs (Guilhermino et al., 2021a), predator cues (Effertz and von Elert, 2017) and cyanobacteria (Cremer et al., 2022), sometimes resulting in increased effects at high light intensity (Effertz and von Elert, 2017; Guilhermino et al., 2021a).

Greater water filtration at high light intensity (Serra et al., 2019) may have increased the uptake of Li from the water, and also from food because microalgae uptake and bioconcentrate Li from the surrounding medium (Kaštánek et al., 2018; Díaz-Alejo et al., 2021; Yücel et al., 2021), leading to more severe toxicity. The synergistic action between high light intensity

and Li resulting in a negative combined effect on *D. magna* population growth rate that becomes much more pronounced with the increase of Li concentration supports this hypothesis. The significant delay of the first brood release, and the reduction of the brood number and the total offspring under exposure to 0.04 mg/L of Li at high light intensity, point to allocation of energy and other resources from growth and reproduction to respond to the chemical stress with negative effects on the population growth rate that was significantly reduced. This is a common response in *D. magna* under stress (e.g., Vandenbrouck et al., 2011; Sengupta et al., 2016). Moreover, Li may have caused energy shortage also by other ways because it disrupts the energy metabolism, decreases energetic reserves and the levels of basic molecules that are needed for growth and reproduction, causes ionic deregulation (Nagato et al., 2013; Tkatcheva et al., 2015; Viana et al., 2020), and downregulates genes that are important for growth and reproduction (Kim et al., 2017). Long-term exposure to Li likely causes energy depletion in *D. magna* (Martins et al., 2022), an effect documented in this species exposed to a nanomaterial containing Li (Bozich et al., 2017; Niemuth et al., 2021). Higher uptake of Li likely also increased its neurotoxicity, including through the disruption of *D. magna* signalling pathways regulating crucial functions, such as the behaviour, somatic growth and reproduction (Kim et al., 2017). Among other possible mechanisms, Li can inhibit the activity of the acetylcholinesterase (AChE) enzyme (Oliveira et al., 2011; Viana et al., 2020; Costa et al., 2021). This enzyme is fundamental to the functioning of the cholinergic system, which is involved in the complex regulation of *D. magna* phototactic behaviour (Bedrossiantz et al., 2021). Previous studies with *D. magna* exposed to pharmaceuticals acting on cholinergic, serotonergic, GABBA-ergic or other systems showed their ability to disrupt *D. magna* phototaxis, other behaviour (e.g., swimming, locomotion, aggregation) and/or its responses to other environmental stimulus, sometimes leading to behavioural and life-history changes (e.g., Campos et al., 2012; Bedrossiantz et al., 2021; Simão et al., 2019). In *D. magna*, Li may also change the levels of melanin (Nagato et al., 2013), which provide protection against intense light and UV radiation, and are regulated according to light intensity and other environmental cues (Scoville and Pfrender, 2010; Stábile et al., 2021). Increased light alters the expression of melanin-related genes in *D. magna* (Cremer et al., 2022). Therefore, Li-induced neurotoxicity may have changed *D. magna* perception of light intensity interfering with its phototactic behaviour and food intake, and/or increased its vulnerability to UV radiation despite the low levels in our experimental conditions. Testing these hypotheses requires investigation on the specific mechanisms potentially involved, which were not in the scope of this study.

4.3. High light intensity increased Li-MPs mixture toxicity, and the interaction was week antagonism or synergism depending on the concentration

High light intensity increased the reproductive toxicity of Li-MPs mixtures to *D. magna* by ~1.3 fold, either based on the concentration of Li or in the concentration of MPs, as indicated by the ratio between the 21-day EC_{50} on living offspring at moderate conditions (0.039 Li + 0.086 mg/L, Martins et al., 2022) and at high light intensity (0.031 Li + 0.067 mg/L). Moreover, at high light intensity, the highest concentration of Li-MPs caused higher parental mortality and reproduction did not occur, leading to population extinction in one generation, an effect that was not observed under exposure to Li-MPs mixtures at moderate light intensity and the same water temperature (20 °C). Therefore, H_{02} was refused and H_{A2} was accepted.

High light intensity interacted slightly antagonistically or synergistically with the chemical stress induced on *D. magna* population growth rate at the lowest or medium concentrations of Li-MPs mixtures, respectively, whereas it acted always synergistically with Li. Under exposure to Li-MPs at high intensity, Li and MPs act antagonistically and the interaction decreased with the concentration. At moderate conditions, Li and MPs acted slightly antagonistically at the lowest concentration of Li-MPs mixtures and synergistically at the medium concentration (Martins et al., 2022). In all the scenarios, the individual effects of the stressors were negative. Together, these findings support the hypothesis of elevated water filtration at high light intensity increasing the uptake of Li and MPs, and show that high light intensity was able to change the interaction between Li and MPs. They also indicate that the toxicity of Li-MPs mixtures increased rapidly, that MPs contributed to the toxicity of Li-MPs mixtures, and that MPs ameliorated the effects of Li, with this role decreasing with the concentration.

D. magna uptakes the tested MPs through ingestion and possibly also through gills during respiration and other routes, and they induce toxicity (Guilhermino et al., 2021a). Li adsorbs to plastic copolymers (Llamas et al., 2013) and several metals adsorb to MPs (Holmes et al., 2012; Tuccori et al., 2019; Yuan et al., 2020). Microalgae uptake Li (Kaštánek et al., 2018; Díaz-Alejo et al., 2021; Yücel et al., 2021) and interact with MPs (Lagarde et al., 2016; Demir-Yilmaz et al., 2022), including the tested MPs (Prata et al., 2020). Therefore, under exposure to Li-MPs mixtures, in addition to the uptake of both Li and MPs from test medium, likely both MPs and microalgae acted as carriers of Li to *D. magna*, whereas under exposure to Li alone only microalgae could have play this role. Despite the importance that the interactions among MPs, Li and microalgae likely had in the toxicity of Li-MPs mixtures, they may also have contributed to the antagonistic action of MPs and Li. During the interval of test medium renewal, the concentrations of MPs in test medium decreased, and aggregates of MPs with microalgae cells were observed on the bottom of the beakers. This likely removed some Li and Li-contaminated food from the water column, as documented for mixtures of other metals and MPs (Yuan et al., 2020; Thi et al., 2021), additionally to the settlement of microalgae that also occurred in treatments with Li alone, contributing to the antagonism. The comparison of Li concentrations in fresh and old test medium, and among treatments with and without MPs indicated no significant differences but the quantity of Li involved may have been too small to be detected because Li concentrations were low, the test medium was changed at each 24 h, and only the total concentrations of Li were determined, still being important regarding the toxicity because the exposure period was long (Martins et al., 2022) and animals accumulate Li (Aral and Vecchio-Sadus, 2008; Viana et al., 2020) and MPs (Hoffchröer et al., 2021).

The lowest concentration of Li-MPs mixtures significantly reduced the somatic growth of *D. magna*, whereas this effect was only observed at 0.08 mg/L of Li alone or at 0.09 mg/L of MPs alone (Guilhermino et al., 2021a). Therefore, the lowest concentration of Li-MPs mixtures was more toxic that its components separately, as also observed at moderate conditions (Martins et al., 2022). The uptake of both Li and MPs likely aggravated the metabolic stress leading to increased allocation of energy, possibly mainly from the somatic growth, as suggested by the lack of significant differences in any of the reproductive parameters compared to Li alone and to the control group at the same light intensity, and the higher 21-day EC_{10} and EC_{20} on living offspring of Li-MPs mixtures compared with those of Li alone. The additional intake of Li through MPs may have also disrupted other mechanisms involved in the somatic growth (Nagato et al., 2013; Kim et al., 2017). The developmental toxicity shows that the role of MPs against Li and any benefits that the possible high food intake may have had were overcome by the toxic effects even at the lowest concentration of Li-MPs mixtures.

Compared to the lowest concentration, at the medium concentration of Li-MPs mixtures, there was more Li and MPs available in test medium, increasing the uptake of both Li and MPs through water filtration. As the decay of MPs in test medium did not augment with the concentration, this likely also increased the proportion of MPs contaminated with MPs, and food contaminated with both MPs and Li because the adsorption of metals to MPs increases with the concentration (Yuan et al., 2020), as well as the interactions between MPs and microalgae (Prata et al., 2018), and the uptake of Li by microalgae (Yücel et al., 2021). The ratio between the number of MPs and the number of microalgae cells (MPs/food ratio) also increased with the concentration of Li-MPs mixtures because the number of cells supplied daily was always the same. High concentration of MPs and MPs/food ratios commonly reduce *D. magna* water filtration and food intake, including beds (Ogonowski et al., 2016; Colomer et al., 2019).

Very small beds often also decrease the somatic growth and reproduction (Jaikumar et al., 2019; Liu et al., 2022; Schwarzer et al., 2022), including the tested ones (Pacheco et al., 2018; Guilhermino et al., 2021a; Martins and Guilhermino, 2018). Decreased water filtration and food intake will also reduce the uptake of Li and MPs. Still, the toxicity could be much higher than under exposure to the lowest concentration of Li-MPs mixtures because the concentrations of MPs and Li were greater, as well as the interactions between them and with food since they increase with the concentration (Prata et al., 2018; Yuan et al., 2020; Yücel et al., 2021). Moreover, the reduction of food would aggravate the metabolic stress. The decrease of the interaction between Li and MPs, the close 21-day EC_{50}s on reproduction of Li alone and Li-MPs mixtures (based on the concentration of Li), the lack of significant differences in the somatic growth and the population growth rate at the medium concentrations, and the extinction of the populations caused by both Li-MPs mixtures and Li alone, support this hypothesis. Compared to the lowest concentration of Li-MPs mixtures, the higher uptake of Li and MPs at the medium and high concentrations likely also increased their interactions with internal targets, such as the AChE enzyme that is inhibited by both Li (Oliveira et al., 2011; Viana et al., 2020; Costa et al., 2021) and the tested MPs (Barboza et al., 2018b), among several other targets and mechanisms where Li (e.g., Nagato et al., 2013; Kim et al., 2017) and MPs (e.g., Yuan et al., 2020; Trotter et al., 2021; Sarasamma et al., 2020; Trotter et al., 2021) act. Other processes may have also contributed to the rapid increase of the toxicity with the concentration of Li-MPs mixtures, such as the time of retention of MPs in *D. magna* gut, which is greater under exposure to high concentrations of MPs and MPs/food ratios than at lower ones, promoting chemical internalization (Ogonowski et al., 2016). Moreover, more MPs in test medium may have increased the number of particles stuck in *D. magna* gills reducing respiration, the binding of particles to the body surface negatively interfering with swimming and other functions, among other physical effects (Eltemsah and Bøhn, 2019), and changed the diel vertical migration (DVM) in the water column, and other activity (Magester et al., 2021). The rapid increase of the toxicity and alterations in the interaction between MPs and other metals was documented in *D. magna* under short-term (Yuan et al., 2020) and long-term exposure (Pacheco et al., 2018).

4.4. Warmer water temperature increased Li and Li-MPs toxicity, and the interaction was always synergism

Based on the ratio between the 21-day EC_{50}s on reproduction and using the values estimated at 20 °C/10830x (Li: 0.039 mg/L; Li-MPs mixtures: 0.039 Li + 0.086 mg/L, Martins et al., 2022), the reproductive toxicity of Li alone to *D. magna* was ~1.2 fold greater at warmer temperature, whereas the reproductive toxicity of Li-MPs mixtures was ~1.4 fold or ~2 fold higher based on the concentrations of Li or MPs, respectively. At the lowest and medium concentrations of Li or MP-Li mixtures, warmer temperature and chemical stress acted synergistically resulting in a negative combined effect on *D. magna* population growth rate. At the medium concentration, Li reduced the population fitness by 63 %, whereas Li-MPs mixtures reduced it by 71 %. The highest concentrations of Li and Li-MP mixtures caused high parental and juvenile mortality, and completely impaired the reproductive success, leading to the extinction of the model populations in one generation. Therefore, warmer temperature increased the long-term toxicity of Li and Li-MPs mixtures leading to the refusal of H_{03} and H_{04}, and acceptance of H_{A3} and H_{A4}. These findings are in line with studies in *D. magna* carried out at the same or close temperatures where warmer temperature increased the long-term toxicity of other metals, such as cadmium (Heugens et al., 2006; Na et al., 2021), copper (Bae et al., 2016), and nickel (Vandenbrouck et al., 2011) to *D. magna*. Increased effects at warmer temperature were also documented in *D. magna* exposed to distinct types of MPs and different ranges of temperature (Sadler et al., 2019; Lyu et al., 2021). The synergistic action between warmer temperature and Li or Li-MPs mixtures reducing the population growth rate is also in agreement with the synergistic action of temperature, MPs and ammonium on *D. magna* filtration capacity, reducing it (Serra et al., 2020).

The synergistic action of warmer temperature and chemical stress resulting in a reduction of the population fitness indicate that the benefits from warmer temperature were overcome by the increased toxicity of Li or Li-MPs mixtures in all the scenarios. At similar conditions of other factors, *D. magna* water filtration, food intake and metabolism are higher at 25 °C than at 20 °C (Burns, 1969; Khan and Khan, 2008). Therefore, warmer temperature likely increased the chemical uptake from the test medium and food, leading to increased body burdens and toxicity. Higher body burden of metals (e.g., Heugens et al., 2006) and MPs (Hoffchröer et al., 2021) at warmer water are documented in D. magna, as well as the toxicity of both metals (Heugens et al., 2006; Vandenbrouck et al., 2011; Bae et al., 2016; Na et al., 2021) and MPs (Sadler et al., 2019; Serra et al., 2020; Hoffchröer et al., 2021; Lyu et al., 2022). Also providing support to the hypothesis is the increased toxicity of Li (Rodríguez et al., 2021) and MP-induced neurotoxicity (Sulukan et al., 2021) at warmer temperature documented in other species. The significant delay of the first brood release and reduction of reproduction caused by the medium concentration of Li and Li-MPs mixtures, suggest allocation of energy to deal with the stress and survive, what is supported by the high parental mortality (60 %) and the lack of reproductive success at the highest concentration. In addition to the potential targets and mechanisms of action already discussed for Li and Li-MPs mixtures at high light intensity, all the stressors (i.e., warmer temperature, Li and MPs) increase the oxidative stress (e.g., Bae et al., 2016; Barboza et al., 2018b; Viana et al., 2020; Liu et al., 2022) and influence stress responses, such as heat shock proteins (Mikulski et al., 2011; Imholf et al., 2017; Kim et al., 2017), what may have contributed to the synergistic action of warmer water and the stress induced by Li and Li-MPs mixtures.

At warmer temperature and compared to Li alone, the somatic growth was significantly reduced at a lower concentration, confirming the higher toxicity of Li-MPs mixtures at low concentrations. Based on the concentrations of Li and in the 21-day EC_{50}s ratio on living offspring, the Li-MPs mixtures were ~1.14 fold more toxic than Li. Compared to MPs alone, the concentrations of MPs in the Li-MPs mixtures and on the 21-day EC_{50} ratio on living offspring, Li-MPs mixtures were ~2.3 fold more toxic than the MPs tested alone (21-day EC_{50} at 25 °C/10830 lx: 0.101 mg/L; Guilhermino et al., 2021a). Moreover, the synergistic action of warmer temperature and chemical stress on *D. magna* population growth rate was more pronounced under exposure to Li-MPs mixtures than under Li alone. These findings highlighting the threats posed by the combined exposure to Li and MPs at warmer temperature.

The comparison of the effects caused by warmer temperature and high light intensity, indicates that the reproductive toxicity of Li, based on the 21-day EC_{50} on reproduction, was ~1.1 fold higher at high light intensity than at warmer temperature. The opposite pattern occurred for Li-MPs mixtures, with slightly higher (~1.1 fold, and the 95 % CL did not overlap) reproductive toxicity at warmer temperature based on the concentration of Li. Based on the concentration of MPs, the reproductive toxicity was ~1.5 fold higher at warmer temperature than at high light intensity. At warmer temperature and the lowest concentration of Li-MP, Li and MPs interact synergistically indicating that MPs did not provide protection against Li or that this role was very low, whereas they did at high light intensity. These findings and others from the literature (e.g., Nieto et al., 2016; Pacheco et al., 2018; Pereira et al., 2017; Ulbing et al., 2019; Im et al., 2020; Serra et al., 2020; Stábile et al., 2021; Wang et al., 2021a; Cremer et al., 2022; Silva et al., 2022) illustrate the complexity of mixture toxicity and the additional challenges of assessing the effects of temperature and light intensity on toxicological interactions due to the influence of several factors and adaptation responses that can lead to different combined effects.

4.5. Relevance for real scenarios

The severe effects of Li and Li-MPs mixtures at high light intensity or warmer temperature, including extinction of the model populations in one generation, confirm that the long-term exposure to environmentally

realistic concentrations of Li or Li-MPs mixtures can reduce significantly the fitness of *D. magna*, even when a considerable amount of Na (~52.5 mg/L) and other ions are simultaneously present in the water, as previously highlighted (Martins et al., 2022). Moreover, the presence of Na and other ions that may ameliorate the effects of Li (e.g., K, Ca, Mg) did not avoid the increase of the toxicity of Li and Li-MPs induced by high light intensity or warmer temperature.

Concentrations of Na in the water lower than those tested (up to 17 mg/L) reduced significantly the toxicity of Li to *Ceriodaphnia dubia* exposed at 25 °C, with the 7-day EC_{50} on reproduction variating between from 0.072 mg/L to >4 mg/L, according to the water concentration of Na (Kszos et al., 2003), therefore higher than the 21-day EC_{50} to *D. magna* reproduction at water temperature of 25 °C (0.032 mg/L). Despite the differences in several experimental conditions, the comparison between the 21-day EC_{50}s suggests distinct sensitivity to Li and/or differences in the combined action of Li and Na between the two species. The concentrations of Li than induced significant effects on *D. magna* reproduction at all the conditions tested were considerably lower than the 2.5 mg/L previously documented (Bozich et al., 2017), pointing to intraspecific differences of sensitivity to Li in *D. magna*. Intraspecific (Hylander et al., 2014; Bae et al., 2016; Sadler et al., 2017) and interspecific (Zhao-Xia et al., 2013; Jaikumar et al., 2019; Drago and Weithoff, 2021) differences of sensitivity to stressors have been documented. They are common in nature, and increase the resilience of populations and communities towards adverse conditions. Nevertheless, the effects induced in real scenarios may have more severe consequences than those observed in the laboratory (Schwarzer et al., 2022).

Regarding the stressors tested in the present study, the irregular shape of many MPs present in the wild may increase their adverse effects, as found in laboratory studies (Ogonowski et al., 2016; An et al., 2021). In many natural freshwater ecosystems, particularly in shallow water ones, the UV radiation from solar light is stronger than in laboratorial bioassays where low UV radiation lamps are commonly used. Often, it triggers adaptation responses *D. magna* and other zooplankton species that may reduce the food intake and/or lead to the ingestion of lower quality food with fitness costs (Storz and Paul, 1998; Ulbing et al., 2019; Stábile et al., 2021). In shallow waters, organisms cannot reduce very much the exposure to intense solar light and UV radiation during the day, as well as to increased water temperature. The increase of urbanization, life-style globalization, and industrialization are changing day/light natural patterns potentially disrupting several physiological functions in animals, including zooplankton species (Maszczyk et al., 2021). In many real scenarios, animals are also exposed to other stress sources, environmental conditions change (Guilhermino et al., 2021a), and these factors also influence interspecific relationships (e.g., Effertz and von Elert, 2017; Kunze et al., 2022) potentially disrupting balances established over time. Warmer water and high light intensity, especially from solar light, also promote the growth of phytoplankton, including cyanobacteria that are food of low quality to *D. magna* (Hiltunen et al., 2021) and other zooplankton species. Increased artificial light can trigger genetic alterations improving the capability of *D. magna* to digest cyanobacteria (Cremer et al., 2022). Nevertheless, this can have adverse consequences for their predators and ecosystems that were not yet investigated. Moreover, warmer water and high light intensity may lead to eutrophication, with decreased oxygen levels in the water, less light reaching medium and lower layers of the water column, and blooms of cyanobacteria and other organisms releasing toxins to the water, among other adverse conditions to life. The potential consequences of such effects include intra- and interspecific biodiversity loss, changes in ecosystem functioning, negative impacts on ecosystem services, such as poor water quality, and negative impacts on public health.

Overall, the findings of the present study highlight the threats of high light intensity and warmer temperature, acting separately, on the long-term toxicity of MPs and Li, and stress the need of further studies at different levels of biological organization to elucidate the mechanisms involved, including under simultaneous exposure to all the stressors, and different environmental conditions. A deeper understanding of the combined effects of environmental stressors is needed to improve our capability of assessing, predicting and managing risks, and increase sustainability.

5. Conclusions

Long-term exposure to environmentally realistic concentrations of Li and Li-MPs mixtures caused parental and juvenile mortality, and significantly decreased the somatic growth and the reproductive success, leading to population fitness reduction in *D. magna*. Compared to the toxicity at moderate water temperature (20 °C) and light intensity (10,830 lx), the effects were much greater at warmer temperature (25 °C) or high light intensity (26,000 lx). Moreover, at high light intensity or warmer temperature, exposure to highest concentration of Li or Li-MPs mixtures caused the complete impairment of the reproductive success leading to the extinction of the populations in one generation. In the scenarios considered, which occur nowadays in regions across the world, the toxicological interaction between Li and MPs changed with chemical concentrations, light intensity and temperature. In most scenarios, high light intensity and chemical stress acted synergistically, whereas warmer temperature and chemical stress always act synergistically.

Among other ecosystem services, zooplankton contributes significantly to water quality, which is crucial to reach the United Nations Sustainable Development Goals, including to reduce the spread of many diseases favoured by poor water quality. The considerable increase of the long-term toxicity of Li and Li-MPs mixtures to *D. magna* promoted by high light intensity or warmer temperature highlight the threats to Global Health in a more polluted and warmer world.

CRediT authorship contribution statement

A. Martins: Methodology – planning of the bioassays; Investigation – carried out the bioassays and determined the concentrations of microplastics in the test medium; Data collection – biological and microplastic concentration data; Writing - Review & Editing.

D. Dias da Silva: Methodology – planning of lithium analyses; Investigation – determination of lithium concentrations in the test medium; Data collection – lithium concentrations in the test medium; Writing – method to determine lithium concentrations, Review & Editing.

R. Silva: Methodology – planning of lithium analyses; Investigation – determination of lithium concentrations in the test medium; Data collection – lithium concentrations in the test medium; Writing – method to determine lithium concentrations, Review & Editing.

F. Carvalho: Methodology – planning of lithium analyses; Investigation – determination of lithium concentrations in the test medium; Data collection – lithium concentrations in the test medium; Resources; Supervision; Funding acquisition; Writing – method to determine lithium concentrations, other contributions, Review & Editing.

L. Guilhermino: Conceptualization; Methodology – Planning of the bioassays and microplastic determinations; Investigation – Data analysis; Supervision; Data curation; Visualization – Figures and Tables; Resources; Project administration; Funding acquisition; Writing – Original draft preparation, Review & Editing.

Data availability

Data will be made available on request.

Declaration of competing interest

None.

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Appendix A. Supplementary data

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