Environmental Pollution 292 (2022) 118363

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Warming, temperature fluctuations and thermal evolution change the effects of microplastics at an environmentally relevant concentration☆

Mengjie Chang [a], Chao Zhang [a,*], Mingyang Li [a], Junyu Dong [a], Changchao Li [a], Jian Liu [a], Julie Verheyen [b], Robby Stoks [b]

[a] Environment Research Institute, Shandong University, Qingdao, 266237, China
[b] Evolutionary Stress Ecology and Ecotoxicology, KU Leuven, Leuven, B-3000, Belgium

ARTICLE INFO

Keywords:
Daily temperature fluctuations
Environmentally relevant concentration
Geographic variation
Global warming
Microplastics
Thermal evolution

ABSTRACT

Microplastics are sometimes considered not harmful at environmentally relevant concentrations. Yet, such studies were conducted under standard thermal conditions and thereby ignored the impacts of higher mean temperatures (MT), and especially daily temperature fluctuations (DTF) under global warming. Moreover, an evolutionary perspective may further benefit the future risk assessment of microplastics under global warming. Here, we investigated the effects of two generations of exposure to an environmentally relevant concentration of polystyrene microplastics (5 μg L^{-1}) under six thermal conditions (2 MT × 3 DTF) on the life history, physiology, and behaviour of *Daphnia magna*. To assess the impact of thermal evolution we thereby compared *Daphnia* populations from high and low latitudes. At the standard ecotoxic thermal conditions (constant 20 °C) microplastics almost had no effect except for a slight reduction of the heartbeat rate. Yet, at the challenging thermal conditions (higher MT and/or DTF), microplastics affected each tested variable and caused an earlier maturation, a higher fecundity and intrinsic growth rate, a decreased heartbeat rate, and an increased swimming speed. These effects may be partly explained by hormesis and/or an adaptive response to stress in *Daphnia*. Moreover, exposure to microplastics at the higher mean temperature increased the fecundity and intrinsic growth rate of cold-adapted high-latitude *Daphnia*, but not of the warm-adapted low-latitude *Daphnia*, suggesting that thermal evolution in high-latitude *Daphnia* may buffer the effects of microplastics under future warming. Our results highlight the critical importance of DTF and thermal evolution for a more realistic risk assessment of microplastics under global warming.

1. Introduction

Microplastics, with particle sizes <5 mm, are ubiquitous in the environment (Rachman, 2018). For example, they have been detected in soil (Ding et al., 2021; Wang et al., 2021), freshwater (Li et al., 2018; Zhang et al., 2018a,b), oceans (Browne et al., 2011; Anderson et al., 2016), and even in distant Arctic ice (Zarfl and Matthies, 2010; Obbard et al., 2014). Microplastics can persist in the environment for a long time and threaten organisms' feeding, growth, reproduction and physiology, and even may result in death (Browne et al., 2013; Lu et al., 2016; Botterell et al., 2019). Currently, one prominent problem in ecotoxicological studies on microplastics is that most exposure concentrations are far greater than the actual concentrations of microplastics in the environment (i.e. 2-7 orders of magnitudes higher, Lenz et al., 2016). Furthermore, due to the low concentrations of environmental microplastics, long-term or even multiple generations of exposure may be needed to detect their effects on organisms (Guven et al., 2018). Recent studies that used multigenerational exposure have indeed shown that also the low environmentally relevant concentrations may cause biological effects. Indeed, three generations of exposure to polystyrene microplastics (1 μg L^{-1}) caused oxidative damage in the water flea *Daphnia pulex* (Liu et al., 2020), and three generations of exposure to polystyrene (2000 particles ml^{-1}) reduced the survival of *D. magna* (Schür et al., 2020). Therefore, investigating the effects of microplastics at environmentally relevant concentrations under a multi-generational exposure scenario is crucial to assess their real ecological risks.

In nature, microplastics is inevitably affected by other environmental stressors, of which warming is one of the most important. Studies have

☆ This paper has been recommended for acceptance by Sarah Harmon.
* Corresponding author.
E-mail address: chaozhang@sdu.edu.cn (C. Zhang).

https://doi.org/10.1016/j.envpol.2021.118363
Received 14 July 2021; Received in revised form 12 September 2021; Accepted 10 October 2021
Available online 11 October 2021
0269-7491/

shown warming and pollutants may interact with each other (Noyes et al., 2009; Moe et al., 2013). Notably, most studies addressing the interactive effects of pollutants and warming only focused on the traditional pollutants, and typically found both pesticides (e.g. Verheyen and Stoks, 2019a) and trace metals (e.g. Zhang et al., 2018a,b) to become more toxic at higher mean temperatures. Instead, "contaminants of emerging concern" such as microplastics have only begun to gain attention very recently in the context of global warming (e.g. Jaikumar et al., 2018; Sadler et al., 2019; Yang et al., 2020; Lyu et al., 2021). Notably, recent studies indicated that the effects of microplastics on organisms may be influenced by temperature. Specifically, in the ecotoxicological model species *Daphnia magna*, the toxicity of microplastics was consistently higher at a higher mean temperature. Indeed, Lyu et al. (2021) found the toxicity of microplastics was more prominent at 30 °C than at 20 °C and 15 °C, and Jaikumar et al. (2018) reported that acute sensitivity to microplastics increased sharply with temperature in *D. magna*. Yet, Sadler et al. (2019) found only limited effects of elevated temperature on the toxicity of microplastics. These results suggest more studies about how higher temperatures affect microplastics are needed. Compared to increases in mean temperature (MT), increases in daily temperature fluctuations (DTF) have received much less attention in global warming research (Vasseur et al., 2014). Increases in DTF may, however, impact fitness more severely compared to increases in MT (Vasseur et al., 2014). This is because higher extreme temperatures are reached during the daily DTF cycle that ask for energetically costly mechanisms to deal with (Colinet et al., 2015). The few studies that integrated DTF into ecotoxicology demonstrated DTF to increase the toxicity of traditional pollutants: pesticides (e.g. Verheyen and Stoks, 2019a) and trace metals (Hallman and Brooks, 2015). Yet, whether and how the effects of microplastics are affected by DTF remains untested.

When studying the interactive effects of pollutants and warming, a geographic perspective, especially the study of populations along thermal gradients, is highly relevant for two reasons. First, populations may show local adaptation along thermal gradients, such as latitudinal gradients, which may reduce the increase in toxicity of pollutants under warming (e.g. for pesticides: Verheyen et al., 2019a, for metals: Dinh Van et al., 2013). Second, contrasting populations from geographic regions with a temperature difference matching a warming scenario, may allow estimating the effects of thermal evolution in the cooler region (for example, a high-latitude region) under future warming by using the current phenotype in the already warmer region (for example, a low-latitude region). Such so-called "space-for-time substitutions" (Stoks et al., 2014; Frenne et al., 2013; Verheyen et al., 2019a) have only been applied few times in ecotoxicology (Verheyen et al., 2019a). The current sensitivity to pollutants under higher temperatures in warm-adapted, low-latitude populations may be used to predict such sensitivity in the future in cold-adapted, high-latitude populations (Verheyen et al., 2019a). Given its powerful prediction ability, "space-for-time substitutions" may add an evolutionary perspective into the impact of microplastics under future warming.

Here, we investigated the effects of transgenerational exposure of polystyrene microplastics at environmentally relevant concentration in the water flea *Daphnia magna*. To explore the effects of microplastics under global warming and the influence of thermal evolution, we tested the single and combined effects of increases in mean temperature and in daily temperature fluctuations on the sensitivity to the microplastics both in low- and high-latitude populations of *D. magna*. Specifically, we tested: (i) if an environmentally relevant concentration of polystyrene microplastics (5 μg L^{-1}) affects *D. magna* after two generations of exposure; (ii) if DTF and a 4 °C increase in MT magnify the effects of polystyrene microplastics; and (iii) if thermal evolution can buffer any magnified effects of polystyrene microplastics under warming. We selected polystyrene microplastics because these have the highest production volume and make up the largest part of plastics pollution in the environment (Andrady and Neal, 2009). We contrasted high-latitude and low-latitude *D. magna* populations to explore the impact of thermal evolution on the sensitivity of polystyrene microplastics under warming in the high-latitude populations. To obtain a detailed picture of the effects, we studied key life history traits (intrinsic growth rate "r", age at maturity, fecundity and somatic growth rate), behavioural trait (swimming speed) and physiological trait (heartbeat rate). Behavioural and physiological endpoints of *D. magna* can improve the detection of pollutant effects at low concentrations where traditional endpoints (e.g. mortality, immobilisation) may not be sensitive enough (Bownik and Pawlik-Skowrońska, 2019; Bownik et al., 2020). Our study design can not only generate results that benefit the ecological risk assessment of microplastics at environmentally relevant concentrations and in currently warmer regions, but can also improve the prediction of its future effects under global warming in currently cooler regions.

2. Materials and methods

2.1. Preparation of microplastics

Polystyrene microplastics (MP) were purchased from BaseLine Chromtech Research Centre, Tianjin, China. The initial concentration of microplastics was 10 g L^{-1} according to the manufacture's information. Before the experiment, the polystyrene microplastics were transferred to a dialysis bag (MWCO 1000 Da) for 3 days to remove additives from the solution. The mean diameter of the dialyzed MP (154.1 ± 2.9 nm) was determined by dynamic light scattering (DLS) (Zetasizer Nano ZS 90, Malvern Instrument, UK). Then the dialysed solution was ultrasonicated in an ultrasonic bath (Shumei KQ5200DE, Kunshan) for 30 min and diluted with MilliQ water to make the stock solution at a nominal concentration of 100 mg L^{-1}. The stock solution was stored at 4 °C under darkness, and was further diluted with MilliQ water to the final concentration of 5 μg L^{-1} for the exposure experiment. Each time a test solution was made, the stock solution was ultrasonicated for 30 min at maximum power to eliminate aggregates.

2.2. Study populations

Three populations of *D. magna* were sampled from shallow ponds at each of two latitudes within Europe. The three low-latitude populations were sampled from southern France (south of Mireval, north of Mireval, and Frontignan), while the three high-latitude populations were from Sweden (Lake Bysjön), Norway (Pond Asklund), and Denmark (Lake Ring). Before the experiment, all *D. magna* clones were cultured in the laboratory under standard conditions (20 ± 1 °C, photoperiod of 16:8 L: D, daily fed 1×10^5 cells mL^{-1} *Acutodesmus obliquus*) in glass flasks filled with dechlorinated tap water for nearly three months. To start the experiment, one clone from each of the six populations was randomly selected. To minimize interference from maternal effects, these clones were individually cultured for two generations (ca. one month) under the standard conditions.

2.3. Experimental design

To investigate the effects of exposure to microplastics, mean temperature and daily temperature fluctuations on high- and low-latitude *D. magna*, a full factorial experiment was set up. High- and low-latitude *D. magna* were exposed to two microplastics treatments (absent and present) at one of the six temperature combinations: 2 MT treatments (20 °C and 24 °C) crossed with DTF treatments (constant = 0 °C, low = 5 °C, high = 10 °C). This resulted in 12 treatments combinations for each latitude: 2 MP × 2 MT × 3 DTF. An exposure concentration of 5 μg L^{-1} of polystyrene was selected as this is within the reported range of environmentally relevant concentrations (1–230 μg L^{-1}, see for example: Dong et al., 2018; Naidoo and Glassom, 2019; Wang et al., 2019; Zhang et al., 2019; Liu et al., 2020). Animals were exposed throughout their life to the microplastics and the medium was

renewed every two days.

The temperature treatments were chosen based on the current temperature regimes of the shallow water bodies (<1 m) that *D. magna* inhabits at the two latitudes: a MT of 20 °C with a maximum DTF of 5 °C at the high latitude (daily cycle going from 17.5 °C to 22.5 °C), and a MT of 24 °C with a maximum DTF of 10 °C at the low latitude (daily cycle going from 19 °C to 29 °C) (see more details about the DTF treatment in Fig. S1). These temperature data were derived from the Flake model (Simmons et al., 2007) and were confirmed with in situ measured temperatures with data loggers (Hobo TidbiT v2 Temp logger) (De Block et al., 2013; Debecker and Stoks, 2019). Experimental water temperature data were collected in the glass jars using an Elitech (GSP-6 thermometer) data logger (see Fig. S1). According to the predictions of the IPCC (Intergovernmental Panel on Climate Change) scenario RCP (Representative Concentration Pathway) 8.5, by the end of this century the mean temperature will increase ~4 °C and the magnitude of DTF will increase ~5 °C (IPCC, 2013). Therefore, the current temperature regime at the low latitude corresponds with the predicted temperature regime by 2100 at the high latitude under global warming. Following Paaijmans et al. (2013), the expected magnitude of DTF at the high latitude under warming scenario RCP 8.5 was estimated using daily temperature ranges obtained from the BSS_CSM 1.1 model (Beijing Climate Center Climate System Model 1.1). More details of the temperature conditions can be found in Verheyen et al. (2019a).

In the first experimental generation, for each *D. magna* clone, sets of 8–12 neonates that were born within 48 h were exposed to one of the 12 treatment combinations. Note that most glass jars had 10 neonates, but due to pipetting errors (two units [0.93%] had 8 neonates, and one unit [0.46%] had 12 neonates). Given only few glass jars started with a different number of neonates and all glass jars were given enough food which is unlikely to have generated density effects. There were three replicates per treatment combination, resulting in 216 experimental units: 6 clones × 2 MP × 2 MT × 3 DTF × 3 replicates. The experimental animals (F1) were reared in 730 mL glass jars containing 500 mL of culture medium in the absence and presence of microplastics. Animals were fed *A. obliquus* daily, 10^5 cells mL^{-1}, and the medium was refreshed every two days. After they released the second brood, 12–15 neonates (mostly 12 neonates, three units [1.39%] had 15 neonates, and four units [1.85%] had 14 neonates) from the third brood were randomly selected to start up the second generation (F2). The released offspring were counted and removed every day to keep densities in each jar constant throughout the experiment. The F2 *D. magna* were reared under the same treatment conditions as their mothers. Total exposure time was 31 days, including 16 days for the first generation and 15 days for the second generation. In the second generation, the response variables were quantified.

2.4. Life history

In the F2 generation, we quantified following key life history traits of *D. magna*: intrinsic growth rate "r", age at maturity, fecundity and somatic growth rate. The intrinsic growth rate was calculated based on the first two broods using the Euler equation:$1 = \int e^{-rx} l_x m_x dx$, where l_x represents the survival up to age x, and m_x the number of offspring released at day x. Age at maturity was defined as the day when more than half of the *D. magna* in a given glass jar were mature (carried brood in their brood pouch). Age at release for first and second clutch were defined as the first day of the mass release of first and second clutch respectively. The fecundity was calculated as the total number of offspring in the first and second clutch divided by the number of mother *Daphnia* present in the glass jar. To determine the somatic growth rate, the body length of *D. magna* when one day old and the day of maturation were measured using a SZ2-ILST stereomicroscope (Olympus, Tokyo, Japan) connected to a Longbase 1610 E camera (Longbase, Qingdao, China). *D. magna* body length was taken as the length from the top of the eyes to the base of the spine. The somatic growth rate was calculated as (body length at maturity – body length on day one)/age at maturity.

2.5. Swimming speed and heartbeat rate

When the F2 *D. magna* matured, two individuals from each glass jar were randomly picked and transferred to separate wells in a 6-well plastic plate to quantify their swimming speed. These animals were video recorded for 1 min (10 frames per second) from above using a GoPro 7 camera that was fixed above the plate. Only two mL culture medium was left in each well to minimize the depth of the medium. Therefore, the vertical swimming of *D. magna* was negligible. Before recording, *D. magna* was given 2 min for acclimation. The swimming speed of *D. magna* was analysed frame by frame using Tracker software following Bownik et al. (2020). As the vertical swimming was negligible, *D. magna* was considered only swimming in two dimensions and the swimming speed was analysed based on x- and y-coordinates. The swimming track left by each *D. magna* individual (interpreted as a mass point in Tracker) was recorded by clicking the mass point in each frame, and the mean velocity was calculated based on the two individuals tested per clone (Graph of swimming speed, see Fig. S2).

To investigate the heartbeat rate, two individuals from each jar were randomly selected and transferred to another six-well plastic plate. Before the video recording started the *D. magna* were given 2 min to acclimatize. The surrounding medium was removed with a pipet to minimize the movement of *D. magna* so that the heartbeat rate could be observed accurately (Bownik et al., 2020). The animals were video-recorded under a stereomicroscope (SZ2-ILST) connected to a camera (Longbase 1610 E) for 1 min. The heartbeat rate was analysed with a KM player (https://en.kmplayer.com/) software by the 0.5x slow playback method.

2.6. Statistical analyses

To test the single and combined effects of exposure to microplastics, MT, DTF and latitude on the response variables, generalized linear models were used. Clone was nested as random factor in latitude. Fisher's LSD post-hoc tests were applied to explore the significant differences among treatment combinations. To specifically test whether the MP had an effect under standard thermal conditions of a typical ecotoxic test we systematically contrasted the control and MP exposure treatment at a mean temperature 20 °C that was kept constant (DTF = 0 °C). P values < 0.05 were considered significant. As there were two-way and three-way interactions, significant differences were shown in a table with P values rather than asterisks on the bar plots. All analyses were conducted using IBM SPSS Statistics 26, and figures were plotted with Origin pro 2018C.

3. Results

3.1. Life history

Survival was high in all treatment combinations (>93%). Microplastics had no effects on any of the tested life history traits under the standard thermal conditions of MT = 20 °C and DTF = 0 °C (contrasts of the control versus MP exposure treatment: all $P > 0.522$). In contrast, several effects of MP exposure on life history were found at the higher MT of 24 °C and/or in the presence of DTF.

Exposure to microplastics decreased the age at maturity (main effect of MP), but only at the high 10 °C DTF (LSD: $P = 0.011$, MP × DTF: Table 1, Fig. 1A). Moreover, while this reduction at 10 °C DTF was at 24 °C present for both latitudes (LSD: all $P < 0.011$), at 20 °C it was only present for the low-latitude clones (LSD: $P = 0.011$) (MP × MT × DTF × Lat, Table 1). As expected, *D. magna* matured earlier at the higher mean temperature (MT: Table 1, Fig. 1A). Low-latitude *D. magna* matured earlier than high-latitude *D. magna* (Lat: Table 1, Fig. 1A), but only at 20 °C combined with 0 °C and 5 °C DTF (LSD: both $P < 0.01$, DTF × MT

Table 1
Results of generalized linear models testing for the effects of microplastics (MP), mean temperature (MT), daily temperature fluctuations (DTF), and latitude (Lat) on life history, swimming speed and heartbeat rate of *D. magna*.

	Age at maturity			Fecundity			Intrinsic growth rate			Somatic growth rate			Swimming speed			Heartbeat rate		
Effects	χ^2	df	*P*	χ^2	df	*P*	χ^2	df	*P*	χ^2	df	*P*	χ^2	df	*P*	χ^2	df	*P*
MP	13.33	1	**<0.001**	6.25	1	**0.012**	12.55	1	**<0.001**	0.24	1	0.625	3.97	1	**0.046**	11.09	1	**0.001**
MT	1080.00	1	**<0.001**	24.31	1	**<0.001**	370.15	1	**<0.001**	122.15	1	**<0.001**	69.19	1	**<0.001**	178.09	1	**<0.001**
DTF	0.80	2	0.670	23.25	2	**<0.001**	72.73	2	**<0.001**	1.33	2	0.514	17.83	2	**<0.001**	2.65	2	0.265
Lat	30.00	1	**<0.001**	12.38	1	**<0.001**	23.27	1	**<0.001**	61.95	1	**<0.001**	3.92	1	**0.048**	3.98	1	**0.046**
MP × MT	0.13	1	0.715	0.51	1	0.476	4.00	1	**0.046**	4.52	1	**0.034**	0.35	1	0.553	1.23	1	0.267
MP × DTF	8.27	2	**0.016**	6.01	2	**0.050**	11.16	2	**0.004**	0.63	2	0.731	5.47	2	0.065	2.59	2	0.274
MP × Lat	0.53	1	0.465	0.33	1	0.565	0.37	1	0.543	0.05	1	0.830	0.04	1	0.841	0.01	1	0.911
MT × DTF	22.40	2	**<0.001**	53.22	2	**<0.001**	2.58	2	0.276	27.09	2	**<0.001**	6.28	2	**0.043**	15.55	2	**<0.001**
MT × Lat	8.53	1	**0.003**	1.82	1	0.178	3.17	1	0.075	5.77	1	**0.016**	53.27	1	**<0.001**	0.94	1	0.333
DTF × Lat	7.20	2	**0.027**	5.00	2	0.082	0.11	2	0.945	14.83	2	**0.001**	3.47	2	0.177	2.51	2	0.286
MP × MT × DTF	7.47	2	**0.024**	2.62	2	0.270	9.99	2	**0.007**	1.34	2	0.512	5.90	2	0.052	0.24	2	0.887
MP × MT × Lat	0.13	1	0.715	7.51	1	**0.006**	5.50	1	**0.019**	0.66	1	0.417	0.01	1	0.934	2.00	1	0.157
MP × DTF × Lat	5.07	2	0.079	0.97	2	0.614	0.84	2	0.657	1.18	2	0.554	0.66	2	0.720	11.44	2	**0.003**
MT × DTF × Lat	7.47	2	**0.024**	2.12	2	0.347	0.07	2	0.964	4.65	2	0.098	0.37	2	0.832	6.61	2	**0.037**
MP × MT × DTF × Lat	7.47	2	**0.024**	5.14	2	0.077	4.59	2	0.101	6.88	2	**0.032**	10.40	2	**0.006**	0.15	2	0.929

× Lat: Table 1, Fig. 1A).

Notably, exposure to microplastics increased the fecundity, yet only under 5 °C DTF (LSD: $P = 0.001$, MP × DTF: Table 1, Fig. 1B). The effects of microplastics were also affected by mean temperature and latitude (MP × MT × Lat: Table 1, Fig. 1B): the microplastics-induced higher fecundity was only significant in high-latitude *D. magna* at 24 °C (LSD: $P = 0.001$). The interaction between mean temperature and DTF (Table 1, Fig. 1B) indicated that at a mean of 20 °C, 5 °C DTF increased the fecundity (LSD: $P < 0.001$), while at a mean of 24 °C, both 5 °C (LSD: $P = 0.007$) and 10 °C DTF (LSD: $P = 0.006$) decreased the fecundity.

While there was a main effect of exposure to microplastics for the intrinsic growth rate, this depended on the mean temperature, DTF and their combination resulting in a significant MP × MT × DTF interaction (Table 1, Fig. 1C). At a mean temperature of 20 °C, expose to microplastics increased the intrinsic growth rate but only at 5 °C DTF (LSD: $P = 0.001$), while at a mean temperature of 24 °C, this occurred only at 10 °C DTF. Importantly, there was a MP × MT × Lat interaction ($P < 0.001$, Table 1, Fig. 1C): the microplastics-induced higher intrinsic growth rate at 24 °C was only significant in high-latitude clones (LSD: $P < 0.001$). In general, the low-latitude *D. magna* had a higher intrinsic growth rate than the high-latitude *D. magna* (Lat: Table 1, Figs. 1C), and 4 °C warming increased the intrinsic growth rate (Table 1, Fig. 1C).

The effect of exposure to microplastics on the somatic growth rate depended jointly on both thermal factors and latitude, resulting in a significant MP × MT × DTF × Lat interaction (Table 1, Fig. 2). At a mean temperature of 24 °C, exposure to microplastics only increased the somatic growth rate in high-latitude *D. magna* under 10 °C DTF (LSD: $P < 0.001$). As expected, the somatic growth rate was higher at the higher mean temperature (MT: Table 1, Fig. 2). Overall, the low-latitude *D. magna* grew faster compared to the high-latitude *D. magna* (Lat: Table 1, Fig. 2).

3.2. Swimming speed and heartbeat rate

When tested under standard thermal conditions (20 °C and DTF = 0 °C), microplastics did not affect the swimming speed (contrast: $P = 0.271$). There was a significant MP × DTF × MT × Lat interaction for swimming speed (Table 1, Fig. 3A). At a mean temperature of 20 °C, exposure to microplastics reduced the swimming speed of low-latitude *D. magna* under 10 °C DTF (LSD: $P = 0.046$), yet increased the swimming speed for low-latitude *D. magna* under 5 °C DTF (LSD: $P = 0.001$).

Under the standard thermal ecotoxic test conditions of 20 °C and 0 °C DTF, microplastics decreased the heartbeat rate (contrast: $P = 0.032$). The full model showed that the microplastics-induced decrease in the heartbeat rate depended on the DTF and latitude (MP × DTF × Lat: Table 1, Fig. 3B). Under 0 °C DTF, microplastics only decreased the heartbeat rate of high-latitude *D. magna* (LSD: $P = 0.007$), while under 10 °C DTF microplastics only decreased the heartbeat rate of low-latitude *D. magna* (LSD: $P < 0.001$). *D. magna* generally had a higher heartbeat rate at the higher mean temperature (main effect of MT: Table 1, Fig. 3B), especially under 10 °C DTF (LSD: $P < 0.001$, MT × DTF: Table 1, Fig. 3B). Low-latitude *D. magna* had a higher heartbeat rate than high-latitude *D. magna* (Table 1, Fig. 3B). The effect of DTF on the heartbeat rate depended both on mean temperature and latitude (MT × DTF × Lat): 5 °C and 10 °C DTF increased it in high-latitude *D. magna* at 20 °C (LSD: $P = 0.001$, $P = 0.003$, respectively), but 10 °C DTF decreased it in both latitudes at 24 °C (LSD: both $P = 0.013$).

4. Discussion

Multigenerational exposure to an environmentally relevant concentration of microplastics was able to change key life history, physiological and behavioural traits of *D. magna*. Notably, these effects were rarely detected under the standard thermal conditions of ecotox tests (20 °C and 0 °C DTF), and were mainly observed at the higher mean temperature and/or under 5 °C and 10 °C DTF. Indeed, at the standard thermal

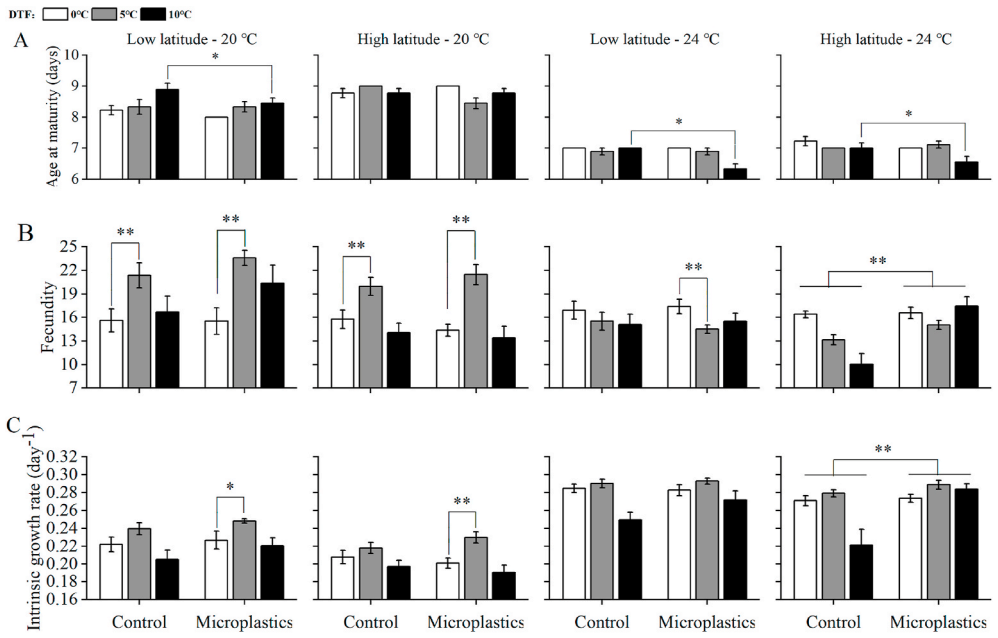

Fig. 1. Effects of microplastics (MP) on life history traits of low-latitude and high-latitude *D. magna* as a function of mean temperature and daily temperature fluctuations (DTF). (A) age at maturity, (B) fecundity, and (C) intrinsic growth rate. Given are means ± 1 SE. Asterisks indicate significant differences between treatments based on LSD tests (*: $p < 0.05$; **: $p < 0.01$).

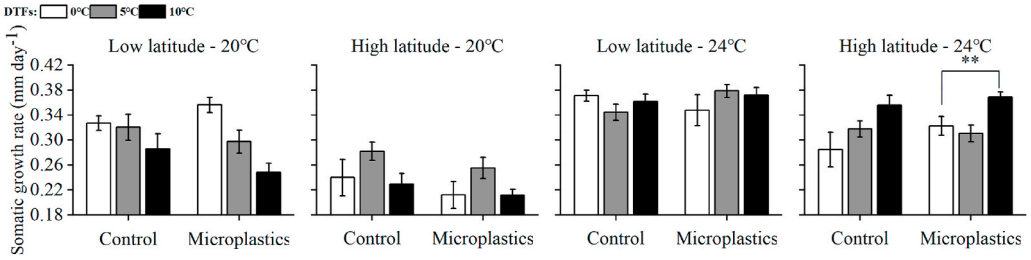

Fig. 2. Effects of microplastics (MP) on somatic growth rate of low-latitude and high-latitude *D. magna* as a function of mean temperature and daily temperature fluctuations (DTF). Given are means ± 1 SE. Asterisks indicate significant differences between different treatments based on LSD tests (*: $p < 0.05$; **: $p < 0.01$).

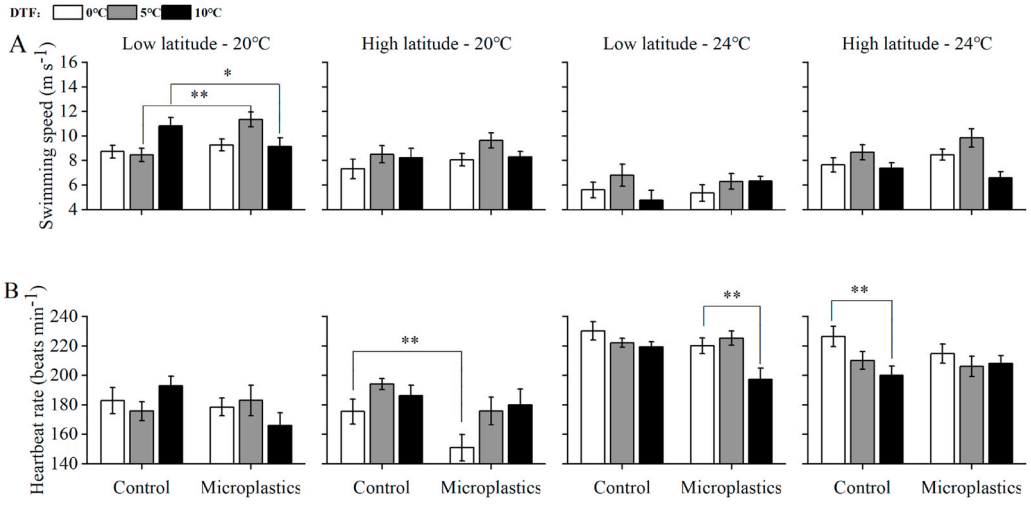

Fig. 3. Effects of microplastics (MP) on swimming speed (A) and heartbeat rate (B) of low-latitude and high-latitude *D. magna* as a function of mean temperature and daily temperature fluctuations (DTF). Given are means ± 1 SE. Asterisks indicate significant differences between treatments based on LSD tests (*: $p < 0.05$; **: $p < 0.01$).

conditions only an effect on heartbeat rate could be observed, while all variables were affected by microplastics under the more challenging thermal conditions. Moreover, capitalizing on a "space-for-time substitution" approach, we for the first time showed that the effects of microplastics under warming may be buffered by gradual thermal evolution, adding an important novel insight for the risk assessment of microplastics under global warming.

Based on our results and the presence of controls without microplastics we can safely conclude that the here used MP particles had a negative effect that is strongly dependent on thermal regimes. Nevertheless, we cannot determine what in particular about microplastics was inducing a biological response. To this end, future work would benefit from including an extra control treatment with natural particles of the same size and shape (Cunningham & Sigwart, 2019; Ogonowski et al., 2018; Yap et al., 2020). It is, however, important to note that the three studies that we know that directly compared the effects of "natural particles" and microplastics in our study species *D. magna* consistently showed that effects of natural particles were smaller than those of microplastics (Ogonowski et al., 2016; Schür et al., 2020; Zimmermann et al., 2020). In general, Ogonowski et al. (2018) showed the LOEC values of microplastics to be significantly lower compared to the values for suspended mineral particles for higher-level responses. Current evidence for the study species therefore suggests our results cannot be simply explained by the effects imposed by small particles in general.

4.1. Effects of microplastics at a low concentration under thermal stress

A key finding was that under standard ecotoxic test conditions (20 °C and 0 °C DTF), exposure to microplastics at an environmentally relevant concentration did not affect any trait, except for heartbeat rate. While under the more challenging thermal conditions (higher mean temperature and/or under DTF), microplastics did affect each tested variable. There is increasing awareness that microplastic exposure studies should be environmentally relevant (Lenz et al., 2016; Wang et al., 2019; Zhang et al., 2019). Yet, the limited number of studies on the effects of microplastics under environmentally relevant concentrations was mostly conducted under standard ecotox thermal conditions and reported no (Guven et al., 2018) or little effects (Zhang et al., 2019; Liu et al., 2020). We also observed that only one trait was directly affected by MP at the standard thermal conditions (constant 20 °C), i.e., the heartbeat rate was decreased. No studies tested the effects of multigenerational exposure to microplastics at a low concentration. Xu et al. (2020) found exposure of one generation to a much higher concentration (50 mg L^{-1}) of polystyrene microplastics also decreased the heartbeat rate of *D. magna*. Though they did not find microplastics in the heart of exposed *D. magna*, the possibility of microplastics penetrating and accumulating in the heart of *D. magna* cannot be excluded (Xu et al., 2020).

Under more challenging thermal conditions, the microplastics induced an earlier maturation, a higher fecundity and a higher intrinsic growth rate. Liu et al. (2020) also showed an increased fecundity and growth rate of *D. magna* exposed to a low concentration of polystyrene microplastics for three generations. The apparently beneficial effect of the microplastics at the low concentration may be related to the hormesis effect where low concentrations of pollutants may stimulate or benefit organisms while negative effects become only apparent at higher concentrations (Mattson, 2008). The increased fecundity might also reflect a strategy where the *D. magna* try to maximize the chance that some offspring survive under microplastics exposure (Liu et al., 2020).

Compared with life history traits, behavioural responses may better reflect the effects of microplastics at environmentally relevant concentrations as they are more sensitive indicators (Bownik and Pawlik--Skowrońska, 2019; Bownik et al., 2020). Nevertheless, also the effects of microplastics on swimming speed were only detected under more challenging thermal conditions. To date, no studies investigated the behavioural responses of *D. magna* in response to multigenerational exposure to low concentration microplastics. Yet, exposure during one generation to a much higher concentrations of polystyrene (1.25 mg L^{-1} and 12.5 mg L^{-1}) enhanced the swimming activity of *D. magna* (De Felice et al., 2019). Our results showed that microplastics significantly increased the swimming speed under 5 °C DTF. An increased swimming activity of *D. magna* in response to pollutants has been interpreted as avoidance behaviour to escape more polluted microsites in water bodies (Lopes et al., 2004) and/or attempts to get rid of the microplastics particles (De Felice et al., 2019). It is noteworthy that the increased swimming speed may increase food ingestion and consequently may have contributed to the here observed increased fecundity as *D. magna* are filter-feeders whose food intake is tightly related to swimming (Enserink et al., 1993).

Another novel finding of our study is that the effects of microplastics depended not only on an increase in mean temperature but also on daily temperature fluctuations. The interactive effects between pollutants and the omnipresent DTF only attracted attention in recent years and the few available studies documented that DTF can magnify the effects of traditional contaminants (e.g. pesticides: Verheyen and Stoks, 2019b; trace metals: Hallman and Brooks, 2015). Though microplastics, as a "contaminant of emerging concern", has received ample attention in recent years, no studies have explored the toxicity of microplastics under more realistic and challenging DTF. We here for the first time showed that the effects of microplastics also depended on DTF. Specifically, only when under a 5 °C DTF cycle, exposure to microplastics increased fecundity and the intrinsic population growth rate; this might partly have been caused by a higher food ingestion linked to the higher swimming speed of (low-latitude) *D. magna*. Notably, 10 °C DTF did not further change the fecundity of *D. magna* exposed to microplastics compared to 0 °C DTF. This may be explained by two reasons: (i) less food was ingested, and (ii) an energy-based trade-off between growth and defence mechanisms against high temperatures. During each 10 °C DTF cycle, a maximum temperature of 29 °C at a mean temperature of 24 °C was encountered by *D. magna* which might have exceeded its optimum temperature, resulting in a lower swimming speed and thus a decreased food intake. This is in line with the study that at the thermal optimum (32 °C) a high 12 °C DTF strongly decreased the survival of *Anopheles stephensi* mosquito larvae compared to a lower 8 °C DTF (Paaijmans et al., 2013).

4.2. Thermal evolution may buffer the effects of microplastics under warming

Low-latitude *D. magna*, living at higher mean temperatures, had evolved a faster life history. Indeed, low-latitude *D. magna* matured earlier, grew faster, and had a higher fecundity and intrinsic growth rate compared to high-latitude *D. magna.* Similarly, Brans and De Meester (2018) found a genetically determined faster pace of life (earlier maturation, increased fecundity and higher population growth rate) in *D. magna* isolated from warmer urban ponds compared to colder rural ponds. Together, this suggests that *D. magna* evolves in response to warmer conditions a faster life history. In the same set of here studied clones, Wang et al. (in prep) also showed across a large range of rearing temperatures that the optimal temperature for intrinsic growth rate was higher in the low-latitude than in the high-latitude clones. This matches the pattern of Geerts et al. (2014) who observed a higher heat tolerance of *D. magna* in low-latitude compared to high-latitude clones. This all points to low-latitude *D. magna* having evolved a higher tolerance to warmer temperatures and a faster life history.

As can be expected, this adaptation to the local thermal conditions also shaped how *D. magna* from both latitudes differently responded to microplastics at the higher mean temperature. Exposure to microplastics at the higher mean temperature increased the fecundity and intrinsic growth rate only for the high-latitude *D. magna*. This may be explained by low-latitude *D. magna* being adapted to the higher mean temperature meaning they may have more energy to deal with the microplastics at

24 °C, thus buffering the effects of microplastics at the higher mean temperature. It is important to note hereby that *D. magna* typically responds to a stressor, such as predation risk, by increasing its intrinsic growth rate (Zhang et al., 2016). Hence, we interpret the lack of a microplastics-induced increase in intrinsic growth rate at 24 °C in low-latitude *D. magna* as indicating they were stressed less by the microplastics at 24 °C than the high-latitude *D. magna*. Alternatively, at 24 °C the high-latitude *D. magna* experienced the microplastics as more stressful than the low-latitude *D. magna* and therefore only high-latitude *D. magna* showed a hormetic response. As the future thermal conditions at the high-latitude sites under global warming by 2100 (IPCC, 2013) are predicted to match the current thermal conditions at the low-latitude sites, the future effects of microplastics on high-latitude *D. magna* under global warming can be predicted based on the current effects of microplastics on low-latitude *D. magna* (Verheyen et al., 2019b). Our results therefore suggest that high-latitude *D. magna* may become able to buffer the effects of microplastics at 24 °C (as current low-latitude *D. magna*) by gradual thermal evolution under future 4 °C warming by 2100.

4.3. Implications for risk assessment of microplastics under global warming

By studying the single and combined effects of 4 °C warming and DTF on the effects of microplastics, we added two novel insights for the risk assessment of microplastics under global warming. First, environmentally relevant concentrations of microplastics may have no observable impact on *D. magna* under widely-used standard thermal conditions, while their effects become distinct under more realistic and challenging thermal conditions, i.e. a higher mean temperature and/or DTF. This is important to consider both for current risk assessment, as there is large spatial variation in these thermal factors (e.g. along latitudinal and urbanisation gradients, Verheyen et al., 2019b) and DTFs are encountered by most organisms in natural environments, and for future risk assessment as both mean temperatures and DTF are expected to increase under global warming (IPCC, 2013). Second, based on a "space-for-time substitution", our results suggest that thermal evolution may buffer the effects of microplastics under warming at the high-latitude sites, highlighting of the importance of an evolutionary perspective for risk assessment of microplastics.

5. Conclusions

Our results demonstrated that microplastics at an environmentally relevant concentration had no effects on the tested variables (expect for heartbeat rate) on *D. magna* under standard thermal conditions. Yet, under more realistic and challenging thermal conditions, microplastics changed each tested variable: an earlier maturation, a higher fecundity and intrinsic growth rate, a decreased heartbeat rate and an increased swimming speed. This highlighted that global warming should be considered in the ecological risk assessment of microplastics. Furthermore, capitalizing on "space-for-time substitution" approach, we showed that high-latitude *D. magna* may buffer the effects of microplastics under warming by gradually thermal evolution, which provided novel insights on the ecological risk assessment of microplastics under global warming. Taken together, our results demonstrated that more realistic thermal conditions (including DTF) and an evolutionary perspective are critical for a more realistic current and future ecological risk assessment of microplastics.

Credit author statement

Mengjie Chang: Conduct the experiment, Methodology, Data curation, Writing- Original draft preparation. Chao Zhang: Conceptualization, Methodology, Writing- Reviewing and Editing, Supervision, Funding acquisition. Mingyang Li: Help with the experiment. Junyu Dong: Help with data curation. Changchao Li: Help with data curation. Jian Liu: Supervision. Julie Verheyen: Writing- Reviewing and Editing. Robby Stoks: Writing- Reviewing and Editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (42007229), the China Postdoctoral Science Foundation (2019M662337), and the Post-doctoral Innovation Research Program of Shandong Province (236346).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2021.118363.

References

Anderson, J.C., Park, B.J., Palace, V.P., 2016. Microplastics in aquatic environments: implications for Canadian ecosystems. Environ. Pollut. 218, 269–280.
Andrady, A.L., Neal, M.A., 2009. Applications and societal benefits of plastics. Philos. Trans. R. Soc., B. 364, 1977–1984.
Botterell, Z.L.R., Beaumont, N., Dorrington, T., Steinke, M., Thompson, R.C., Lindeque, P. K., 2019. Bioavailability and effects of microplastics on marine zooplankton: a review. Environ. Pollut. 245, 98–110.
Bownik, A., Pawlik-Skowrońska, B., 2019. Early indicators of behavioral and physiological disturbances in *Daphnia magna* (Cladocera) induced by cyanobacterial neurotoxin anatoxin-a. Sci. Total Environ. 695, 133913.
Bownik, A., Ślaska, B., Dudka, J., 2020. Cisplatin affects locomotor activity and physiological endpoints of *Daphnia magna*. J. Hazard Mater. 384, 121259.
Brans, K.I., De Meester, L., 2018. City life on fast lanes: urbanization induces an evolutionary shift towards a faster lifestyle in the water flea *Daphnia*. Funct. Ecol. 32, 2225–2240.
Browne, M.A., Crump, P., Niven, S.J., Teuten, E., Tonkin, A., Galloway, T., Thompson, R., 2011. Accumulation of microplastic on shorelines worldwide: sources and sinks. Environ. Sci. Technol. 45, 9175–9179.
Browne, M.A., Niven, S.J., Galloway, T.S., Rowland, S.J., Thompson, R.C., 2013. Microplastic moves pollutants and additives to worms, reducing functions linked to health and biodiversity. Curr. Biol. 23, 2388–2392.
Colinet, H., Sinclair, B.J., Vernon, P., Renault, D., 2015. Insects in fluctuating thermal environments. Annu. Rev. Entomol. 60, 123–140.
Cunningham, E.M., Sigwart, J.D., 2019. Environmentally accurate microplastic levels and their absence from exposure studies. Integr. Comp. Biol. 59, 1485–1496.
De Block, M., Pauwels, K., Van Den Broeck, M., De Meester, L., Stoks, R., 2013. Local genetic adaptation generates latitude-specific effects of warming on predator-prey interactions. Global Change Biol. 19, 689–696.
De Felice, B., Sabatini, V., Antenucci, S., Gattoni, G., Santo, N., Bacchetta, R., Ortenzi, M. A., Parolini, M., 2019. Polystyrene microplastics ingestion induced behavioral effects to the cladoceran *Daphnia magna*. Chemosphere 231, 423–431.
Debecker, S., Stoks, R., 2019. Pace of life syndrome under warming and pollution: integrating life history, behavior, and physiology across latitudes. Ecol. Monogr. 89, e1332.
Ding, L., Wang, X., Ouyang, Z., Chen, Y., Wang, X., Liu, D., Liu, S., Yang, X., Jia, H., Guo, X., 2021. The occurrence of microplastic in Mu Us Sand Land soils in northwest China: different soil types, vegetation cover and restoration years. J. Hazard Mater. 403, 123982.
Dinh Van, K., Janssens, L., Debecker, S., De Jonge, M., Lambret, P., Nilsson-Örtman, V., Bervoets, L., Stoks, R., 2013. Susceptibility to a metal under global warming is shaped by thermal adaptation along a latitudinal gradient. Global Change Biol. 19, 2625–2633.
Dong, S., Qu, M., Rui, Q., Wang, D., 2018. Combinational effect of titanium dioxide nanoparticles and nanopolystyrene particles at environmentally relevant concentrations on nematode Caenorhabditis elegans. Ecotoxicol. Environ. Saf. 161, 444–450.
Enserink, L., de la Haye, M., Maas, H., 1993. Reproductive strategy of *Daphnia magna*: implications for chronic toxicity tests. Aquat. Toxicol. 25, 111–123.
Frenne, P., Graae, B.J., Rodríguez Sánchez, F., Kolb, A., Chabrerie, O., Decocq, G., Kort, H., Schrijver, A., Diekmann, M., Eriksson, O., Gruwez, R., Hermy, M., Lenoir, J., Plue, J., Coomes, D.A., Verheyen, K., 2013. Latitudinal gradients as natural laboratories to infer species' responses to temperature. J. Ecol. 101, 784–795.

Geerts, A.N., De Meester, L., Stoks, R., 2014. Heat tolerance and its evolutionary potential along a latitudinal gradient in *Daphnia magna*. Evol. Ecol. Res. 16, 517–528.
Guven, O., Bach, L., Munk, P., Dinh, K.V., Mariani, P., Nielsen, T.G., 2018. Microplastic does not magnify the acute effect of PAH pyrene on predatory performance of a tropical fish (*Lates calcarifer*). Aquat. Toxicol. 198, 287–293.
Hallman, T.A., Brooks, M.L., 2015. The deal with diel: temperature fluctuations, asymmetrical warming, and ubiquitous metals contaminants. Environ. Pollut. 206, 88–94.
Ipcc, C.C., 2013. The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge (United Kingdom) and New York (USA).
Jaikumar, G., Baas, J., Brun, N.R., Vijver, M.G., Bosker, T., 2018. Acute sensitivity of three Cladoceran species to different types of microplastics in combination with thermal stress. Environ. Pollut. 239, 733–740.
Lenz, R., Enders, K., Nielsen, T.G., 2016. Microplastic exposure studies should be environmentally realistic. Proc. Natl. Acad. Sci. Unit. States Am. 113, E4121–E4122.
Li, J., Liu, H., Paul Chen, J., 2018. Microplastics in freshwater systems: a review on occurrence, environmental effects, and methods for microplastics detection. Water Res. 137, 362–374.
Liu, Z., Cai, M., Wu, D., Yu, P., Jiao, Y., Jiang, Q., Zhao, Y., 2020. Effects of nanoplastics at predicted environmental concentration on *Daphnia pulex* after exposure through multiple generations. Environ. Pollut. 256, 113506.
Lopes, I., Baird, D.J., Ribeiro, R., 2004. Avoidance of copper contamination by field populations of *Daphnia longispina*. Environ. Toxicol. Chem. 23, 1702–1708.
Lu, Y., Zhang, Y., Deng, Y., Jiang, W., Zhao, Y., Geng, J., Ding, L., Ren, H., 2016. Uptake and accumulation of polystyrene microplastics in zebrafish (*Danio rerio*) and toxic effects in liver. Environ. Sci. Technol. 50, 4054–4060.
Lyu, K., Cao, C., Li, D., Akbar, S., Yang, Z., 2021. The thermal regime modifies the response of aquatic keystone species Daphnia to microplastics: evidence from population fitness, accumulation, histopathological analysis and candidate gene expression. Sci. Total Environ. 783, 147154.
Mattson, M.P., 2008. Hormesis defined. Ageing Res. Rev. 7, 1–7.
Moe, S.J., De Schamphelaere, K., Clements, W.H., Sorensen, M.T., Van den Brink, P.J., Liess, M., 2013. Combined and interactive effects of global climate change and toxicants on populations and communities. Environ. Toxicol. Chem. 32, 49–61.
Naidoo, T., Glassom, D., 2019. Decreased growth and survival in small juvenile fish, after chronic exposure to environmentally relevant concentrations of microplastic. Mar. Pollut. Bull. 145, 254–259.
Noyes, P.D., McElwee, M.K., Miller, H.D., Clark, B.W., Van Tiem, L.A., Walcott, K.C., Erwin, K.N., Levin, E.D., 2009. The toxicology of climate change: environmental contaminants in a warming world. Environ. Int. 35, 971–986.
Obbard, R.W., Sadri, S., Wong, Y.Q., Khitun, A.A., Baker, I., Thompson, R.C., 2014. Global warming releases microplastic legacy frozen in Arctic Sea ice. Earth's Future 2, 315–320.
Ogonowski, M., Schür, C., Jarsén, Å., Gorokhova, E., 2016. The effects of natural and anthropogenic microparticles on individual fitness in Daphnia magna. PLoS One 11, e155063.
Ogonowski, M., Gerdes, Z., Gorokhova, E., 2018. What we know and what we think we know about microplastic effects - a critical perspective. Curr. Opin. Environ. Sci. Health 1, 41–46.
Paaijmans, K.P., Heinig, R.L., Seliga, R.A., Blanford, J.I., Blanford, S., Murdock, C.C., Thomas, M.B., 2013. Temperature variation makes ectotherms more sensitive to climate change. Global Change Biol. 19, 2373–2380.
Rachman, C.M., 2018. Microplastics research - from sink to source. Science 360, 28–29.
Sadler, D.E., Brunner, F.S., Plaistow, S.J., 2019. Temperature and clone-dependent effects of microplastics on immunity and life history in *Daphnia magna*. Environ. Pollut. 255, 113178.
Schür, C., Zipp, S., Thalau, T., Wagner, M., 2020. Microplastics but not natural particles induce multigenerational effects in *Daphnia magna*. Environ. Pollut. 260, 113904.
Simmons, A., Uppala, S.M., Dee, D., Kobayashi, S., 2007. ERA-Interim: new ECMWF reanalysis products from 1989 onwards. ECMWF Newsletter 110, 25–35.
Stoks, R., Geerts, A.N., De Meester, L., 2014. Evolutionary and plastic responses of freshwater invertebrates to climate change: realized patterns and future potential. Evol. Appl. 7, 42–55.
Vasseur, D.A., DeLong, J.P., Gilbert, B., Greig, H.S., Harley, C.D.G., McCann, K.S., Savage, V., Tunney, T.D., O'Connor, M.I., 2014. Increased temperature variation poses a greater risk to species than climate warming. Proc. R. Soc. B. 281, 20132612.
Verheyen, J., Delnat, V., Stoks, R., 2019a. Increased daily temperature fluctuations overrule the ability of gradual thermal evolution to offset the increased pesticide toxicity under global warming. Environ. Sci. Technol. 53, 4600–4608.
Verheyen, J., Tuzun, N., Stoks, R., 2019b. Using natural laboratories to study evolution to global warming: contrasting altitudinal, latitudinal, and urbanization gradients. Curr. Opin. Insect Sci. 35, 10–19.
Verheyen, J., Stoks, R., 2019a. Shrinking body size and physiology contribute to geographic variation and the higher toxicity of pesticides in a warming world. Environ. Sci. Technol. 53, 11515–11523.
Verheyen, J., Stoks, R., 2019b. Current and future daily temperature fluctuations make a pesticide more toxic: contrasting effects on life history and physiology. Environ. Pollut. 248, 209–218.
Wang, J., Li, J., Liu, S., Li, H., Chen, X., Peng, C., Zhang, P., Liu, X., 2021. Distinct microplastic distributions in soils of different land-use types: a case study of Chinese farmlands. Environ. Pollut. 269, 116199.
Wang, Y., Zhang, D., Zhang, M., Mu, J., Ding, G., Mao, Z., Cao, Y., Jin, F., Cong, Y., Wang, L., Zhang, W., Wang, J., 2019. Effects of ingested polystyrene microplastics on brine shrimp, Artemia parthenogenetica. Environ. Pollut. 244, 715–722.
Xu, E.G., Cheong, R.S., Liu, L., Hernandez, L.M., Azimzada, A., Bayen, S., Tufenkji, N., 2020. Primary and secondary plastic particles exhibit limited acute toxicity but chronic effects on *Daphnia magna*. Environ. Sci. Technol. 54, 6859–6868.
Yang, Y., Guo, Y., O Brien, A.M., Lins, T.F., Rochman, C.M., Sinton, D., 2020. Biological responses to climate change and nanoplastics are altered in concert: full-Factor screening reveals effects of multiple stressors on primary producers. Environ. Sci. Technol. 54, 2401–2410.
Yap, V.H.S., Chase, Z., Wright, J.T., Hurd, C.L., Lavers, J.L., Lenz, M., 2020. A comparison with natural particles reveals a small specific effect of PVC microplastics on mussel performance. Mar. Pollut. Bull. 160, 111703.
Zarfl, C., Matthies, M., 2010. Are marine plastic particles transport vectors for organic pollutants to the Arctic? Mar. Pollut. Bull. 60, 1810–1814.
Zhang, C., Jansen, M., De Meester, L., Stoks, R., 2016. Energy storage and fecundity explain deviations from ecological stoichiometry predictions under global warming and size-selective predation. J. Anim. Ecol. 85, 1431–1441.
Zhang, C., Jansen, M., Smolders, E., De Meester, L., Stoks, R., 2018a. Stoichiometric responses to nano ZnO under warming are modified by thermal evolution in *Daphnia magna*. Aquat. Toxicol. 202, 90–96.
Zhang, C., Jeong, C., Lee, J., Wang, D., Wang, M., 2019. Transgenerational proteome plasticity in resilience of a marine copepod in response to environmentally relevant concentrations of microplastics. Environ. Sci. Technol. 53, 8426–8436.
Zhang, K., Shi, H., Peng, J., Wang, Y., Xiong, X., Wu, C., Lam, P.K.S., 2018b. Microplastic pollution in China's inland water systems: a review of findings, methods, characteristics, effects, and management. Sci. Total Environ. 630, 1641–1653.
Zimmermann, L., Göttlich, S., Oehlmann, J., Wagner, M., Völker, C., 2020. What are the drivers of microplastic toxicity? Comparing the toxicity of plastic chemicals and particles to Daphnia magna. Environ. Pollut. 267, 115392.