Environmental Pollution 255 (2019) 113178

Contents lists available at ScienceDirect

Environmental Pollution

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

Temperature and clone-dependent effects of microplastics on immunity and life history in *Daphnia magna*☆

Daniel E. Sadler, Franziska S. Brunner*, Stewart J. Plaistow

Institute of Integrative Biology, University of Liverpool, Biosciences Building, Crown Street, L69 7ZB Liverpool, United Kingdom

ARTICLE INFO

Article history:
Received 28 June 2019
Received in revised form
3 September 2019
Accepted 4 September 2019
Available online 5 September 2019

Keywords:
Daphnia
Nanoplastics
Thermal stress
Genetic variation
Multivariate phenotype

ABSTRACT

Microplastic (MP) pollution is potentially a major threat to many aquatic organisms. Yet we currently know very little about the mechanisms responsible for the effects of small MPs on phenotypes, and the extent to which effects of MPs are modified by genetic and environmental factors. Using a multivariate approach, we studied the effects of 500 nm polystyrene microspheres on the life history and immunity of eight clones of the freshwater cladoceran *Daphnia magna* reared at two temperatures (18 °C/24 °C). MP exposure altered multivariate phenotypes in half of the clones we studied but had no effect on others. In the clones that were affected, individuals exposed to MPs had smaller offspring at both temperatures, and more offspring at high temperature. Differences in response to MP exposure were unrelated to differences in particle uptake, but were instead linked to an upregulation of haemocytes, particularly at high temperature. The clone-specific, context-dependent nature of our results demonstrates the importance of incorporating genetic variation and environmental context into assessments of the impact of plastic particle exposure. Our results identify immunity as an important mechanism underpinning genetically variable responses to MP pollution and may have major implications for predicting consequences of MP pollution.

1. Introduction

Pollution is one of the largest contributors to biodiversity loss (Young et al., 2016). Plastic pollution is an especially pressing issue (United Nations Environment Programme, 2014) with large amounts accumulating in diverse environments (Barnes et al., 2009). Macroplastic waste has negative effects on megafauna causing suffocation and poisoning (Carr, 1987; Fowler, 1987; Gall and Thompson, 2015). However, there is increasing evidence that microplastics (MPs) and nanoplastics (NPs) also significantly affect wildlife and ecosystems (Browne et al., 2015; Rist et al., 2017; Thompson et al., 2004; Yu et al., 2018). MPs are plastic particles <5 mm (Rochman, 2018) which can be either primary, resulting from disposal of industrial MPs and microbeads from cosmetic products, or secondary, resulting from the degradation of macromolecules through microbial degradation, UV radiation, and physical factors including temperature exposure and wave action (Song et al., 2017). MPs fragment further into NPs; for which size definitions vary between <100 nm and <1000 nm (Gigault et al., 2018; Koelmans et al., 2015). NPs and smaller MPs could be more problematic than larger MPs due to different ecotoxicological properties and their relatively larger surface area (Chae and An, 2017; Jeong et al., 2016; Rios Mendoza et al., 2018; Triebskorn et al., 2019), as well as the fact that smaller particles accumulate more readily (Browne et al., 2008; Rist et al., 2017). Despite their likely importance, these small plastic particles are understudied, partly due to the difficulty of quantifying them in the natural environment (Koelmans et al., 2015).

Most studies on the impacts of MPs and NPs have focused on the marine environment, leaving the freshwater environment understudied (Besseling et al., 2017; de Sá et al., 2018; Scherer et al., 2017). Freshwater systems are likely to be subject to plastic pollution effects as most plastics are introduced into the oceans from streams and rivers (Dris et al., 2015; Rochman, 2018) and MPs and NPs have been found to be widespread in freshwater systems (Wagner et al., 2014). Indeed, particles can even be found in secluded systems such as subalpine lakes (Imhof et al., 2013). Small plastic particles easily transfer from species to species through trophic transfer (Chae et al., 2018) but have a particularly strong effect on planktonic organisms due to gut blockage and damage

☆ This paper has been recommended for acceptance by Maria Cristina Fossi.

* Corresponding author
E-mail address: fbrunner@liverpool.ac.uk (F.S. Brunner).

https://doi.org/10.1016/j.envpol.2019.113178

(Chae et al., 2018; Cole et al., 2013; Foley et al., 2018; Mattsson et al., 2015). This however appears to generate diverse effects on life history traits, with some studies showing negative effects of plastic particles on growth and reproduction (Besseling et al., 2014; Cole et al., 2015; Jemec et al., 2016; Liu et al., 2019b; Ogonowski et al., 2016), other studies finding no effect on life history traits (Canniff and Hoang, 2018; Imhof et al., 2017; Rist et al., 2017) and a meta-analysis showing a detectable negative effect only on zooplankton survival (Foley et al., 2018). The mixed results have been explained by differences in particle size (Rist et al., 2017; Scherer et al., 2017), particle morphology (Frydkjær et al., 2017), type of plastic (Jemec et al., 2016) and age of the organism (Liu et al., 2018). However, the role that genetic variation and environmental context play in creating this variation is currently unknown. Furthermore, most studies on plastic particle exposure have focussed on univariate traits such as survival or growth (e.g. Jaikumar et al., 2018) or considered multiple traits independently (e.g. Rist et al., 2017). But organisms act as integrated entities, meaning physiological traits covary and should be studied as a connected multivariate phenotype (Pigliucci and Preston, 2004). Univariate approaches may miss subtle but relevant phenotypic shifts and cannot detect effects on life history trait integration (Plaistow and Collin, 2014).

In *D. magna*, integrated life histories and their plastic expression are genetically variable (Plaistow and Collin, 2014). As a result, responses to plastic particles may also be genetically variable. Imhof et al. (2017) examined the effects of MPs on *Daphnia magna* using three different clones, each from one population in either Germany, Belgium or France. They showed variation in the effect that MPs had on growth and reproduction between different clones examined. However, no study has yet examined within population variation in response to small plastic particles. Genetic variation in responses to plastic particle exposure could help to explain some of the mixed results previously demonstrated in single genotype studies (e.g. Besseling et al., 2014; Rist et al., 2017), and would represent evolutionary potential of responses to small plastic particles.

The effects of NPs and MPs on life histories are usually studied in one environment (Bundschuh et al., 2016), with only a few studies focusing on the context dependence of plastic particle effects (Aljaibachi and Callaghan, 2018; Jaikumar et al., 2018; Welden and Cowie, 2016). However, it seems obvious that environmental variation could affect the uptake and exposure time to plastic particles which may then alter the cost of plastic particle exposure. For example, an increase in temperature increases feeding rate, therefore increasing the amount of ingested particles (Burns, 1969). Microplastic uptake has been shown to be influenced by food availability and quality (Aljaibachi and Callaghan, 2018; Prater et al., 2018), proximity to land (Welden et al., 2018), water velocity, and wave activity (Reisser et al., 2015; Welden et al., 2018). Moreover, Jaikumar et al. (2018) demonstrated that sensitivity to NPs in cladocerans increased with temperature in species from temperate climates. Given that freshwaters are particularly vulnerable to climate change (Woodward et al., 2010) and multiple stressors tend to have altered and often exacerbated effects in combination (Folt et al., 1999; Galic et al., 2018; Wan et al., 2018), it is important to consider potential synergistic effects of temperature changes and plastic pollution on freshwater systems.

The effect that plastic particle exposure has on life histories is often attributed to gut blockage, decreased feeding rates, and a reduction in resource availability (Bergami et al., 2016; Chae et al., 2018; Cole et al., 2013). However, smaller particles may also affect other areas of an organism's phenotype. For example, xenobiotics such as plastic particles can induce an immune response in invertebrates (Auffret and Oubella, 1997; Triebskorn et al., 2019; Von Moos et al., 2012) which may also generate a shift in resource allocation, even if resource availability is not reduced. Consequently, plastic particles can affect the immune system and may alter susceptibility to parasites (Greven et al., 2016) and potentially divert energy from other physiological processes.

Daphnia magna is a freshwater cladoceran which feeds through active filtration and passive uptake, actively filtering particles as small as 200 nm, dependent on filtering apparatus size (Brendelberger, 1991), and as large as 80 μm (Burns, 1968) as well as passively taking up smaller particles (Gerritsen et al., 1988). Its alternative sexual and parthenogenetic reproduction allow the maintenance of clonal lines (Ebert, 2005). It is also a model organism for ecotoxicology, including studies on microplastics (Rist et al., 2017; Rochman, 2018). In order to better understand the context-dependent effects that MPs might have on populations, we studied the effect of small MPs (500 nm polystyrene beads) on the multivariate life history phenotypes of eight *D. magna* clones sampled from a single population and reared at two different temperatures (18 °C and 24 °C). To investigate the causal mechanisms responsible for MP exposure effects, we used confocal microscopy to look at how treatment and clone influenced the uptake of MPs and a haemocyte-count assay to examine how treatment and clone influenced *D. magna* immunity.

2. Materials and methods

2.1. Study organism

For this experiment, eight *D. magna* clones (BMH2, BMH22, BMH30, BMH38, BMH42, BMH58, BMH122, and BMH175) of *D. magna* were taken from laboratory lines isolated from Brown Moss Nature Reserve (52°57′01.2″N 2°39′05.6″W) and maintained at 21 °C in an incubator with a 14: 10 light: dark photoperiod for 1 year. Throughout the experiment, *D. magna* were kept individually in 150 ml jars of ASTM, which was supplemented with organic Marinure solution replaced every second day (Baird et al., 1989). Individuals were fed *ad libitum* (200,000 cells/ml of the alga *Chlorella vulgaris* each day) throughout both conditioning and experimental phases.

2.2. Microplastics

Carboxylate-modified polystyrene beads with fluorescent red colouring (505 nm excitation, 550 emission) were purchased from Sigma-Aldrich (Dorset, UK, Article No L3280) with a statutory average particle size of 500 nm in a 2.5% w/v aqueous suspension. These particles can be classified as small microplastics or nanoplastics depending on the used size definition (Gigault et al., 2018; Koelmans et al., 2015). Average particle size was confirmed with confocal microscopy and ImageJ to measure particle size (575 ± 18.9 nm, $n = 100$). The supplied suspension was diluted to 1.5 g/L with deionized water as a working solution. This working solution was vortexed each time before mixing an aliquot into the experimental jars, equivalent to 1 mg/L or 1.46×10^7 particles/L. The concentration in suspension was assessed using haemocytometer counts from 6 separately prepared jars. A concentration of $1.25 \pm 0.205 \times 10^6$ particles/L, or 85.6 ± 14.0 μg/L, suggests that many fewer particles were in suspension compared to the concentration added to the jar, resulting in a suspension concentration that was close to predicted environmental levels (Lenz et al., 2016). However, *D. magna* are also grazers (Siehoff et al., 2009) and may therefore have consumed more particles when feeding at the bottom of jars.

2.3. Laboratory experiment – the effect of plastics, temperature and clone on life history

Before the main experiment, *Daphnia* clones were conditioned at 21 °C (Fig. S1) for two generations to reduce potentially confounding maternal effects (Plaistow et al., 2015; Plaistow and Collin, 2014). The offspring from the second F3 clutch were then used in the experiment. Neonates were exposed to one of two temperature treatments; high temperature (24 °C) or low temperature (18 °C). Within each temperature half of the *D. magna* were exposed to a fresh preparation of small MPs every other day as the water was changed. The other half acted as a control and were not exposed to MPs (Figs. S1 and C). 1 mg/L is at the lower end of concentrations commonly used for testing the effects of MPs on organisms and 85.6 μg/L is below concentrations commonly tested (Besseling et al., 2014; Chae et al., 2018; Rist et al., 2017). Environmental concentrations of MPs below 1 μm are currently unknown due to the difficulty in determining environmental concentrations of smaller particles, with estimates extrapolating from measured MP concentrations suggesting average concentrations between 1 pg/L and 1 μg/L (Koelmans et al., 2015; Lenz et al., 2016). Our tested concentration in suspension is predicted to be at the upper end of current environmental concentrations of MPs in freshwater (Besseling et al., 2014) but lower than MP concentrations in strongly polluted locations (Li et al., 2018). For each combination of the 8 clones and 4 treatments, 5 replicates were used, with each replicate containing 1 animal, for a total of 160 experimental animals. 6 animals died during the experiment and haemocyte counts were not collected for 5 individuals because of mistakes in the extraction process. Full data was therefore available from 149 individuals, only this data was used in the data analyses. All individuals were kept at a 14: 10 light: dark photoperiod throughout. As with the conditioning, all individuals were fed *ad libitum* (200,000 cells/ml of the alga each day).

2.3.1. Life history

Life history data were collected using daily observations until individuals produced their second clutch, as described in Plaistow and Collin (2014). Body sizes were measured using the software ImageJ (Schneider et al., 2012). Multivariate life history data consisted in: length at maturity (mm), length at second clutch (mm), age at maturity (days), age at second clutch (days), juvenile growth rate ((length at maturity -length as neonate)/age at maturity), adult growth rate ((length at second clutch - length at maturity)/(age at second clutch-age at maturity)), mean fecundity (mean number of offspring between clutch 1 and 2), average offspring size (mean taken across 5 neonates from clutch 1 and 5 neonates from clutch 2 of each experimental *Daphnia*).

2.3.2. Immunity

Immunity was analysed using haemocyte counts. At the end of the life history analysis, individuals were placed in clean ASTM for 5 min to remove any excess MPs on the carapace, then placed on a glass plate with 4 μl of ice cold buffer solution (98 mM NaOH, 186 mM NaCl, 17 mM EDTA and 41 mM citric acid, pH adjusted to 4.5; following Auld et al. (2012)). Hearts were then pierced with a 20-gauge syringe (care was taken not to pierce the gut), causing the haemolymph to seep into the buffer. 2 μl of the haemolymph-buffer mixture was transferred into a Neubauer haemocytometer ($0.0025\ mm^2 \times 0.1$ mm) and haemocytes were counted and converted to cells/μl of solution. Individuals were then frozen at −20 °C in 24-well plates for later microscope imaging.

2.3.3. Microplastic imaging

MP imaging was performed using a Zeiss LSM 710 confocal laser scanning microscope using the 458 nm laser to observe the fluorescently labelled polystyrene beads (λex ~575 nm; λem ~610 nm) in the *D. magna* individuals. *D. magna* were defrosted and mounted on a slide. The 10x objective lens was used to capture multiple images in a z-stack. These were combined into a 3D image of the gut and transmitted light images were overlaid to visualise both the gut and the whole individual (see Fig. 1). Image J (Schneider et al., 2012) was then used to analyse the amount of fluorescence emitted by the particles using the mean density of particle fluorescence present in the entire area of the individual, as recorded by confocal microscopy. The mean density was compared across treatments and clones.

2.3.4. Statistical analysis

All analyses were performed in R version 3.5.1 (R Core Team, 2017). Permutational multivariate analysis of variance (perMANOVA) was performed to test for clonal variation in multivariate life history responses to treatments (Temperature x MP exposure x Clone) and for treatment effects in each clone (Temperature x MP exposure). Principal component analysis (PCA) was used to visualise the multivariate phenotypes.

To better understand the trait shifts underlying multivariate phenotype changes, we used three-way analyses of variance (ANOVA) on each life history parameter, immunity, as well as the uptake of MPs. In these ANOVAs, we set temperature, MP exposure, their two-way interaction and clone as fixed factors. Furthermore, influences of quantitative MP uptake on univariate life history traits were assessed using linear models with temperature and clone as a random factors, using the *lmer* and *lmerTest* functions from the lme4 and lmerTest packages, respectively (Bates et al., 2015; Kuznetsova et al., 2015).

See Supplement for additional details on statistics.

3. Results

3.1. The effect of microplastics, temperature and clone on life history

3.1.1. Multivariate phenotype changes

Clones varied significantly in their responses to temperature

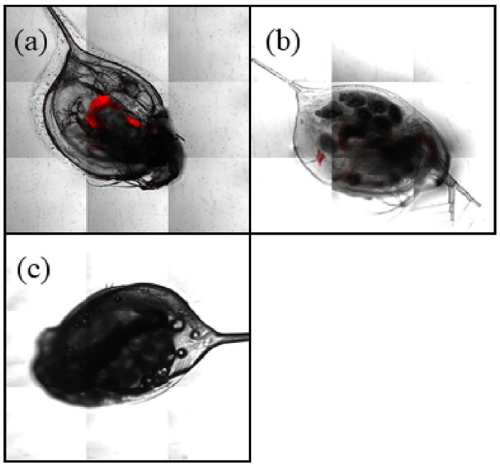

Fig. 1. Confocal microscopy images of *D. magna* showing MPs taken up during exposure fluorescing in red, mostly concentrated in the gut. Transition microscopy image was overlaid with the confocal image to show where in the *Daphnia* the particles were present, confocal images were standardised for exposure. (a) High uptake of MPs, (b) low uptake of MPs, and (c) control exposure (no MPs). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

and MP exposure (perMANOVA, Temperature: MP: Clone interaction effect, $F_{7,117} = 1.66$, $p = 0.047$; Table S1). PCA plots visualise the differences in life history variation between treatments within each clone (Fig. 2). Clones 2 and 122 showed an effect of MP x temperature (Table S2), whilst clones 30 and 58 showed an independent effect of MPs (Table S2). These effects are visualised by the non-overlapping of ellipses in PCA plots (Fig. 2). Conversely clones 22, 38, 42 and 175 showed no differences in life history variation with MP treatment (Table S2). The elongated ellipses evident in most clones in the PCA are accounted for by large variation in haemocyte counts and average clutch number across replicates, particularly across PC2 in individuals exposed to high temperature and MPs (Fig. 2).

Across clones, temperature drove consistent differences in multivariate phenotypes (Table S2, Fig. 3). PC1 is primarily associated with this temperature effect and explained 40.3% of the total phenotypic variation. Individuals with high PC1 scores were individuals reared at 24 °C with higher juvenile and adult growth rates and faster development (Fig. 3a and c, Table S3). By contrast, individuals with lower PC1 scores, reared at 18 °C, grew more slowly and took longer to mature. Individuals with high PC2 scores are again individuals reared at 24 °C with higher growth rate in juveniles (but not in adults), greater length at maturity, and greater reproductive output (Fig. 3a and c, Table S3). PC2 explained 21.7% of the phenotypic variation. PC3 (13.5% of variation) represents an offspring size versus offspring number trade-off but was mostly unaffected by MP treatment (Fig. 3b and d, Table S3). In contrast, individuals with high PC4 scores (10.3%) were MP exposed individuals that had substantially increased haemocyte counts (Fig. 3b and d, Table S3).

3.1.2. Individual life history traits

To investigate the traits underlying the observed shifts in multivariate phenotypes further we used analyses of variance with MPs, temperature, their interaction and clone as fixed factors. Offspring reared at 24 °C grew faster as juveniles and adults (Table 1, Fig. S2). While animals at both temperatures matured at the same size, offspring reared at 18 °C matured later and were both older and larger than individuals reared at 24 °C at clutch 2 (Table 1, Fig. S2). As a result, they tended to produce more, smaller offspring than individuals reared at 24 °C (Table 1, Fig. S2). However, reproductive traits were also influenced by MPs. Exposed individuals had smaller offspring, irrespective of temperature (Table 1, Fig. S2), and significantly greater clutch sizes at 24 °C but not at 18 °C (Table 1, Fig. S2). All other life history traits were unaffected by MP exposure but all traits measured varied with clone (Table 1).

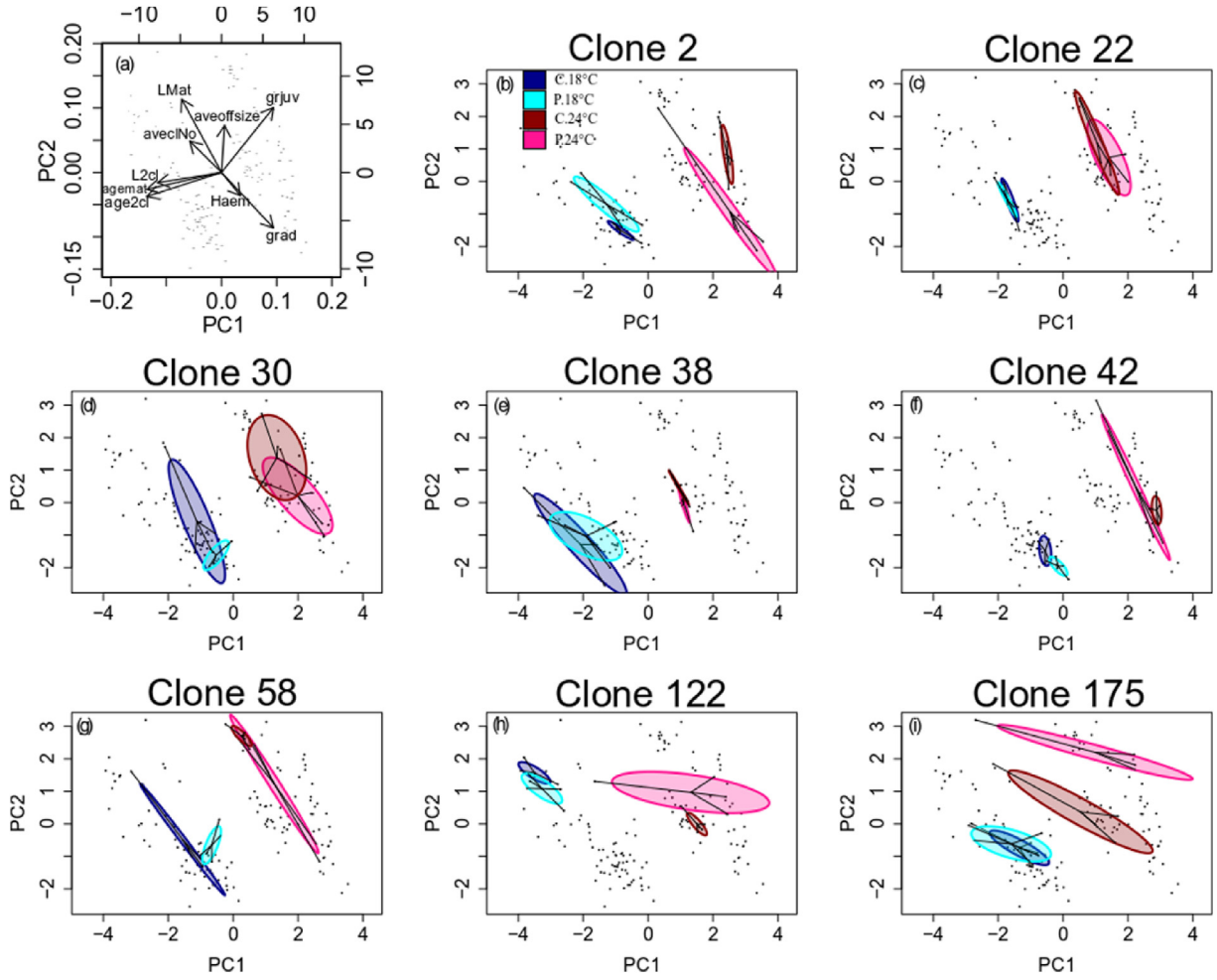

Fig. 2. Treatment effects on individual clones shown in Principal component space (a) Biplot for the first two principal components (PC1 and PC2) indicating correlations of life history traits and contributions of traits to PC axes (b)–(i) PCA plots showing the effect of small MP exposure (P vs C) and temperature (18 °C vs 24 °C) for each clone. Ellipses indicate 95% confidence intervals around the group centroids. Lines indicate distance of each individual from respective group centroid.

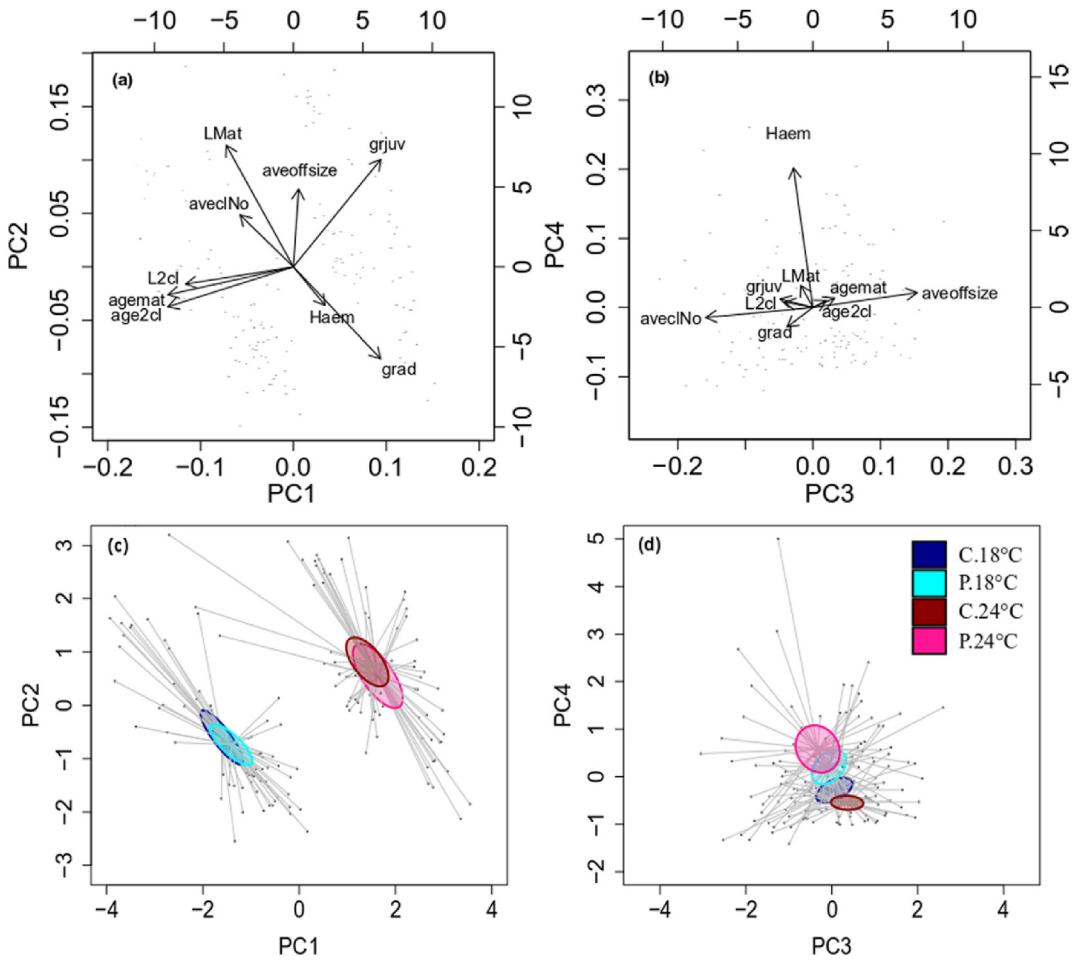

Fig. 3. Principal Component Analysis of life history parameters across clones. Contributions to principal component space is shown in biplots (a) PC1 (40.3% of data variation) vs PC2 (21.7%), (b) and PC3 (13.5%) vs PC4 (10.3%). 95% confidence intervals of group means are plotted for MP exposure (P) and control (C) at two temperatures (18 °C and 24 °C). Lines indicate distance of each individual from respective group centroid.

3.2. Effects of microplastics on haemocyte number

Haemocyte numbers on average increased under MP exposure and this effect was modulated by temperature (MP:Temperature effect, $F_{1,138} = 8.521$, $p = 0.004$, Table 1, Fig. S3), with a 3-fold increase in haemocyte counts under MP exposure in high temperature conditions and a 1.6-fold increase in low temperature conditions. Clonal identity also affected haemocyte counts (Clone effect, $F_{7,138} = 5.602$, $p < 0.001$). Some clones showed significant responses to higher temperatures by further increasing immune cells in MP treatments (e.g. Clone 58; Fig. 4) whilst other clones had the highest immune activity at lower temperatures and MP exposure (e.g. Clone 22, Fig. 4) or showed no response to temperature, only to MP exposure (e.g. Clone 2; Fig. 4). Two clones showed no significant change in haemocyte counts across all treatments (Clones 30 and 42, Fig. 4). Temperature had no effect on haemocyte counts in absence of MPs in any clone.

3.3. Microplastic ingestion

All exposed individuals showed a large uptake of MPs measured by fluorescence (Fig. 5), whilst control exposed individuals showed no fluorescence when examined using confocal microscopy (Fig. 1). There was a significant interaction of clonal variation and temperature on MP density (Temperature: Clone; $F_{7,51} = 2.966$, $p = 0.011$, see Fig. 5). This was driven by an increased MP density at 18 °C in clone 58 compared to other clones at 18 °C, whereas no clone changed the ingested MP density with temperature (Fig. 5). When testing for effects on life history traits, MP uptake had a weak negative effect on *Daphnia* length at second clutch ($F_{1,63} = 4.283$, $p = 0.043$).

4. Discussion

Plastic pollution is a pressing issue (Horton et al., 2017; Windsor et al., 2019). Small MP may be especially detrimental to organisms (Chae and An, 2017; Koelmans et al., 2015) but studies investigating the effects of these particles on phenotypes frequently generate mixed results (Foley et al., 2018). This could be because plastic particle studies often ignore genetic variation and environmental context. Using a multivariate approach, we show here that the effect of small microplastics on life histories is variable across genotypes, and context-dependent, generating a larger response at higher temperatures in responding genotypes. The average life history response to small MPs consisted in a decline in offspring size at both temperatures and an increase in fecundity only at higher temperature. This response was unrelated to differences in MP uptake but was instead linked to increased numbers of immune cells under MP exposure, particularly at high temperature.

Life history traits are clonally variable in *Daphnia* (Baird et al.,

Table 1
Summary of individual life history trait analyses by ANOVAs. To meet the assumptions of the ANOVA, boxcox transformations were performed where indicated by Shapiro Wilks or Leven's test. Lambda values are listed for transformed variables.

LH Parameter	Treatment	Df	F Value	P Value
Adult growth (grad)	Microplastic	1	0.059	0.808
	Temperature	1	12.54	**<0.001**
	Clone	7	3.709	**0.001**
	Microplastic: Temperature	1	0.044	0.834
	Residuals	138		
Juvenile growth (grjuv)	Microplastic	1	1.750	0.188
	Temperature	1	538.7	**<0.001**
	Clone	7	11.17	**<0.001**
	Microplastic: Temperature	1	0.595	0.442
	Residuals	138		
Average number of offspring in one clutch (aveclNo)	Microplastic	1	1.006	0.318
	Temperature	1	8.869	**0.003**
	Clone	7	21.57	**<0.001**
	Microplastic: Temperature	1	4.038	**0.046**
	Residuals	138		
Avg size of offspring (aveoffsize)	Microplastic	1	6.448	**0.012**
Transformed	Temperature	1	17.28	**<0.001**
Lambda = −2.390	Clone	7	8.861	**<0.001**
	Microplastic: Temperature	1	1.210	0.273
	Residuals	138		
Length at maturity (LMat)	Microplastic	1	0.342	0.560
Transformed	Temperature	1	0.013	0.910
Lambda = −5.899	Clone	7	6.537	**<0.001**
	Microplastic: Temperature	1	1.133	0.289
	Residuals	138		
Length at second clutch (L2cl)	Microplastic	1	0.026	0.872
	Temperature	1	133.4	**<0.001**
	Clone	7	4.558	**<0.001**
	Microplastic: Temperature	1	0.450	0.503
	Residuals	138		
Age at maturity (agemat)	Microplastic	1	0.719	0.398
	Temperature	1	396.8	**<0.001**
	Clone	7	9.492	**<0.001**
	Microplastic: Temperature	1	0.310	0.587
	Residuals	138		
Age at second clutch (age2cl)	Microplastic	1	0.143	0.706
	Temperature	1	723.1	**<0.001**
	Clone	7	10.16	**<0.001**
	Microplastic: Temperature	1	0.433	0.511
	Residuals	138		
Haemocyte count (Haem)	Microplastic	1	47.60	**<0.001**
Transformed	Temperature	1	1.295	0.257
Lambda = 0.101	Clone	7	5.626	**<0.001**
	Microplastic: Temperature	1	8.521	**0.004**
	Residuals	138		

1990), as confirmed by the significant primary clone effect in all parameters we measured. It is therefore not surprising that the effect of MP exposure on phenotypes also varied between genotypes. While MP exposure induced shifts in multivariate phenotypes in four of the clones we observed (Clones 2, 30, 58 & 122), the other four clones were relatively resistant to the effects of MP exposure showing no, or only marginal, differences in multivariate phenotype (Clones 22, 42, 38 & 175). In the clones that were affected, the primary response to MP exposure observed was an upregulation in haemocyte numbers which is primarily a response to parasite infections. However, MPs can induce immune responses similar to parasites, including immune cell activation (Greven et al., 2016), changes in stress and immune gene regulation (Détrée and Gallardo-Escárate, 2018; Liu et al., 2018) and immune cell recruitment (Brown et al., 2001). Host defence mechanisms against parasites are generally genetically variable (Haldane, 1949; Schmid-Hempel, 2003), including in *Daphnia* (Carius et al., 2001; Duffy and Sivars-Becker, 2007). Genetic variation in host resistance mechanisms can be generated by frequency-dependent selection (Haldane, 1949) and variable cost-benefit ratios of resistance (Auld et al., 2013; Sheldon and Verhulst, 1996). Such evolved variation in the reactivity of the *Daphnia*'s immune system may underlie the genetic variation in immune responses to MPs which we observed in this study. Immune responses like increased production of immune cells (our study) or regulatory changes of stress response genes (Imhof et al., 2017; Liu et al., 2019b) divert resources from other functions such as reproduction or growth (Détrée and Gallardo-Escárate, 2018), potentially explaining further variation in life histories (Sheldon and Verhulst, 1996).

A crucial next step towards understanding MP and NP effects in natural environments is the investigation of population level effects rather than focusing on a single genotype as the majority of studies do (Windsor et al., 2019). Here we demonstrate the variability of responses to MPs across multiple genotypes from the same population. Our results help to explain why some single genotype studies have observed effects of MP or NP exposure on phenotypes (Besseling et al., 2014; Cole et al., 2015; Greven et al., 2016; Jemec et al., 2016; Ogonowski et al., 2016), whereas others have not (Aljaibachi and Callaghan, 2018; Canniff and Hoang, 2018; Rist et al., 2017). Differences in responses to plastics exposure have been attributed to differences in particle size (Rist et al., 2017; Scherer et al., 2017), morphology (Frydkjær et al., 2017), type of plastic

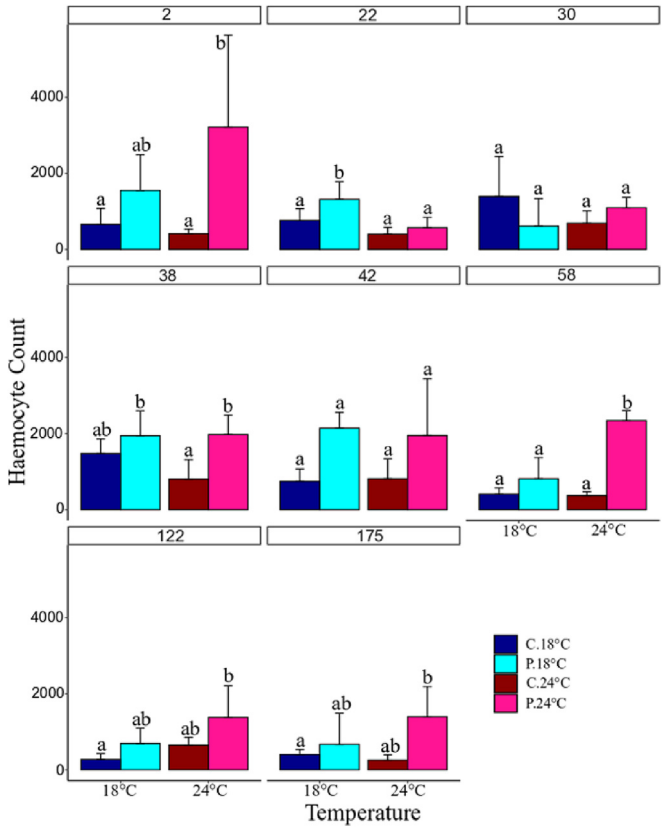

Fig. 4. Haemocyte number (mean ± S.D.) of *D. magna* within each clone (2, 22, 30, 38, 42, 58, 122, 175), at high temperature (24 °C) and low temperature (18 °C), in control (C= No MPs) and MP (P = microplastic exposure) treatments. Different letters indicate significant differences between treatments using TukeyHSD.

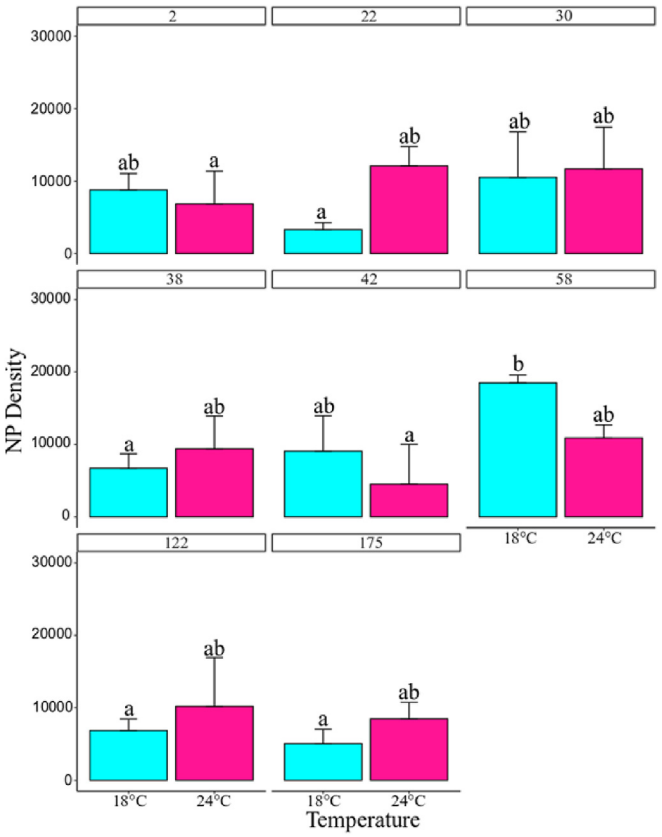

Fig. 5. Microplastic (MP) density (mean ± S.D.) in *D. magna* of each of 8 clones (numbered 2–175), in high temperature (24 °C) and low temperature (18 °C) treatments. Different letters indicate significant differences between clones and treatments, using TukeyHSD ($P < 0.05$).

(Jemec et al., 2016) as well as age of the *Daphnia* (Liu et al., 2018). Our findings suggest that genetic variation is also a factor explaining mixed results, not only between (Imhof et al., 2017) but also within populations. As a result, we suggest that MP and NP studies should incorporate phenotypic responses from multiple genotypes before drawing conclusions about the effects that the MPs will have on natural populations. Genetic variation in responses to MP exposure within a population also demonstrates the potential for evolutionary change, which may be crucial for population persistence in highly contaminated ecosystems.

Environmental differences, for example in temperature or food availability, can change the toxicity of chemicals (Heugens et al., 2001), the uptake and exposure to plastic particles (Aljaibachi and Callaghan, 2018; Rist et al., 2017), and the physiological cost of exposure (Aljaibachi and Callaghan, 2018; Jaikumar et al., 2018). Using a multivariate approach, we were able to show that individuals had some consistent responses to MP exposure at 18 °C and 24 °C; increasing their haemocyte counts and decreasing the size of the offspring they produced. However, the effect on haemocyte numbers was doubled at 24 °C compared to 18 °C. This might explain why exposure to MPs increased the number of offspring produced at 24 °C but had no effect on offspring number at 18 °C. Our results support previous studies which have demonstrated that the sensitivity of *D. magna* to MP exposure increases with temperature (Jaikumar et al., 2018). Such increased impacts of MPs on aquatic keystone species at higher temperatures are particularly concerning given that shallow freshwater ecosystems have little buffering capacity against climate change (Woodward et al., 2010). Combined stressors can have additive, synergistic or antagonistic effects (Folt et al., 1999), so it is crucial to study relevant combinations of stressors that are found in natural environments. It is also noteworthy that synergistic effects of stressors can disappear over evolutionary time. For example, Zhang et al. (2018) showed that the synergistic stress effect of ZnO exposure and increased temperature disappeared after the tested lake *D. magna* population had adapted to increasing average and peak temperatures.

Increased sensitivity to contaminants in filter feeders at higher temperatures is often attributed to higher metabolic rates (Heugens et al., 2003; Jaikumar et al., 2018), which correlate with sensitivity (Baas and Kooijman, 2015), and to an increased particle exposure, because we expect higher temperature to increase feeding rate (McMahon, 1965). In this study, we found no evidence for a general increase in MP uptake at warmer temperatures. This could be a result of rapid clearance of MPs from the gut, preventing particle build-up (Ogonowski et al., 2016). However, we also found that a higher temperature increased haemocyte counts in MP exposed animals but not control treatment animals; that fecundity increased in MP exposed animals only at higher temperature and that individuals which took up more MPs were smaller when reaching their second clutch. Together, these findings suggest that the increased sensitivity to MP exposure at higher temperatures was due to the temperature sensitivity of an MP-triggered immune response and the effect this had on the relative allocation of resources to adult growth and fecundity. This finding is consistent with previous *Daphnia* studies that have demonstrated that temperature is an important factor influencing costs of infection (Mitchell et al., 2005; Vale et al., 2008), resistance mechanisms

(Garbutt et al., 2014) and the likelihood and progression of epidemics (Auld and Brand, 2017; Hall et al., 2006; Vale et al., 2011). The shift from continued adult growth towards increased fecundity after a strong activation of the immune system at 24 °C could be the result of fecundity compensation, i.e. a shift to increased earlier reproduction in the face of a (perceived) threat to survival which is known to occur in *D. magna* (Ebert et al., 2004; Vale and Little, 2012). The fact that MP exposure interferes with *D. magna* immunity may have consequences for their ability to respond to parasites, as resources for immune responses may be depleted resulting in increased susceptibility (Lochmiller and Deerenberg, 2000) or alternatively, the already mounted immune response could confer increased resistance. Both haemocyte upregulation and fecundity compensation are known responses of *D. magna* to their common and well-studied parasite *Pasteuria ramosa* (Auld et al., 2012; Ebert et al., 2004). If NPs impose selection pressures on those same traits, coevolutionary dynamics between *Daphnia* and their parasites could be disrupted, reducing the *Daphnia*'s parasite resistance over evolutionary time. The proposed mechanisms are consistent with previous findings that adaptation to pesticides can reduce parasite resistance in *Daphnia* (Jansen et al., 2011).

Many studies have demonstrated that xenobiotics, such as plastics, induce an immune response in invertebrates (Auffret and Oubella, 1997; Liu et al., 2019a; Von Moos et al., 2012). In *Daphnia*, nanoplastics have also been shown to affect stress response gene expression (Liu et al., 2019b), which may be part of the response pathway to the cellular immune response we have shown here. The effect that MP or NP exposure has on life histories is normally attributed to gut blockage, decreased feeding rates, and a reduction in resource availability (Bergami et al., 2016; Chae et al., 2018; Cole et al., 2013; Wright et al., 2013). However, by quantifying multivariate phenotypes, immunity and MP uptake simultaneously, our results demonstrate that in *D. magna* at least, the effect that MPs have on life histories could be explained by a shift in resource allocation following an induced immune response. We were able to confirm that the effects of MPs on the phenotype are both clone-specific and context-dependent. This may explain the variable results previously demonstrated in MP studies that have used single clones and simple univariate phenotypes (e.g. Besseling et al., 2014; Rist et al., 2017). Our results further highlight the importance of considering further environmental factors when evaluating the toxicity of MPs and NPs (Bundschuh et al., 2016; Koelmans et al., 2015). The intensification of MP effects on *Daphnia* immunity and life histories at higher temperatures strongly suggests that plastic particle contamination and climate change may have synergistic effects on this important freshwater grazer.

Acknowledgments

This study was supported by the Institute of Integrative Biology, Liverpool University. Special thanks to Dr Alan Reynolds and Stephen Price for laboratory and field assistance. Further thanks go to Dr Marco Marcello and the centre of cellular imaging for assistance in confocal microscopy and to two anonymous reviewers for their insightful comments which improved this paper. SJP and FSB were supported by NERC Highlight grant NE/N016017/1 to SJP.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2019.113178.

References

Aljaibachi, R., Callaghan, A., 2018. Impact of polystyrene microplastics on *Daphnia magna* mortality and reproduction in relation to food availability. PeerJ 6, e4601. https://doi.org/10.7717/peerj.4601.

Auffret, M., Oubella, R., 1997. Hemocyte aggregation in the oyster Crassostrea gigas: in vitro measurement and experimental modulation by xenobiotics. Comp. Biochem. Physiol. Physiol. 118, 705–712. https://doi.org/10.1016/S0300-9629(97)00017-0.

Auld, S.K.J.R., Brand, J., 2017. Simulated climate change , epidemic size , and host evolution across host − parasite populations. Glob. Chang. Biol. https://doi.org/10.1111/gcb.13769.

Auld, S.K.J.R., Graham, A.L., Wilson, P.J., Little, T.J., 2012. Elevated haemocyte number is associated with infection and low fitness potential in wild Daphnia magna. Funct. Ecol. 26, 434–440. https://doi.org/10.1111/j.1365-2435.2011.01959.x.

Auld, S.K.J.R., Penczykowski, R.M., Housley Ochs, J., Grippi, D.C., Hall, S.R., Duffy, M.A., 2013. Variation in costs of parasite resistance among natural host populations. J. Evol. Biol. 26, 2479–2486. https://doi.org/10.1111/jeb.12243.

Baas, J., Kooijman, S.A.L.M., 2015. Sensitivity of animals to chemical compounds links to metabolic rate. Ecotoxicology 24, 657–663. https://doi.org/10.1007/s10646-014-1413-5.

Baird, D.J., Barber, I., Calow, P., 1990. Clonal variation in general responses of Daphnia magna straus to toxic stress. I. Chronic life-history effects. Funct. Ecol. 4, 399. https://doi.org/10.2307/2389602.

Baird, D.J., Soares, A.M.V.M., Girling, A., Barber, I., 1989. The long-term maintenance of Daphnia magna Straus for use in ecotoxicity tests: problems and prospects. In: Proc. First Eur. Conf. Ecotoxicol..

Barnes, D.K.A., Galgani, F., Thompson, R.C., Barlaz, M., 2009. Accumulation and fragmentation of plastic debris in global environments. Philos. Trans. R. Soc. B 364, 1985–1998. https://doi.org/10.1098/rstb.2008.0205.

Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using {lme4}. J. Stat. Softw. 67, 1–48. https://doi.org/10.18637/jss.v067.i01.

Bergami, E., Bocci, E., Vannuccini, M.L., Monopoli, M., Salvati, A., Dawson, K.A., Corsi, I., 2016. Nano-sized polystyrene affects feeding, behavior and physiology of brine shrimp Artemia franciscana larvae. Ecotoxicol. Environ. Saf. 123, 18–25. https://doi.org/10.1016/j.ecoenv.2015.09.021.

Besseling, E., Quik, J.T.K., Sun, M., Koelmans, A.A., 2017. Fate of nano- and microplastic in freshwater systems: a modeling study. Environ. Pollut. 220, 540–548. https://doi.org/10.1016/j.envpol.2016.10.001.

Besseling, E., Wang, B., Lürling, M., Koelmans, A.A., 2014. Nanoplastic affects growth of S. obliquus and reproduction of D. magna. Environ. Sci. Technol. 48, 12336–12343. https://doi.org/10.1021/es503001d.

Brendelberger, H., 1991. Filter mesh size of cladocerans predicts retention efficiency for bacteria. Limnol. Oceanogr. 36, 884–894. https://doi.org/10.4319/lo.1991.36.5.0884.

Brown, D.M., Wilson, M.R., MacNee, W., Stone, V., Donaldson, K., 2001. Size-dependent proinflammatory effects of ultrafine polystyrene particles: a role for surface area and oxidative stress in the enhanced activity of ultrafines. Toxicol. Appl. Pharmacol. 175, 191–199. https://doi.org/10.1006/taap.2001.9240.

Browne, M.A., Dissanayake, A., Galloway, T.S., Lowe, D.M., Thompson, R.C., 2008. Ingested microscopic plastic translocates to the circulatory system of the mussel, Mytilus edulis (L.). Environ. Sci. Technol. 42, 5026–5031. https://doi.org/10.1021/es800249a.

Browne, M.A., Underwood, A.J., Chapman, M.G., Williams, R., Thompson, R.C., Franeker, J.A. Van, 2015. Linking effects of anthropogenic debris to ecological impacts. Proc. R. Soc. Biol. Sci. 282.

Bundschuh, M., Seitz, F., Rosenfeldt, R.R., Schulz, R., 2016. Effects of nanoparticles in fresh waters: risks, mechanisms and interactions. Freshw. Biol. 61, 2185–2196. https://doi.org/10.1111/fwb.12701.

Burns, C.W., 1969. Relation between filtering rate, temperature, and body size in four species of Daphnia. Limnol. Oceanogr. 14, 693–700. https://doi.org/10.4319/lo.1969.14.5.0693.

Burns, C.W., 1968. The relationship between body size of filter-feeding cladocera and the maximum size of particles ingested. Limnol. Oceanogr. 13, 675–678.

Canniff, P.M., Hoang, T.C., 2018. Microplastic ingestion by Daphnia magna and its enhancement on algal growth. Sci. Total Environ. 633, 500–507. https://doi.org/10.1016/j.scitotenv.2018.03.176.

Carius, H.J., Little, T.J., Ebert, D., 2001. Genetic variation in a host-parasite association: potential for coevolution and frequency- dependent selection. Evolution (N. Y). 55, 1136–1145.

Carr, A., 1987. Impact of nondegradable marine debris on the ecology and survival outlook of sea turtles. Mar. Pollut. Bull. 18, 352–356. https://doi.org/10.1007/s10717-014-9625-y.

Chae, Y., An, Y.J., 2017. Effects of micro- and nanoplastics on aquatic ecosystems: current research trends and perspectives. Mar. Pollut. Bull. 124, 624–632. https://doi.org/10.1016/j.marpolbul.2017.01.070.

Chae, Y., Kim, D., Kim, S.W., An, Y.J., 2018. Trophic transfer and individual impact of nano-sized polystyrene in a four-species freshwater food chain. Sci. Rep. 8, 1–11. https://doi.org/10.1038/s41598-017-18849-y.

Cole, M., Lindeque, P., Fileman, E., Halsband, C., Galloway, T.S., 2015. The impact of polystyrene microplastics on feeding, function and fecundity in the marine copepod Calanus helgolandicus. Environ. Sci. Technol. 49, 1130–1137. https://doi.org/10.1021/es504525u.

Cole, M., Lindeque, P., Fileman, E., Halsband, C., Goodhead, R., Moger, J., Galloway, T.S., 2013. Microplastic ingestion by zooplankton. Environ. Sci. Technol. 47, 6646–6655. https://doi.org/10.1021/es400663f.

de Sá, L.C., Oliveira, M., Ribeiro, F., Rocha, T.L., Futter, M.N., 2018. Studies of the

effects of microplastics on aquatic organisms: what do we know and where should we focus our efforts in the future? Sci. Total Environ. 645, 1029–1039. https://doi.org/10.1016/j.scitotenv.2018.07.207.
Détrée, C., Gallardo-Escárate, C., 2018. Single and repetitive microplastics exposures induce immune system modulation and homeostasis alteration in the edible mussel Mytilus galloprovincialis. Fish Shellfish Immunol. 83, 52–60. https://doi.org/10.1016/j.fsi.2018.09.018.
Dris, R., Gasperi, J., Rocher, V., Saad, M., Renault, N., Tassin, B., 2015. Microplastic contamination in an urban area: a case study in Greater Paris. Environ. Chem. https://doi.org/10.1071/EN14167.
Duffy, M.A., Sivars-Becker, L., 2007. Rapid evolution and ecological host-parasite dynamics. Ecol. Lett. 10, 44–53. https://doi.org/10.1111/j.1461-0248.2006.00995.x.
Ebert, D., 2005. Ecology, Epidemiology and Evolution of Parasitism in Daphnia, Evolution. https://doi.org/10.1108/02634501111102760.
Ebert, D., Carius, H.J., Little, T., Decaestecker, E., 2004. The evolution of virulence when parasites cause host castration and gigantism. Am. Nat. 164, S19–S32. https://doi.org/10.1086/424606.
Foley, C.J., Feiner, Z.S., Malinich, T.D., Höök, T.O., 2018. A meta-analysis of the effects of exposure to microplastics on fish and aquatic invertebrates. Sci. Total Environ. 631–632, 550–559. https://doi.org/10.1016/j.scitotenv.2018.03.046.
Folt, C.L., Chen, C.Y., Moore, M.V., Burnaford, J., 1999. Synergism and antagonism among multiple stressors. Limnol. Oceanogr. 44, 864–877. https://doi.org/10.4319/lo.1999.44.3_part_2.0864.
Fowler, C., 1987. Marine debris and northern Fur seals: a case study. Mar. Pollut. Bull. 18, 326–335. https://doi.org/10.1016/S0025-326X(87)80020-6.
Frydkjær, C.K., Iversen, N., Roslev, P., 2017. Ingestion and egestion of microplastics by the cladoceran Daphnia magna: effects of regular and irregular shaped plastic and sorbed phenanthrene. Bull. Environ. Contam. Toxicol. 99, 655–661. https://doi.org/10.1007/s00128-017-2186-3.
Galic, N., Sullivan, L.L., Grimm, V., Forbes, V.E., 2018. When things don't add up: quantifying impacts of multiple stressors from individual metabolism to ecosystem processing. Ecol. Lett. 21, 568–577. https://doi.org/10.1111/ele.12923.
Gall, S.C., Thompson, R.C., 2015. The impact of debris on marine life. Mar. Pollut. Bull. 92, 170–179. https://doi.org/10.1016/j.marpolbul.2014.12.041.
Garbutt, J.S., Scholefield, J.A., Vale, P.F., Little, T.J., 2014. Elevated maternal temperature enhances offspring disease resistance in Daphnia magna. Funct. Ecol. 28, 424–431. https://doi.org/10.1111/1365-2435.12197.
Gerritsen, J., Porter, K.G., Strickler, J.R., 1988. Not by sieving alone: observations of suspension feeding in Daphnia. Bull. Mar. Sci. 43, 366–376.
Gigault, J., Halle, A. ter, Baudrimont, M., Pascal, P.Y., Gauffre, F., Phi, T.L., El Hadri, H., Grassl, B., Reynaud, S., 2018. Current opinion: what is a nanoplastic? Environ. Pollut. 235, 1030–1034. https://doi.org/10.1016/j.envpol.2018.01.024.
Greven, A.C., Merk, T., Karagöz, F., Mohr, K., Klapper, M., Jovanović, B., Palić, D., 2016. Polycarbonate and polystyrene nanoplastic particles act as stressors to the innate immune system of fathead minnow (Pimephales promelas). Environ. Toxicol. Chem. 35, 3093–3100. https://doi.org/10.1002/etc.3501.
Haldane, J.B.S., 1949. Disease and evolution, reprint from La Ricerca Scientifica Supplemento 19, 1949. La Ric. Sci. Suppl. 19, 1–11.
Hall, S.R., Tessier, A.J., Duffy, M.A., Huebner, M., Cáceres, C.E., 2006. Warmer does not have to mean sicker: temperature and predators can jointly drive timing of epidemics. Ecology 87, 1684–1695. https://doi.org/10.1890/0012-9658(2006)87[1684:WDNHTM]2.0.CO;2.
Heugens, E.H.W., Hendriks, A.J., Dekker, T., Van Straalen, N.M., Admiraal, W., 2001. A review of the effects of multiple stressors on aquatic organisms and analysis of uncertainty factors for use in risk assessment. Crit. Rev. Toxicol. 31, 247–284. https://doi.org/10.1080/20014091111695.
Heugens, E.H.W., Jager, T., Creyghton, R., Kraak, M.H.S., Hendriks, A.J., Van Straalen, N.M., Admiraal, W., 2003. Temperature-dependent effects of cadmium on Daphnia magna: accumulation versus sensitivity. Environ. Sci. Technol. 37, 2145–2151. https://doi.org/10.1021/ES0264347.
Horton, A.A., Walton, A., Spurgeon, D.J., Lahive, E., Svendsen, C., 2017. Microplastics in freshwater and terrestrial environments: evaluating the current understanding to identify the knowledge gaps and future research priorities. Sci. Total Environ. 586, 127–141. https://doi.org/10.1016/j.scitotenv.2017.01.190.
Imhof, H.K., Ivleva, N.P., Schmid, J., Niessner, R., Laforsch, C., 2013. Contamination of beach sediments of a subalpine lake with microplastic particles. Curr. Biol. 23, R867–R868. https://doi.org/10.1016/j.cub.2013.09.001.
Imhof, H.K., Rusek, J., Thiel, M., Wolinska, J., Laforsch, C., 2017. Do microplastic particles affect Daphnia magna at the morphological, life history and molecular level? PLoS One 12, 1–20. https://doi.org/10.5061/dryad.9d84j.
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. https://doi.org/10.1016/j.envpol.2018.04.069.
Jansen, M., Stoks, R., Coors, A., van Doorslaer, W., de Meester, L., 2011. Collateral damage: rapid exposure-induced evolution of pesticide resistance leads to increased susceptibility to parasites. Evolution (N. Y). 65, 2681–2691. https://doi.org/10.1111/j.1558-5646.2011.01331.x.
Jemec, A., Horvat, P., Kunej, U., Bele, M., Kržan, A., 2016. Uptake and effects of microplastic textile fibers on freshwater crustacean Daphnia magna. Environ. Pollut. 219, 201–209. https://doi.org/10.1016/j.envpol.2016.10.037.
Jeong, C.B., Won, E.J., Kang, H.M., Lee, M.C., Hwang, D.S., Hwang, U.K., Zhou, B., Souissi, S., Lee, S.J., Lee, J.S., 2016. Microplastic size-dependent toxicity, oxidative stress induction, and p-JNK and p-p38 activation in the monogonont Rotifer (Brachionus koreanus). Environ. Sci. Technol. 50, 8849–8857. https://doi.org/10.1021/acs.est.6b01441.
Koelmans, A.A., Besseling, E., Shim, W.J., 2015. Nanoplastics in the aquatic environment. Critical review. In: Bergmann, M., Gutow, L., Klages, M. (Eds.), Marine Anthropogenic Litter. Springer, Cham. https://doi.org/10.1007/978-3-319-16510-3.
Kuznetsova, A., Bruun Brockhoff, P., Haubo Bojesen Christensen, R., 2015. lmerTest: Tests in Linear Mixed Effects Models.
Lenz, R., Enders, K., Nielsen, T.G., 2016. Microplastic exposure studies should be environmentally realistic. Proc. Natl. Acad. Sci. 113, E4121–E4122. https://doi.org/10.1073/pnas.1606615113.
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. https://doi.org/10.1016/j.watres.2017.12.056.
Liu, Z., Cai, M., Yu, P., Chen, M., Wu, D., Zhang, M., Zhao, Y., 2018. Age-dependent survival, stress defense, and AMPK in Daphnia pulex after short-term exposure to a polystyrene nanoplastic. Aquat. Toxicol. 204. https://doi.org/10.1016/j.aquatox.2018.08.017.
Liu, Z., Yu, P., Cai, M., Wu, D., Zhang, M., Chen, M., Zhao, Y., 2019a. Effects of microplastics on the innate immunity and intestinal microflora of juvenile Eriocheir sinensis. Sci. Total Environ. 685, 836–846. https://doi.org/10.1016/j.scitotenv.2019.06.265.
Liu, Z., Yu, P., Cai, M., Wu, D., Zhang, M., Huang, Y., Zhao, Y., 2019b. Polystyrene nanoplastic exposure induces immobilization, reproduction, and stress defense in the freshwater cladoceran Daphnia pulex. Chemosphere 215, 74–81. https://doi.org/10.1016/j.chemosphere.2018.09.176.
Lochmiller, R.L., Deerenberg, C., 2000. Trade-offs in evolutionary immunology: just what is the cost of immunity? Oikos 88, 87–98.
Mattsson, K., Hansson, L.A., Cedervall, T., 2015. Nano-plastics in the aquatic environment. Environ. Sci. Process Impacts 17, 1712–1721. https://doi.org/10.1039/c5em00227c.
McMahon, J.W., 1965. Some physical factors influencing the feeding behavior of Daphnia magna (Straus). Can. J. Zool. 43, 603–611.
Mitchell, S.E., Rogers, E.S., Little, T.J., Read, A.F., 2005. Host-parasite and genotype-by-environment interactions: temperature modifies potential for selection by a sterilizing pathogen. Evolution (N. Y). 59, 70–80. https://doi.org/10.1554/04-526.
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, 1–20. https://doi.org/10.1371/journal.pone.0155063.
Pigliucci, M., Preston, K. (Eds.), 2004. Phenotypic Integration: Studying the Ecology and Evolution of Complex Phenotypes. Oxford University Press, New York.
Plaistow, S.J., Collin, H., 2014. Phenotypic integration plasticity in Daphnia magna: an integral facet of G × E interactions. J. Evol. Biol. 1913–1920. https://doi.org/10.1111/jeb.12443.
Plaistow, S.J., Shirley, C., Collin, H., Cornell, S.J., Harney, E.D., 2015. Offspring provisioning explains clone-specific maternal age effects on life history and life span in the water flea, Daphnia pulex. Am. Nat. 186, 376–389. https://doi.org/10.1086/682277.
Prater, C., Wagner, N.D., Frost, P.C., 2018. Seasonal effects of food quality and temperature on body stoichiometry, biochemistry, and biomass production in Daphnia populations. Limnol. Oceanogr. 63, 1727–1740. https://doi.org/10.1002/lno.10803.
R Core Team, 2017. R: A Language and Environment for Statistical Computing.
Reisser, J., Slat, B., Noble, K., Du Plessis, K., Epp, M., Proietti, M., De Sonneville, J., Becker, T., Pattiaratchi, C., 2015. The vertical distribution of buoyant plastics at sea: an observational study in the North Atlantic Gyre. Biogeosciences 12, 1249–1256. https://doi.org/10.5194/bg-12-1249-2015.
Rios Mendoza, L.M., Karapanagioti, H., Álvarez, N.R., 2018. Micro(nanoplastics) in the marine environment: current knowledge and gaps. Curr. Opin. Environ. Sci. Heal. 1, 47–51. https://doi.org/10.1016/j.coesh.2017.11.004.
Rist, S., Baun, A., Hartmann, N.B., 2017. Ingestion of micro- and nanoplastics in Daphnia magna – quantification of body burdens and assessment of feeding rates and reproduction. Environ. Pollut. 228, 398–407. https://doi.org/10.1016/j.envpol.2017.05.048.
Rochman, C.M., 2018. Microplastics research - from sink to source. Science 360, 28–29.
Scherer, C., Brennholt, N., Reifferscheid, G., Wagner, M., 2017. Feeding type and development drive the ingestion of microplastics by freshwater invertebrates. Sci. Rep. 7, 1–9. https://doi.org/10.1038/s41598-017-17191-7.
Schmid-Hempel, P., 2003. Variation in immune defence as a question of evolutionary ecology. Proc. R. Soc. Biol. Sci. 270, 357–366. https://doi.org/10.1098/rspb.2002.2265.
Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ : 25 years of image analysis. Nat. Methods 9, 671–675. https://doi.org/10.1038/nmeth.2089.
Sheldon, B.C., Verhulst, S., 1996. Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. Trends Ecol. Evol. 11, 317–321.
Siehoff, S., Hammers-Wirtz, M., Strauss, T., Ratte, H.T., 2009. Periphyton as alternative food source for the filter-feeding cladoceran Daphnia magna. Freshw. Biol. 54, 15–23. https://doi.org/10.1111/j.1365-2427.2008.02087.x.
Song, Y.K., Hong, S.H., Jang, M., Han, G.M., Jung, S.W., Shim, W.J., 2017. Combined effects of UV exposure duration and mechanical abrasion on microplastic fragmentation by polymer type. Environ. Sci. Technol. 51, 4368–4376. https://doi.org/10.1021/acs.est.6b06155.
Thompson, R.C., Olsen, Y., Mitchell, R.P., Davis, A., Rowland, S.J., John, A.W.G.,

McGonigle, D., Russell, A.E., 2004. Lost at sea: where is all the plastic? Science 304, 838. https://doi.org/10.1126/science.1094559.
Triebskorn, R., Braunbeck, T., Grummt, T., Hanslik, L., Huppertsberg, S., Jekel, M., Knepper, T.P., Krais, S., Müller, Y.K., Pittroff, M., Ruhl, A.S., Schmieg, H., Schür, C., Strobel, C., Wagner, M., Zumbülte, N., Köhler, H.R., 2019. Relevance of nano- and microplastics for freshwater ecosystems: a critical review. Trends Anal. Chem. 110, 375–392. https://doi.org/10.1016/j.trac.2018.11.023.
United Nations Environment Programme, 2014. UNEP Year Book 2014: Emerging Issues in Our Global Environment, Emerging Issues in Our Global Environment. UNEP Division of Early Warning and Assessment, Nairobi, Kenya. https://doi.org/10.1007/s11377-015-0024-x.
Vale, P.F., Little, T.J., 2012. Fecundity compensation and tolerance to a sterilizing pathogen in Daphnia. J. Evol. Biol. 25, 1888–1896. https://doi.org/10.1111/j.1420-9101.2012.02579.x.
Vale, P.F., Stjernman, M., Little, T.J., 2008. Temperature-dependent costs of parasitism and maintenance of polymorphism under genotype-by-environment interactions. J. Evol. Biol. 21, 1418–1427. https://doi.org/10.1111/j.1420-9101.2008.01555.x.
Vale, P.F., Wilson, A.J., Best, A., Boots, M., Little, T.J., 2011. Epidemiological, evolutionary, and coevolutionary implications of context-dependent parasitism. Am. Nat. 177, 510–521. https://doi.org/10.1086/659002.
Von Moos, N., Burkhardt-Holm, P., Köhler, A., 2012. Uptake and effects of microplastics on cells and tissue of the blue mussel Mytilus edulis L. after an experimental exposure. Environ. Sci. Technol. 46, 11327–11335. https://doi.org/10.1021/es302332w.
Wagner, M., Scherer, C., Alvarez-Muñoz, D., Brennholt, N., Bourrain, X., Buchinger, S., Fries, E., Grosbois, C., Klasmeier, J., Marti, T., Rodriguez-Mozaz, S., Urbatzka, R., Vethaak, A.D., Winther-Nielsen, M., Reifferscheid, G., 2014. Microplastics in freshwater ecosystems: what we know and what we need to know. Environ. Sci. Eur. 26, 1–9. https://doi.org/10.1186/s12302-014-0012-7.
Wan, J.-K., Chu, W.-L., Kok, Y.-Y., Lee, C.-S., 2018. Distribution of microplastics and nanoplastics in aquatic ecosystems and their impacts on aquatic organisms, with emphasis on microalgae. Rev. Environ. Contam. Toxicol. 237, 133–158.
Welden, N.A.C., Abylkhani, B., Howarth, L.M., 2018. The effects of trophic transfer and environmental factors on microplastic uptake by plaice, Pleuronectes plastessa, and spider crab, Maja squinado. Environ. Pollut. 239, 351–358. https://doi.org/10.1016/j.envpol.2018.03.110.
Welden, N.A.C., Cowie, P.R., 2016. Environment and gut morphology influence microplastic retention in langoustine, Nephrops norvegicus. Environ. Pollut. 214, 859–865. https://doi.org/10.1016/j.envpol.2016.03.067.
Windsor, F.M., Durance, I., Horton, A.A., Thompson, R.C., Tyler, C.R., Ormerod, S.J., 2019. A catchment perspective of plastic pollution. Glob. Chang. Biol. https://doi.org/10.1111/j.1365-294X.2010.04833.x.
Woodward, G., Perkins, D.M., Brown, L.E., 2010. Climate change and freshwater ecosystems: impacts across multiple levels of organization. Philos. Trans. R. Soc. Biol. Sci. 365, 2093–2106. https://doi.org/10.1098/rstb.2010.0055.
Wright, S.L., Rowe, D., Thompson, R.C., Galloway, T.S., 2013. Microplastic ingestion decreases energy reserves in marine worms. Curr. Biol. 23, R1031–R1033. https://doi.org/10.1016/j.cub.2013.10.068.
Young, H.S., McCauley, D.J., Galetti, M., Dirzo, R., 2016. Patterns, causes, and consequences of anthropocene defaunation. Annu. Rev. Ecol. Evol. Syst. 47, 333–358. https://doi.org/10.1146/annurev-ecolsys-112414-054142.
Yu, P., Liu, Z., Wu, D., Chen, M., Lv, W., Zhao, Y., 2018. Accumulation of polystyrene microplastics in juvenile Eriocheir sinensis and oxidative stress effects in the liver. Aquat. Toxicol. 200, 28–36. https://doi.org/10.1016/j.aquatox.2018.04.015.
Zhang, C., Jansen, M., De Meester, L., Stoks, R., 2018. Thermal evolution offsets the elevated toxicity of a contaminant under warming: a resurrection study in *Daphnia magna*. Evol. Appl. 1–12. https://doi.org/10.1111/eva.12637.