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Chapter 7

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REVIEW: EFFECTS OF MICROPLASTIC ON ZOOPLANKTON SURVIVAL AND SUBLETHAL RESPONSES

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Abstract Microplastics (MPs) are a prolific contaminant in aquatic ecosystems across the globe. Zooplankton (including holoplankton and meroplankton) play vital ecological roles in marine and freshwater ecosystems and have been shown to readily consume MPs. The present review uses 88 pieces of published literature to examine and compare the effects of MPs on survival, growth, development, feeding rate, swimming speed, reproduction, organ damage and gene expression of different groups of zooplankton, including copepods, daphnids, brine shrimp, euphausids, rotifers and the larvae of fishes, sea urchins, molluscs, barnacles, decapods and ascidians. Among the groups studied, daphnids and copepods are the most sensitive to MPs, with their feeding rate and fecundity significantly decreased at environmentally relevant MP concentrations. This might adversely affect daphnid and copepod populations in the long term. In contrast, molluscs, barnacles, brine shrimp and euphausids appear to be more tolerant to MPs. No clear impacts on survival, development time, growth or feeding rate can be observed in these zooplankton groups at any of the MP concentrations tested, suggesting that these groups might become more dominant with prolonged exposure to MP pollution. Leachates derived from MPs can induce severe abnormality in bivalve and sea urchin embryos. MPs have prominent effects on survival and fecundity of F_1 offspring in bivalves, copepods and daphnids, indicating that MPs could incite transgenerational effects and drastically affect sustainability in zooplankton populations.

Introduction

The invention of plastics has had a vast societal and environmental impact (Thompson et al. 2009). Since the material was introduced in 1907, plastic production has increased continuously, rising from 47 million tons in 1975 to 335 million tons in 2016; the plastic industry has now become one of the largest manufacturing sectors in the world (Plastic Europe 2017). However, mismanagement has led to inordinate amounts of plastic waste ending up in the natural environment. Owing to its durability, plastic debris accumulates in the environment, where it poses a threat to a wide range of biota (Thompson et al. 2009).

After entering the natural environment, plastic debris is subjected to fragmentation via UV degradation and physio-chemical and biological processes, eventually breaking down into microscopic pieces, termed microplastics (MPs) (Thompson et al. 2004). The definition of a MP is still under debate, with many different definitions proposed in, for example, Koelmans (2015) and Mendoza et al. (2018). Here, we define a MP as a piece of plastic 0.1 μm–1 mm in diameter in accordance with Hartmann et al. (2019). MPs are a prolific marine contaminant, accounting for more than 90% of all marine plastic debris (Eriksen et al. 2014). Recent studies have shown the presence of MPs in freshwater and marine environments, including the coral reefs (Hall et al. 2015), open oceans (Eriksen et al. 2014), deep oceans (Van Cauwenberghe et al. 2013) and polar regions (Waller et al. 2017, Peeken et al. 2018). Despite their widespread occurrence within the natural environment, the effects of MPs on marine and freshwater ecosystems remain poorly understood (Thompson et al. 2004, Andrady 2011, Law & Thompson 2014, Shim & Thomposon 2015).

Zooplankton encompass both freshwater and marine holoplankton and meroplankton, which exhibit very different life histories. Holoplankton (e.g. copepods and daphnids) spend their entire life as plankton. On the other hand, meroplankton (e.g. bivalve and sea urchin larvae) only spend part of their life as plankton and become either nekton or benthos in later developmental stages. Numerous organisms, including mammals, seabirds, bivalves, fish and zooplankton, have been reported to ingest MPs (Egbeocha et al. 2018). High MP to zooplankton ratios have been documented in the north Pacific gyre and Mediterranean Sea (Moore et al. 2001, Collignon et al. 2012), and both holoplankton and meroplankton have been reported to ingest MPs in the field (Desforges et al. 2015, Sun et al. 2017, Steer et al. 2017) and laboratory studies (e.g. Cole et al. 2013, Setälä et al. 2014). As a primary consumer, zooplankton graze on phytoplankton and transfer energy to higher trophic levels along the food chain and are therefore considered essential sources of prey for numerous marine organisms. They also play a vital role in nutrient cycling by feeding in surface water and packaging the organic matter into dense faeces which facilitate the transport of carbon and nutrients to the deep sea (Turner 2015). Thus, any negative impact MPs have on zooplankton has the potential to subsequently affect different trophic levels and key ecological processes within the marine environment.

One of the controversial issues in MP ecotoxicological studies is the concentration of MP used often far exceed the levels documented in the marine environment (Lenz et al. 2016). Current MP concentrations reported in the field typically range from 1×10^{-3} to 1×10^{-6} mg L^{-1} (Lenz et al. 2016). However, concentrations orders of magnitude higher than field concentrations are often used in laboratory studies to assess the impacts of MPs (Lee et al. 2013, Rehse et al. 2016). As a result, the impacts of MP derived from such high concentrations may never happen in the real environment. It is possible that these laboratory studies are not representative and might overestimate the effects of MPs, although they may still provide important insights into the mechanisms by which MP can cause toxicity.

There is presently no detailed review on the effect different sizes and concentrations of virgin or chemically coated MPs have on survival and sublethal health responses (e.g. growth, development, feeding and swimming behaviours, reproduction, gene expression from transcriptome analysis and organ damages) of individual groups of zooplankton. While a recent detailed review assessed the factors affecting the bioavailability of MPs to marine zooplankton, including size, shape, colour, polymer type, density, age, abundance and aggregation (Botterell et al. 2018), the relative sensitivities of different zooplankton groups to MPs have never been compared before.

The present study reviews and compares the impact of MPs (polymer type, size, concentration and shape) on eight of the most commonly assessed biological endpoints – survival, development, growth, feeding rate, swimming speed, reproduction, organ damage and gene expression – in a range of zooplankton taxa, including holoplankton (copepods, daphnids, brine shrimp, euphausids and rotifers), and meroplankton (larvae of fishes, sea urchins, molluscs, barnacles, decapods and ascidians). We further compared the relative sensitivity among these zooplankton groups for different endpoints. In particular, we reviewed the effects of MPs at concentrations that are relevant to real environments (0–1 mg L^{-1}) and at the high concentrations used under laboratory conditions which are beyond the concentration in the natural environment. This review attempts to give insight into which biological traits and zooplankton groups are more sensitive to MPs (at both environmentally relevant concentrations and high concentrations in laboratory conditions) and could therefore act as a potential indicator for MP pollution in the environment. Finally, we identify the knowledge gaps based on present MP studies on zooplankton.

Abbreviations

Some chemical terms and polymer types are explained subsequently with their abbreviations. The full names of these terms and their abbreviations used in this review are listed in Table 1.

Methods

Published articles evaluating effects of MP on zooplankton were searched for on Science Citation Index (SCI) journals, Google Scholar and the ISI Web of Science using a combination of keywords and Boolean operators (i.e. AND), including microplastic, zooplankton, larvae, fish, copepod, sea urchin, bivalve, gastropod, barnacle, daphnid, brine shrimp, crustacean and rotifer. Eight of the most frequently evaluated endpoints – mortality, development, growth, feeding rate, swimming speed, reproduction, organ damage and gene expression – were extensively reviewed. A total of 88 articles were identified, covering the following zooplankton groups: Holoplankton: copepods, daphnids, brine shrimp, euphausids and rotifers; Meroplankton: the larvae of fishes, sea urchins, bivalves, gastropods, barnacles, decapods and ascidians. In each zooplankton group, the eight endpoints were discussed according to 1) developmental stage (gametal, embryonic, larval or adult stage), 2) transgenerational effects (offspring generation) and 3) the type of MP (virgin MPs or those that had interacted with chemicals).

Microplastic mass calculations

Published literature used a variety of concentration units, such as beads mL^{-1} and mg L^{-1}. For standardisation purposes, studies whose concentration unit was based on the number of particles (beads L^{-1}) were transformed to units of mass (mg L^{-1}). First, the volume (V) of spherical MPs (i.e. beads) was calculated using the formula $V = 4/3\ \pi r^3$, where radius (r) was ascertained from the diameter of the particle. For fibrous MPs, the volume (V) of fibre MPs was calculated using the cylindrical volume formula $V = \pi r^2 h$, where radius (r) and height (h) were ascertained from the diameter and length of the fibre. Literature using fragmented MPs and only reporting the number of particles (beads L^{-1}) cannot be transformed to units of mass (mg L^{-1}). Hence, those studies were described in the context but were excluded from the analysis. Next, the volume of the MP particles was multiplied by the density (ρ) of the specific polymer to obtain the mass (M) of a single MP. Finally, the mass of a single MP was multiplied by the particle concentration (beads mL^{-1}), as reported in the literature, to give the mass of MP per millilitre (g mL^{-1}), with units converted to ascertain the mass per litre (mg L^{-1}).

Table 1 Full names and abbreviations of the terms used in this review

Category	Full names (abbreviations)
Plastics	Microplastic (MP), polyethylene (PE), low-density polyethylene (LDPE), high-density polyethylene (HDPE), polystyrene (PS), polystyrene coated with carboxylic groups (PS-COOH), polystyrene coated with amine groups (PS-NH_2), polyvinylchloride (PVC), polymethyl methacrylate (PMMA), polyamide (PA), polycarbonate (PC), polyethylene terephthalate (PET), polylactide (PLA), acrylonitrile-burtdiene-styrene terpolymer (ABS), polyoxymethylene homopolymer (POM) and styreneacrylonitrile copolymer (SAN)
Additives	Polychlorinated biphenyl (PCB), polycyclic aromatic hydrocarbon (PAH), phenanthrene (Phe), benzo[a]pyrene (BaP), triclosan (TCS), bisphenol A (BPA), 17 α-ethynylestradiol (EE2) and benzophenone-3 (BP-3)
Toxicological terms	Concentration lethal to 50% of a population (LC_{50}), concentration at which an effect is observed in 50% of a population (EC_{50}), lowest observed effect concentration (LOEC) and no effect concentration (NEC)

Calculating percentage change of microplastics

Since all of the studies reviewed here were based on different treatments, there was a need to standardise them all to facilitate comparisons. To compare the percentage change of biological endpoints in the presence of MP, the measured value of animals in the control was subtracted by that in the MP treatment and then divided by the value given in the control and then data multiplied by −1 so adverse effects were shown as negative data:

$$\text{Percentage change}\,(\%) = \frac{X - Y}{X} \times 100 \times -1$$

X = measured value of the control
Y = measured value of MP treatments

For experiments based on virgin MPs, the measured values that were used to calculate the percentage change in each endpoint are as follows: 1) survival: survival rate, hatching rate or fertilisation rate; 2) development: development time; 3) morphological normality or abnormality; 4) growth: body length, width, arm length or weight; 5) feeding rate: ingestion rate (no. of algae/*Artemia* nauplii consumed) or carbon biomass uptake; 6) swimming speed: swimming velocity, maximum swimming velocity or distance travelled in a period; and 7) reproduction: total number of offspring produced, number of offspring produced per brood or egg production rate. Literature that did not use the measured values listed here was excluded from percentage change analysis. Of the 88 articles reviewed, data from 74 papers were included in the percentage change calculation. To compare the effects of size and concentration, MPs were assigned to one of three size classes: 0.1–10, >10–100 and >100 μm; the concentration was categorised into four groups: 0–1, >1–10, >10–100, >100 mg L^{-1}. The mean percentage change with one standard deviation (1SD) was calculated for each size class of MPs at different concentrations. MP concentrations at 0–1 mg L^{-1} are consistent with those documented in the field (Lenz et al. 2016). Thus, the observed effects under this concentration are considered environmentally relevant. For concentrations >1 mg L^{-1}, these are considered higher than have been observed in the natural environment, and therefore the effects potentially exaggerate the impacts of MPs.

When investigating transgenerational effects of MP on zooplankton, measured values used for survival, development, growth, normality, feeding rate, swimming speed and reproduction were the same as previously described. Note that when evaluating transgenerational effects, we combined all the values of different MP sizes and concentrations together, predominantly due to the small number of studies on these effects. Because the interactions between MPs and chemicals are complex, we did not calculate the mean percentage change in interactive effects between MPs and chemicals, but their effects are discussed in context. All literature in the present studies is listed in the supplementary information (Table A1–9).

Survival

Holoplankton

Copepods

Larvae and juveniles MPs (0.1–10 μm) rarely had lethal effects on copepod naupliar larvae. The percentage change in survival was <5% (Figure 1A). Lee et al. (2013) observed that neither acute (96 hours) nor chronic (14 days) exposure to 0.5 and 6 μm polystyrene (PS) MPs (0.125–25 mg L^{-1}) had an observable lethal effect on MP-exposed *Tigriopus japonicus* (Harpacticoida) naupliar larvae. All MP treatments resulted in over 80% survival, including controls (82%) (Figure 1A). Similarly, PS MPs (1–6 μm, 1–10 mg L^{-1}) did not decrease the survival of *Tigriopus fulvus* (LOEC >10 mg L^{-1}). In calanoid copepods, PS (4–6 μm) and polyvinylchloride (PVC) MPs (20 μm) did not affect survival in *Acartia clausi* (LOEC >30 mg L^{-1}) (Beiras et al. 2018, 2019). Because Beiras et al. (2018) only reported LOEC values, data from this study were not included in the percentage change analysis. The lack of impact again indicates that MPs of size 0.1–10 μm rarely had lethal effects on copepods (Figure 1A).

Adults Exposure to virgin 0.1–10 μm and >10–100 μm MPs had no observable impacts on the survival of adult calanoid and harpacticoid copepods that have been studied (Figure 1A,B). In the copepod *T. japonicus*, long-term exposure (14 days) to 0.5 and 6 μm PS MPs did not affect the adult survival rate (up to 80%) at any concentrations tested (0–25 mg L^{-1}) (Lee et al. 2013) (Figure 1A). Similarly, survival rates of calanoid copepods *Calanus helgolandicus* and *Calanus finmarchicus* exposed to 20.0 μm (0.33 mg L^{-1}) and 15 μm (0.095 and 0.95 mg L^{-1}) PS MPs were not significantly different from that of the control (Cole 2014, Cole et al. 2015, Vroom et al. 2017). Both controls (82%) and MP treatments (81%) reached over 80% survival rate (Figure 1B). No study has evaluated the effect of MPs >100 μm on copepods; thus, no data were included in this size class during the percentage change analysis.

Microplastic-chemical interactions Co-exposure to MPs with chemicals might decrease the survival of organisms, but the extent to which it is toxic appears to be chemically dependent. Co-exposure to polyethylene (PE) MPs (43.5 mg L^{-1}) and triclosan (TCS), a synthetic antimicrobial

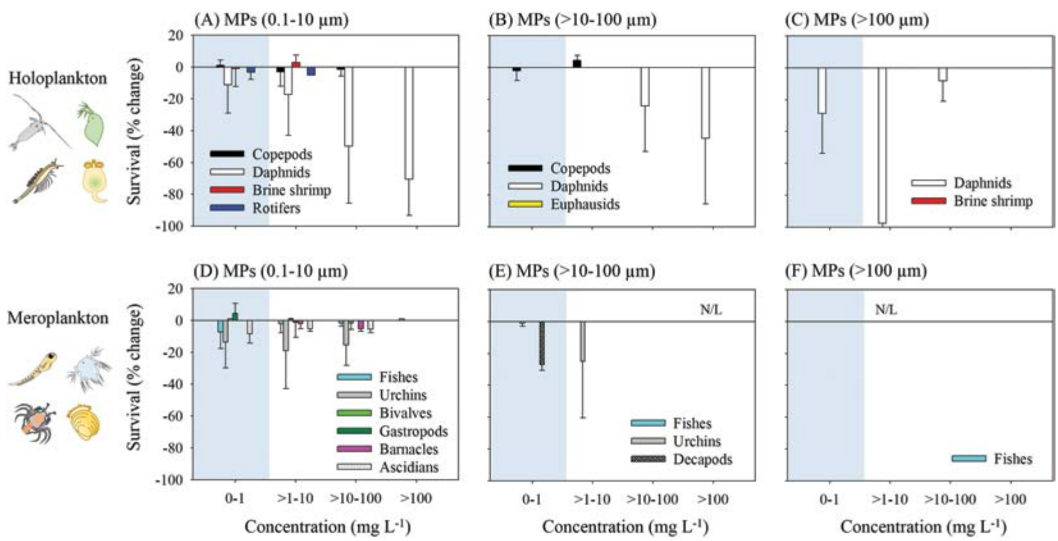

Figure 1 Percentage change in survival (mean + 1SD %) of (A–C) holoplankton and (D–F) meroplankton in MP treatments when compared to controls. For literature used for all groups of zooplankton, refer to supplementary Table A1. A negative percentage change means a decrease amount of the value in MP treatment compared to that of the control and vice versa. Note: In figure (A), no data are available on copepods (>10^2 mg L^{-1}), rotifers (>10 mg L^{-1}) and euphausids (all concentrations). In figure (B), no data are available on brine shrimp and rotifers at all concentrations, except for daphnids (0–1 mg L^{-1}) and euphausids (>10 mg L^{-1}). In figure (C), no data are available for copepods, euphausids and rotifers at all concentrations, except for brine shrimp (0–10, >10^2 mg L^{-1}). In figure (D), no data are available for urchins (>10^2 mg L^{-1}), bivalves (>10^2 mg L^{-1}), gastropods (>10 mg L^{-1}), barnacles (>10^2 mg L^{-1}), ascidians (>10^2 mg L^{-1}) and decapods (all concentrations). In figure (E), no data are available for bivalves, gastropods, barnacles, ascidians at all concentrations, except for fishes (0–1, >10^2 mg L^{-1}), urchins (>10 mg L^{-1}) and decapods (>1 mg L^{-1}). In figure (F), no data are available for urchins, bivalves, gastropods, barnacles, decapods and ascidians at all concentrations, except for fishes (>1–10 mg L^{-1}). Note: light blue background indicates the concentration where environmentally relevant, and white background indicates high laboratory concentration, which does not appear in the environment at the moment. N/L = no data available.

agent, significantly lowered LC_{50} (109.6 ± 0.01 μg L^{-1}) compared to TCS alone (157.9 ± 0.01 μg L^{-1}) in the calanoid copepod *Acartia tonsa* (Syberg et al. 2017). Bejgarn et al. (2015) assessed the toxicity of leachate from plastic products and found that 8 of the 21 plastic materials tested (38%) (<1 mm; 100 g L^{-1}) caused acute toxicity, with PVC and polyurethane (PUR) leachates seeming to have higher toxicities. Exposure to MPs and leachates derived from commercial PVC products significantly reduced the calanoid copepod *A. clausi* survival by 60%–90% (Beiras et al. 2019). In contrast, benzophenone-3 (BP-3) was less toxic: the lowest observed effect concentration (LOEC) was higher than the highest concentration used (10 mg L^{-1} PE MPs [1–6 μm] spiked with 20 μg L^{-1} BP-3), suggesting that BP-3 had no clear impact on the survival of *T. fulvus* and *A. tonsa* (Beiras et al. 2018).

Transgenerational effect The offspring produced by MP-exposed copepods died at a significantly higher rates than the controls, although their MP-exposed parents were not affected (both Calanoida and Harpacticoida). In *T. japonicus*, exposure to 0.5 μm PS MPs (25 mg L^{-1}) significantly decreased the survival of the F_1 generation to 35%, but not the F_0 generation (survival over 80%) (Lee et al. 2013). Hatching success of eggs produced by PS MP-exposed (20 μm; 0.33 mg L^{-1}) *C. helgolandicus* was ~22% lower than that of the control (Cole 2014, Cole et al. 2015). Polyethylene terephthalate (PET) MPs (<11 μm; 14.44 mg L^{-1}) significantly reduced the population size of the copepod *Parvocalanus crassirostris* by around 40% compared to controls after 24 days of exposure (Heindler et al. 2017). These results suggest that MP exposure might have transgenerational effects, reducing the fitness of their offspring. Nevertheless, the size of MPs might affect these results. For example, 6 μm PS MPs (0.125–25 mg L^{-1}) affected neither parental nor offspring survival rates (over 70% survival) (Lee et al. 2013). Thus, the calculated mean percentage change was only ~10% (Figure 2A). However, the study number is still small, and further investigations are highly recommended.

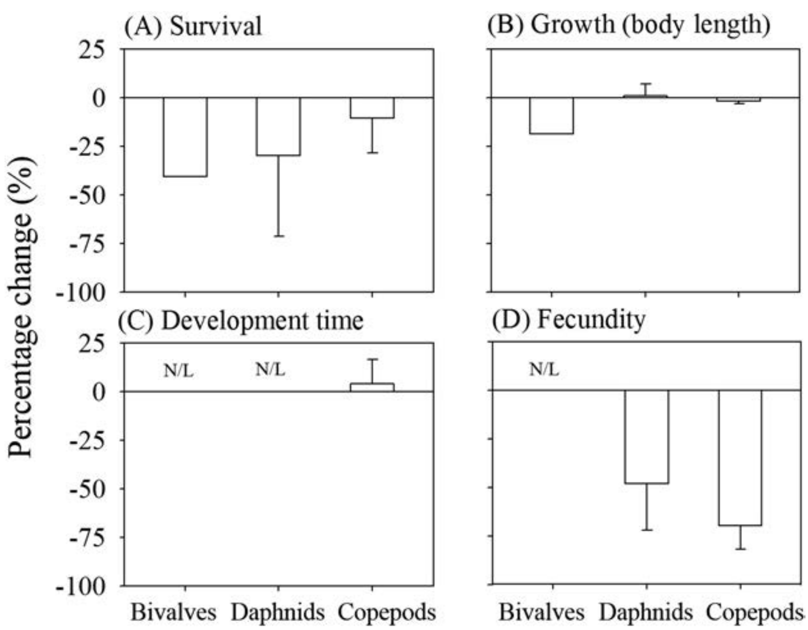

Figure 2 Percentage change in (A) survival, (B) growth (body length), (C) development time and (D) fecundity (mean + 1SD %) of F_1 offspring in MP treatments when compared to controls. N/L = no data available. For literature used for all groups of zooplankton, refer to supplementary Table A2. Note: No data are available for brine shrimp, euphausids, rotifers, fishes, urchins, gastropods, barnacles, decapods and ascidians for transgenerational effects of MPs.

Daphnids

Since daphnids are the most extensively studied organisms for MP toxicity tests, there are plenty of studies evaluating the effects of MP on daphnid survival. This section is divided into three parts: 1) the effect of food particles, 2) MP shape and 3) species.

(1) *Presence of food particles*: The presence of food particles appears to be an important factor in determining MPs' effect on mortality. MPs had no clear effect when food particles were present in the solution. On the other hand, MP significantly increased mortality in the absence of food. In acute toxicity tests, the organisms are usually not fed during the exposure. At the beginning of exposure, MPs had no observable effect on survival if the exposure time was less than 72 hours. For instance, neither PS MPs (1–15 μm) nor polyamide (PA) Ps (15–20 μm; 25–250 mg L^{-1}) were toxic to *Daphnia magna* after 72 hours of exposure (Ma et al. 2016, Puranen Vasilakis 2017, Horton et al. 2018, Rehse et al. 2018). All of the controls and MP treatments reached over 90% survival. One exception was the study by Zhang et al. (2019), who found that *D. magna* survival significantly decreased in a dose-dependent manner after 48 hours of exposure to PS MPs (1 and 10 μm; 0.1–600 mg L^{-1}). However, the toxicity rose with increasing exposure time. Both PET (~5 μm; 0.1–10000 mg L^{-1}) and high-density polyethylene (HDPE) MPs (1 μm; 12.5–400 mg L^{-1}) significantly decreased daphnid (*D. magna*) survival from 20% to 100%, compared to 100% survival in controls, after 96 hours of exposure (Rehse et al. 2016, Gerdes et al. 2018), despite yielding no observed effect at 48 hours. Jaikumar et al. (2018) also compared the toxicity of different exposure times and found a strong time-dependent correlation in which toxicity was higher after 96 hours of exposure. These studies largely contributed to the high percentage decrease in survival observed upon exposure to 0.1–10 μm MPs (Figure 1A).

On the other hand, in chronic-exposure experiments, food particles are added to keep animals alive. If food particles were present in the solution, MPs had minor or negligible effects on daphnid survival. For example, none of the three *D. magna* clones tested had increased mortality after exposure to two mixtures of MPs (PA + polycarbonate [PC] + PET + PVC and acrylonitrile-burtdiene-styrene terpolymer [ABS] + PVC + polyoxymethylene homopolymer ([POM] + styreneacrylonitrile copolymer [SAN]) for 20–22 days (Imhof et al. 2017). No significant effect on survival was found for either PE (63–75 μm; 25–100 mg L^{-1}) or PS (1.25 μm, 2–8 mg L^{-1}; 1–5 μm, 4.5 mg L^{-1}) MP-exposed *D. magna* (Canniff & Hoang 2018, Gorokhova et al. 2018, Tang et al. 2019). Similarly, unknown types of MPs did not cause any clear mortality in *D. magna* (1–5 μm) (0.1 mg L^{-1}; 12.86 mg L^{-1}) (Martins & Guilhermino 2018, Gerdes et al. 2019). All the groups generally attained over 90% survival in these studies (Figure 1A,B). However, some studies found elevated mortality, although these increases were relatively minor. For example, mortality only increased slightly (less than 30%) in unknown plastic type (30%; 1–5 μm; 2 mg L^{-1}) and PS (26%; 1–5 μm; 0.65 mg L^{-1}) exposed *D. magna* (Puranen Vasilakis 2017, Pacheco et al. 2018) (Figure 1A). These results highlight that the presence of food might effectively offset the negative effects of MP. This is further supported by the study of Aljaibachi & Callaghan (2018), who found that low food concentration, not MP ingestion, was the main cause of mortality.

(2) *MP shape*: In contrast to spherical MPs, irregular-shaped MPs (fragments and fibres) significantly reduced the survival of MP-exposed animals. There was, however, variation among studies. Some studies showed that irregular-shaped MPs had higher toxicity than spherical. For example, survival was lower in daphnids (*D. magna*) exposed to irregularly shaped MP (2.6 ± 1.8 μm, 1.19 mg L^{-1}; 0.8 day survival) compared to controls (2.9 day survival) and spherical MP-exposed animals (2.4 day survival) (Ogonowski et al. 2016). Frydkjær et al. (2017) observed only 12%–40% survival (95% in control) after exposure to PE fragmented MPs (*D. magna*; 10–75 μm; 10–5000 mg L^{-1}). Similarly, PET microfibres (60–1400 μm, 12.5–100 mg L^{-1}; 100−400 μm; 0.13–0.24 mg L^{-1}) also decreased survival by 10%–100% in *D. magna* and *Ceriodaphnia dubia* (Jemec et al. 2016, Ziajahromi et al. 2017). The decreased survival observed in these studies led to a high percentage decrease in survival observed in all three size classes of MPs (Figure 1A–C). The

decreased survival could be explained by the formation of aggregates of MP which might cause internal damage during gut passage or interfere with swimming in MP-exposed animals. In contrast, Kokalj et al. (2018) found no clear effect of MP fragments and fibres on daphnids. Neither spherical nor irregular MPs (including fragment and fibre) (PE and PET; 63.05–264 μm; 100 mg L^{-1}) resulted in any mortality (0%) in *D. magna* (Kokalj et al. 2018). Since the size and types of MPs were similar among these studies, the discrepancies might be explained by other factors such as morphological characters which could affect the toxicity of MPs as well.

(3) *Species: C. dubia* appeared to be more sensitive to MP pollution than the model species *D. magna*. Acute exposure (48 hours) to PE MPs (1−4 μm) and PET fibres (100−400 μm) decreased *C. dubia* survival by 10%–100%. No survival (0%) was observed at low MP concentrations, over 0.24 and 8.04 mg L^{-1} for fibres and beads, respectively (Ziajahromi et al. 2017). Similar sizes and concentrations of MPs have never been documented to cause 0% survival in *D. magna*, suggesting that the MPs' toxicity is species specific and that *C. dubia* is more sensitive than *D. magna*. This species-specific sensitivity caused the non-concentration dependent trend in Figure 1C. The data on MPs >100 μm at concentrations ≤10 mg L^{-1} were calculated from the study by Ziajahromi et al. (2017), which used *C. dubia* as a model species, whereas all data at concentrations >10 mg L^{-1} came from studies using *D. magna* (Jemec et al. 2016, Rehse et al. 2016). Thus, the high sensitivity of *C. dubia* peaked the percentage change to nearly 100% at >1–10 mg L^{-1}, and then the percentage change decreased afterward because of the high resistance of *D. magna*. However, the number of studies is still small, so further investigations are needed before drawing a strong conclusion.

Microplastic-chemical interactions Leachates derived from MPs may have toxic chemicals and can be a hazard to biota. However, 100% survival was observed when *D. magna* were exposed to leachates derived from PET fibres (60–1400 μm; 12.5–100 mg L^{-1}) (Jemec et al. 2016). These chemicals may have been at a level too low to cause observable impacts. Moreover, it has been suggested that MPs would concentrate hydrophobic chemicals from the environments and have detrimental effects on biota. However, this hypothesis is currently under debate. Some studies found that MPs and chemicals have synergistic effects. Frydkjær et al. (2017) found that irregular PE MPs (10–75 μm) were a good vector for phenanthrene (Phe) and adding MPs (EC_{50}: 0.14 mg L^{-1}) was more toxic than adding the same concentration of Phe (EC_{50}: 0.47 mg L^{-1}). In contrast, other studies showed that MP did not increase the toxicity of chemicals. Co-exposure to PS MPs and pesticides (dimethoate and deltamethrin) neither increased nor decreased the toxicity of the two pesticides. The probabilities of normal mobility for *D. magna* were similar between treatments with or without MP (0.57 and 0.2 for dimethoate and deltamethrin, respectively) (Horton et al. 2018). Exposure to PS MPs and Phe (5, 10 and 15 μm; 2.5–50 mg L^{-1}) did not decrease *D. magna* survival (Ma et al. 2016). The EC_{50} of Phe (0.59 ± 0.05 mg L^{-1}) did not shift significantly in the presence of MPs (0.66 mg L^{-1}). In some cases, MP presence even lowered the toxicity of contaminants. Treatments with bisphenol A (BPA) and the addition of PA MPs (15–20 μm; 200 mg L^{-1}) reduced immobilisation by 20% compared to daphnids that were treated with BPA alone (Rehse et al. 2018). Adding 1 mg L^{-1} of PS MPs (0.1 μm) increased *D. magna* survival by 45% compared to those that were exposed to the same concentration of polychlorinated biphenyl (PCB)-18 (640 μg L^{-1}) (Lin et al. 2019). The toxicity of nickel (Ni; EC_{50} 3.85 mg L^{-1}) was lower when PS MPs (0.19 μm) were presented in combination with Ni (EC_{50} 17.72 mg L^{-1}) (Kim et al. 2017). These studies suggest that the toxicity of chemicals might decrease when co-exposed with MPs. Since toxicity largely depends on both the type of polymer and the interacting chemicals, more studies are needed to assess the interactive effects on various polymers and chemicals.

Transgenerational effect Although MPs did not affect survival in the *D. magna* F_0 generation, continuous MP exposure to the F_1 generation had transgenerational effects on their offspring (Figure 2A). Decreased survival was observed in F_1 offspring if they were continually exposed to MPs.

No survival (0%) was even found in the first brood of F_1 offspring, with all offspring rapidly dying within 1–4 days of MP exposure (1–5 µm; 0.1 mg L^{-1}). Even the survival rate of the third brood of F_1 offspring decreased by 20% compared to controls (Martins & Guilhermino 2018). Bosker et al. (2019) also found that after 21 days of PS MP exposure (1–5 µm; 4.69 mg L^{-1}), the population size of *D. magna* significantly decreased by 26% compared to that of the control. These studies suggest that long-term exposure to MPs across generations might drastically decrease *D. magna* populations.

If the F_1 offspring were no longer exposed to MPs, however, survival appeared to recover with time, with 100% survival observed in F_1 and the subsequent generations (F_2 and F_3) if they were moved to clean water immediately after birth (*D. magna*) (Martins & Guilhermino 2018). Similarly, offspring survival rates were generally over 90% in all treatments in other studies (Ogonowski et al. 2016, Aljaibachi & Callaghan 2018). These studies suggest that negative transgenerational effects of MPs can be offset with enough recovery time, although some sublethal effects will still last for several generations (see 'Development and growth' and 'Reproduction' in the present review).

Brine shrimp

Larvae Brine shrimp larvae appeared to be highly tolerant to MPs. No significant change in survival was observed in any of the studies, regardless of the size, shape or type of MP used (Figure 1A,C). Short-term exposure to spherical (PS; 1 and 9.9 µm; 0.1 mg L^{-1}), irregular and fibre MPs (PE and PET; 100–300 µm; 100 mg L^{-1}) did not affect survival (100%) in nauplius larvae of *Artemia franciscana* and an unknown *Artemia* sp. (Katzenberger 2015, Kokalj et al. 2018). Similarly, 100% survival was observed in PS MP-exposed *A. franciscana* (0.1 µm; 0.001–10 mg L^{-1}) (Gambardella et al. 2017). Even prolonged exposure to PS MPs (10 µm; 0.00055–5.54 mg L^{-1}) over 10 days had no significant impact on nauplii survival (100%) of *Artemia parthenogenetica* (Wang et al. 2019).

Adults Survival of adult *A. franciscana* was not affected by 1–5 µm MPs (0.4–1.6 mg L^{-1}) at any tested concentrations after 44 days of exposure (Peixoto et al. 2019). The percentage decreases in survival were lower than 5% at all the concentrations tested, suggesting that brine shrimp are quite resistant to MPs (Figure 1A).

Microplastic-chemical interactions Chemical-coated MPs also did not have any observable impact on brine shrimp larvae. PS MPs (1 and 9.9 µm; 0.1 mg L^{-1}) coated with bisphenol A did not affect survival of *Artemia* sp. after 24 hours of exposure (Katzenberger 2015). *Artemia* sp. nauplii take up and store benzo[a]pyrene (BaP) in yolk droplets when being exposed to BaP-spiked PE MPs (1–5 and 10–20 µm), suggesting that MP could function as a vector for transferring BaP (Batel et al. 2016). However, the study did not evaluate the potential toxicological effects of BaPs on *Artemia* sp. nauplii. Sinche (2010) studied the interaction between PS MP and phenol. The LC_{50} values of adult *Artemia* in the PS MP (3 µm; 100–300 mg L^{-1}) addition group (102.9 mg L^{-1}) were greater than those in the group without MPs (90.90 mg L^{-1}), suggesting that phenol toxicity decreased when MPs were present in the solutions. Sinche (2010) suspected that MP could uptake phenol present in the organism's gut, making the phenol less available to the animal and therefore lowering the toxicity.

Euphausids

MPs of size >10–100 µm do not seem to affect adult euphausid survival, with 100% survival observed in both short-term (24 hours) and long-term (10 days) PE MP-exposure (*Euphausia superba*) at all concentrations tested (27–32 µm; 0.042–1.68 mg L^{-1}) (Dawson et al. 2018a,b) (Figure 1B).

Rotifers

MPs (0.1–10 µm) did not have an observable lethal effect on rotifers (Figure 1A). No significant effect was observed on survival in 0.1 µm PS MP-exposed rotifers (*Brachionus plicatilis*) at any concentrations tested (0.01–10 mg L^{-1}) after 24 and 48 hours of exposure (Gambardella et al. 2018);

all treatments had survival >95%. Similarly, exposure to 4–6 μm PE MP did not have any significant effect on *B. plicatilis*, although 1–4 μm PE MP slightly decreased survival of *B. plicatilis* at 1 mg L^{-1} (LOEC = 1) (Beiras et al. 2018). In contrast, the lifespans of *Brachionus koreanus* exposed to high concentrations of 0.5 μm PS MPs (1, 10 and 20 mg L^{-1}) were shorter by ~1.6 days compared to controls. Population size of *B. koreanus* was largely reduced by ~8%–62% after 12 days of exposure (Jeong et al. 2016). However, lifespan was not included in percentage change analysis.

Microplastic-chemical interactions Benzophenone-3–spiked PE MPs proved to have no toxicity in copepod, mussel and sea urchin larvae (Beiras et al. 2018). Similarly, no significant effect was found on survival in BP-3 coated MP-exposed (0.01–10 mg L^{-1}) rotifers (*B. plicatilis*) at any of the BP-3 concentrations tested (0.2 and 20 μg L^{-1}) (LOEC >10 mg L^{-1}) (Beiras et al. 2018).

Meroplankton

Fishes

Embryos The survival rate of fish embryos appeared to be unaffected by virgin MPs (>10–100 μm) (Figure 1E). The hatching success of zebrafish embryos (*Danio rerio*) was not impacted, even when exposed to high concentrations of PE MPs (10–45 μm; 5 and 20 mg L^{-1}). All the treatments and controls reached nearly 100% hatching success after 5 days of MP exposure (LeMoine et al. 2018).

Larvae MPs had no detrimental effect on fish larvae regardless of the species tested. The percentage decrease in survival was <10% in all three size classes of MPs (Figure 1D–F). Exposure to virgin MPs did not reduce survival in the larval stages of zebrafish (*D. rerio*; PS, 45 μm, 1 mg L^{-1}), Japanese rice fish (*Oryzias latipes*; PE, 4–6 μm, 1–10 mg L^{-1}), fathead minnows (*Pimephales promelas*; PE, 212–500 μm, 0.07–140 mg L^{-1}), sheepshead minnows (*Cyprinodon variegatus*; PE, 150–180 μm, 250 mg L^{-1}) or three-spine stickleback (*Gasterosteus aculeatus*; PS, 1 and 9.9 μm, 5.3–530 mg L^{-1}) (Katzenberger 2015, Chen et al. 2017, Beiras et al. 2018, Choi et al. 2018, Malinich et al. 2018). Irregularly shaped MPs did not affect survival of MP-exposed larvae either. The survival rate of zebrafish *(D. rerio*; low-density polyethylene (LDPE) 0–18 μm, 0.500 mg L^{-1}), silver barb (*Barbodes gonionotus*; PVC, 40–300 μm, 1.0 mg L^{-1}) and sheepshead minnow (*Cyprinodon variegatus*; PE, 6–350 μm, 250 mg L^{-1}) larvae were not impacted by fragmented MPs (Karami et al. 2017, Choi et al. 2018, Romano et al. 2018). Both MP-treated and control groups in these studies reached over 70% survival. These results suggest that virgin MPs rarely have lethal impacts on fish larvae, regardless of the MPs' size, shape, polymer type and concentration used (Figure 1D–F).

One exception is larvae of the European sea bass (*Dicentrarchus labrax*), where the survival rate was 13% lower in the PE MP-treated group (<45 μm; 12 mg per gram of diet) than that of the control (Mazurais et al. 2015). The accumulation of MP debris observed in the gastrointestinal tract of dead larvae might be the reason mortality increased, suggesting that European sea bass might be more vulnerable to MP pollution than other species. The concentration unit used in Mazurais et al. (2015) was mass (mg) per gram of diet and cannot be transformed to the unit used in present study (mg L^{-1}); thus, their results were not included in the percentage change analysis.

Microplastic-chemical interactions As for the interaction between MPs and chemicals, the toxicity is largely dependent on the incorporated chemicals. Exposure to PE MPs (4–6 μm; 10 mg L^{-1}) coated with 0.2 and 20 μg L^{-1} BP-3 decreased embryonic survival to 82% and 42%, respectively (compared to 90% in controls), and reduced the hatching rate by 12% and 52% in Japanese rice fish embryos (*Oryzias melastigma*), respectively (Beiras et al. 2018). The decreased survival can be explained by the toxicity of BP-3 and long exposure time (14 days). In addition, three-spine stickleback larvae (*G. aculeatus*) fed with *Artemia* sp. previously exposed to 9.9 μm

PS MPs and a high concentration of bisphenol A (3200 μg L^{-1}) had a 78% survival rate compared to 100% in controls, whereas the same concentration of BPA had no clear effects (Katzenberger 2015). In contrast, exposure to BaP-coated PE MPs (BaP: 10 mM; MP: 1–5 and 10–20 μm) did not result in any lethal effect in zebrafish embryos (*D. rerio*) (Batel et al. 2018), although there was evidence that BaP moved into the fish tissue. It may be that the BaP transferred to embryos was too low to be lethal. Co-exposure to PS MPs (1 mg L^{-1}) and EE2 (2 and 20 μg L^{-1}) did not affect zebrafish (*D. rerio*) survival (Chen et al. 2017). These results suggest that the combined effect of MP and chemical might be more detrimental than either MPs or chemicals alone, but the toxicity level depends on the chemicals incorporated.

Sea urchins

Gametes Virgin MPs of size 0.1–10 μm decreased sea urchin gamete survival (Figure 1D). Decreases in fertilisation success by 42%–30% were observed in PS MP-exposed (6 μm; 0.12–12 mg L^{-1}) sea urchin gametes (*Paracentrotus lividus*), suggesting that MP exposure interfered with the fertilisation process (Martínez-Gómez et al. 2017).

Larvae Sea urchin embryos develop into free-swimming and ciliated larvae called pluteus larvae, which start to feed 36–48 hours post fertilisation (hpf). In *Tripneustes gratilla* larvae, exposure to virgin 10−45 μm PE MPs at 300 beads mL^{-1} (3.46 mg L^{-1}) decreased survival rate by ~40%, although it was not significant (0.012–1.2 mg L^{-1}) (Kaposi et al. 2014) (Figure 1E). In contrast, in *P. lividus*, the survival in both MP and control treatments generally reached 90% at all concentrations tested after exposure to 0.1 μm PS MPs (0.01–10 mg L^{-1}; 24 hours) (Gambardella et al. 2018). Similarly, no significant difference was found in *P. lividus* survival rates between 10 μm PS MPs (0.125–25 mg L^{-1}) and the control treatments after 72 hours of exposure (Messinetti et al. 2018). Moreover, various sizes of PS MPs (4–6, 11–13, 11–15, <40 μm; 1–100 mg L^{-1}) did not induce severe lethality in *P. lividus* (LOEC ≥100 mg L^{-1}) (Beiras et al. 2018), but only LOEC values were reported, so this study was not included in the percentage change analysis. These studies suggest that the larval stage of *T. gratilla* might be relatively more sensitive to MPs than that of *P. lividus*. Due to the variation among studies, the mean percentage decrease in survival did not exceed 20% at any concentration tested (Figure 1D).

Microplastic-chemical interactions All the toxicity studies reviewed here used the sea urchin *P. lividus* as a model organism, with the majority of the studies finding no clear impacts. PE MPs (4–40 μm, 1 and 10 mg L^{-1}) spiked with the toxic chemical benzophenone-3 did not reduce embryo survival, despite the high concentrations of BP-3 used in the study (LOEC higher than 10 mg L^{-1}, MPs coated with 20 μg L^{-1} BP-3) (Beiras et al. 2018). PS MPs did not increase toxicity of 4-n-nonylphenol (NP), either. The EC_{50} of neither starved nor fed *P. lividus* larvae were significantly affected by the addition of MPs (1 and 10 mg L^{-1}; 67.6–83.7, 158.8–171.1 μg L^{-1}) compared to treatments without MPs (64.3, 190.9 μg L^{-1}) (Beiras & Tato 2019). These studies indicate that the chemical-coated MPs tested had no detrimental lethal impacts on the early stages of sea urchin *P. lividus*.

Bivalves

Gametes MPs of size 0.1–10 μm had limited effects on the gametal stage of bivalves (Figure 1D). The fertilisation rates were all over 90% in both 2 μm and 0.5 μm PS MP (0.1–25 mg L^{-1}) treated oyster gametes (*Crassostrea gigas*), except for animals that were treated with 0.5 µm MPs at 25 mg L^{-1} (~86%) (Tallec et al. 2018).

Larvae Exposure to PS MPs (1–4, 4–6, 6–8.5, 11–13, <40 μm; 20–100 mg L^{-1}) did not affect the survival of the mussel *Mytilus galloprovincialis*. The LOEC of these MP sizes was generally higher than 100 mg L^{-1} (Beiras et al. 2018); however, since they only reported LOEC, this study was not included in the percentage change analysis. Although Cole & Galloway (2015) found no effect of MPs on oyster larvae (*C. gigas*) metamorphosis (1 and 10 μm; 0.001–0.06 mg L^{-1}), but they did not report mortality. A high proportion of *C. gigas* larvae still successfully underwent metamorphosis (over 86%) when exposed to high concentrations of PS MPs (2 μm; 0.1–25 mg L^{-1}) (Tallec et al. 2018) (Figure 1D). However, the lack of a clear effect might be related to short exposure time in this study (24 hours). Overall, early stages of bivalves are quite resistant to MPs of size 0.1–10 μm. The percentage change in survival was lower than 5% for all concentrations tested (Figure 1D). No study was found for >10–100 and >100 μm MPs; thus, no data are included for these size classes.

Microplastic-chemical interactions Combined effects of MPs and chemicals seem to be species specific. Neither carboxylic- (COOH) nor amino- (NH_2) coated PS MPs affected the survival of oyster gametes (*C. gigas*). No significant difference was found in the percentage of dead gametes of *C. gigas* (oocytes and spermatozoa) after 5 hours of exposure to 0.1 μm PS-COOH and PS-NH_2 MPs (0.1–10 mg L^{-1}) (González-Fernández et al. 2018). In the mussel *M. galloprovincialis*, BP-3–spiked PE MPs (4–6, 11–13 μm) did not decrease their larval survival. The LOEC was >10 mg L^{-1} in both low and high BP-3–coated MP treatments (0.2 and 20 μg L^{-1}) (Beiras et al. 2018). In contrast, PS-COOH and PS-NH_2 MPs (0.15–0.2 μm; 0.02–2 mg L^{-1}) significantly decreased embryonic hatching rate and larval metamorphosis rate by 5.79%–39.5% and 4.46%–43.2%, respectively, in the clam *Meretrix meretrix* (Luan et al. 2019).

Transgenerational effect MPs have a clear transgenerational effect on the survival of F_1 oyster larvae (Figure 2A). The survival of D-larvae produced by MP-exposed female oysters (*C. gigas*) (29.6 ± 0.3%) was significantly lower compared to that of the control animals (49.8 ± 1.6%). The decrease in larval quality might be explained by the reduction in sperm and oocyte quality observed in parental generation (Sussarellu et al. 2016).

Gastropods

Larvae Like in bivalves, gastropod larval survival was unaffected by 0.1–10 μm MPs (Figure 1D). Exposure to PS MPs (2–5 μm; 0.0002–3.33 mg L^{-1}) did not increase larval mortality in the slipper limpet, *Crepidula onyx*. The mortality rate was similar between controls (~1 individual day^{-1}) and MP treatments (~1.5 individuals day^{-1}) (Lo & Chan 2018), suggesting that MPs have limited lethal effects on mollusc larvae. There were no studies using >10–100 and >100 μm MPs, so no data were present on these size classes.

Barnacles

Nauplius and cypris larvae Both the naupliar and cypris stages of barnacle larvae were resistant to 0.1–10 μm MPs, with a calculated percentage change in survival lower than 10% at all concentrations tested (Figure 1D). The survival of *Amphibalanus amphitrite* stage II naupliar larvae reached over 90% after exposure to 0.1 μm PS and polymethyl methacrylate (PMMA)MPs for 48 and 24 hours (0.001–10 mg L^{-1}; 5–25 ppm) (Gambardella et al. 2017, Bhargava et al. 2018). Moreover, metamorphosis of *A. amphitrite* cypris larvae appeared to be unaffected by the presence of PMMA MPs (0.18 μm; 1–25 ppm) either (Bhargava et al. 2018), but percentage of metamorphosis was not quantified in this study. Overall, these studies suggest that barnacle larvae are quite resistant to MPs (0.1–10 μm).

Decapods

Larvae Larvae of the grass shrimp *Palaemonetes pugio* tended to be relatively sensitive to PE MPs. Exposure to 38 and 59 μm PE MPs (1–0.01 mg L^{-1}; 0.05–0.0005 mg L^{-1}) decreased survival by ~30%, while 100% survival was observed in the control (Weinstein 2015). The higher sensitivity of grass shrimp larvae resulted in decreased percentage change at 0–1 mg L^{-1} observed in Figure 1E.

Ascidians

Embryos Survival of early developmental stages of ascidians were unaffected by MPs. The survival of 1 and 10 μm PS MP-exposed (0.125–25 mg L^{-1}) ascidian embryos (*Ciona robusta*) reached over 80% in all MP treatments and the control after 18 hours of exposure (from two cells to larval stage) (Messinetti et al. 2018, 2019) (Figure 1D).

Larvae The survival from larval stage to stage four juveniles also did not significantly differ between 1 and 10 μm PS MP treatments (0.125–25 mg L^{-1}) and the control in ascidian larvae (*C. robusta*). Survival was generally higher than 90% in all treatment groups (Messinetti et al. 2018, Messinetti et al. 2019) (Figure 1D).

Comparing the effect of microplastic on survival among zooplankton groups under environmentally relevant and high laboratory concentrations

MPs of size 0.1–10 μm have been the most studied in relation to survival of zooplankton. The mean percentage decrease in survival for all zooplankton groups is <20% upon exposure to 0.1–10 μm MPs at 0–1 mg L^{-1} (environmentally relevant concentration) (Figure 1A,D). Comparing all the zooplankton groups examined, sea urchins and daphnids are more susceptible to mortality, suggesting that these organisms might be the more sensitive to 0.1–10 μm MPs. Especially daphnids suffered over 50% decrease in survival at concentrations >10 mg L^{-1} (Figure 1A). However, these detrimental effects are only observed at very high concentrations (>1 mg L^{-1}). In contrast, the percentage decrease in survival for bivalves, gastropod, barnacles, brine shrimp, euphausid, fishes, rotifers and ascidians is generally <10% at both environmental and laboratory concentrations (Figure 1A,D), suggesting that these groups are relatively tolerant to MPs. Similarly, for MPs of size >10–100 μm, there is a trend that sea urchins and daphnids are more sensitive than others at laboratory concentrations (>1 mg L^{-1}) but not at environmental concentrations (0–1 mg L^{-1}) (Figure 1B,E). Decapod larvae show high sensitivity as well, although this again appears to be species specific (Figure 1E). As for MPs >100 μm, daphnids are the most susceptible group, with decreases in survival observed at both environmental and laboratory concentrations (Figure 1C,F). But the number of studies on this size class is relatively small for zooplankton, presumably given these are on the upper size spectra of what can be consumed by organisms of this size. Overall, MPs did not induce severe mortality to all the zooplankton groups at environmentally relevant concentrations (0–1 mg L^{-1}), suggesting that lethal effects would rarely occur under natural conditions. Of all the groups examined, sea urchins, daphnids and shrimp larvae are the most affected groups in zooplankton, while molluscs and other crustaceans – including copepods, barnacles, brine shrimp and euphausids – show high survival when exposed to MPs regardless of size.

The combined effects of MPs and chemicals could either enhance or decrease toxicity. We observed that interactive effects are complex and depend on both polymer type and the chemicals' properties. Due to the small number of studies, it is difficult to compare which zooplankton groups are more tolerant at this stage. In addition, decreases in survival were observed in the offspring produced by MP-exposed copepods, bivalves and daphnids, suggesting that MPs might have transgenerational effects and potentially affect zooplankton populations in the long term (Figure 2A). This may be the case because of the additives and monomers leached from virgin MPs. Cole et al. (2019) detected several additive chemicals such as stabilisers, lubricants and by-products incorporated in virgin nylon MPs used in MP toxicity studies. Long-term exposure to small quantities of the additives and monomers leached from virgin MPs might cause health impacts such as disrupting endocrine chemicals on exposed zooplankton (Cole et al. 2019). Their study suggests that observed health effects not only stem from the physical properties of MPs but also the chemicals present in the polymer matrix.

Development and growth

Holoplankton

Copepods

MPs of size 0.1–10 μm did not severely delay the development time from nauplii to matured adults in copepods (Figure 3A). Neither the Calanoida nor Harpacticoida studied suffered developmental impacts. Development time of *Tigriopus japonicus* (Harpacticoida) from nauplius to matured adult (~15.2 days) was not significantly different to controls (~15 days) after exposure to a very high concentration (25 mg L^{-1}) of 0.5 and 6 μm PS MPs (Lee et al. 2013). Similarly, a calanoid copepod's (*Paracyclopina nana*) development time (~10.8 days) did not differ from those of controls (~11.8 days) after being exposed to the same size of PS MPs (0.5 and 6 μm; 20 mg L^{-1}) (Jeong et al. 2017). In contrast, Cole et al. (2019) found that juvenile copepod (*Calanus helgolandicus*) exposed to nylon fibres (10 × 30 μm, 0.14 mg L^{-1}) and granules (10–30 μm, 0.24 mg L^{-1}) moulted significantly earlier

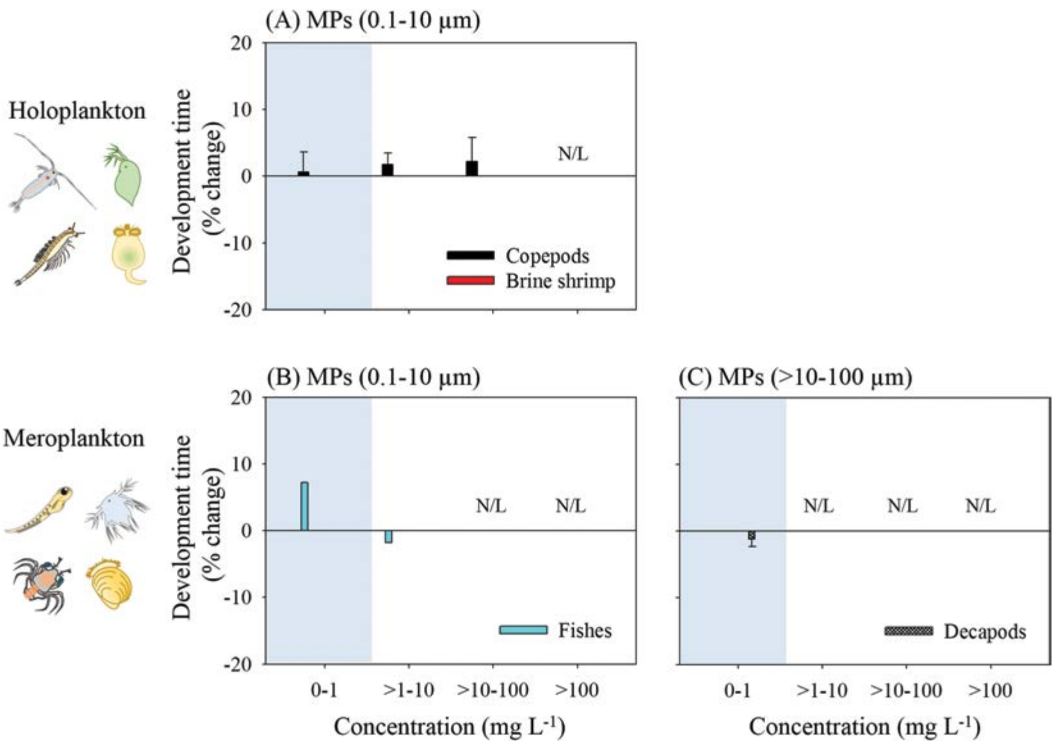

Figure 3 Percentage change in development time (mean + 1SD %) of (A) holoplankton and (B, C) meroplankton in MP treatments when compared to controls. For literature used for all groups of zooplankton, refer to supplementary Table A3. A negative percentage change means a decrease amount of the value in MP treatment compared to that of the control and vice versa. Note: In figure (A), no data are available for daphnids, euphausids and rotifers at all concentrations, except for copepods (>10^2 mg L^{-1}) and brine shrimp (>10 mg L^{-1}). No data are available for holoplankton for MPs of size >10–100 μm and >100 μm. In figure (B), no data are available for urchins, bivalves, gastropods, barnacles, decapods and ascidians at all concentrations, except for fishes (>10 mg L^{-1}). In figure (C), no data are available for fishes, urchins, bivalves, gastropods, barnacles and ascidians at all concentrations, except for decapods (>1 mg L^{-1}). No data are available for meroplankton for MPs >100 μm. Note: light blue background indicates the concentration where environmentally relevant, and white background indicates high laboratory concentration, which does not appear in the environment at the moment. N/L = no data available.

than copepods in the control treatment. The premature moulting might relate to compounds detected in nylon MPs which could cause endocrine disruption (Cole et al. 2019). This study did not, however, mention the exact development time of copepods and was thus was not included in percentage change analysis. For MPs' effects on growth, prosome length of *Calanus finmarchicus* juveniles, adult males and females was not significantly affected after being exposed to nylon granules (10–30 μm, 0.24 mg L^{-1}) and fibres (10 × 30 μm, 0.14 mg L^{-1}) (Cole et al. 2019) (Figure 4B).

Transgenerational effect MPs have transgenerational effects on growth as well (Figure 2B). Cole et al. (2015) found that PS MP-exposed (20 μm; 0.33 mg L^{-1}) *C. helgolandicus* produced significantly smaller eggs than those of the control after four (MP: 180.4 μm; control: 185.1 μm) and six days (MP: 179.5 μm; control: 183.4 μm) of exposure, but the effect was relatively mild, and thus the calculated percentage decrease was low (Figure 2B).

Even though there was no apparent impact observed in the F_0 generation, a significant developmental delay in the F_1 generation was found in 0.5 μm PS MP-treated copepods, although this only occurred at high MP concentrations (Figure 2C). Development time of 25 mg L^{-1} MP-treated copepods was ~17.5 days, compared to only ~14.5 days in controls, suggesting that MPs could affect

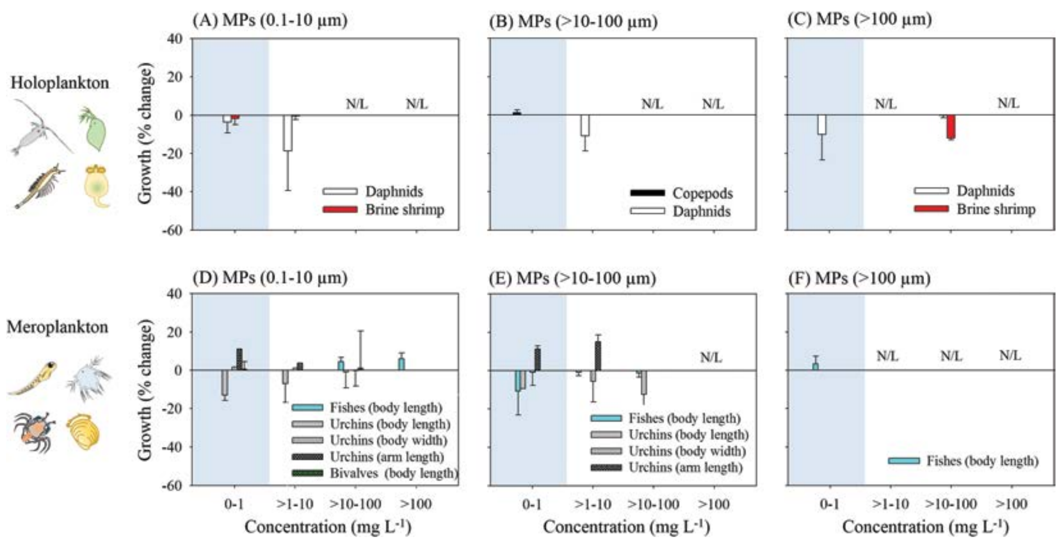

Figure 4 Percentage change in growth (body length, body width, arm length) (mean + 1SD %) of (A–C) holoplankton and (D–F) meroplankton in MP treatments when compared to controls. For literature used for all groups of zooplankton, refer to supplementary Table A4. A negative percentage change means a decreased amount of the value in MP treatment compared to that of the control and vice versa. Note: In figure (A), no data are available for copepods, rotifers and euphausids at all concentrations, except for daphnids (>10 mg L^{-1}) and brine shrimp (>10 mg L^{-1}). In figure (B), no data are available for brine shrimp, euphausids and rotifers at all concentrations, except for daphnids (0–1, >10 mg L^{-1}) and copepods (>1 mg L^{-1}). In figure (C), no data are available for copepods, euphausids and rotifers at all concentrations, except for daphnids (>1–10, >10^2 mg L^{-1}) and brine shrimp (0–10, >10^2 mg L^{-1}). In figure (D), no data are available for fishes (0–10 mg L^{-1}), urchins (body length, body width and arm length) (>10^2 mg L^{-1}), bivalves (>1 mg L^{-1}) and gastropods, barnacles, decapods and ascidians at all concentrations. In figure (E), no data are available for fishes (>10^2 mg L^{-1}), urchins (body length) (>1–10, >10^2 mg L^{-1}), urchins (body width and arm length) (>10 mg L^{-1}) and bivalves, gastropods, barnacles, decapods and ascidians at all concentrations. In figure (F), no data are available for urchins (body length, body width and arm length), bivalves, gastropods, barnacles, decapods and ascidians at all concentrations, except for fishes (>1 mg L^{-1}). Note: light blue background indicates the concentration where environmentally relevant, and white background indicates high laboratory concentration, which does not appear in the environment at the moment. N/L = no data available.

naupliar development time across generations (Lee et al. 2013). However, no clear transgenerational effect was observed in copepods exposed to 6 μm PS MPs in the same study (Lee et al. 2013). Due to the variation between sizes, the mean percentage increase was lower than 10% (Figure 2C). This result highlights that the adverse impacts of MP exposure could extend to the offspring of MP-exposed parents and could potentially last for several generations.

Daphnids

Most of the studies showed that MPs of size 0.1–100 μm had no significant impact on *Daphnia magna* body length. For instance, polymers including PE (1–10 μm), PS (1–5 μm; 0.0046–4.6 mg L^{-1}; 19.8 μm, 2.1–9.2 mg L^{-1}), PLA (1–4 μm), unknown type of MPs (1–5 μm, 0.2–2.0 mg L^{-1}; 12.86 mg L^{-1}; 0.001–1 mg L^{-1}) and plastic mixture (PA + PC + PET + PVC, ABS + fPVC + POM + SAN) did not affect body length of *D. magna* and *D. pulex* (Imhof et al. 2017, Puranen Vasilakis 2017, Aljaibachi & Callaghan 2018, Pacheco et al. 2018, Martins & Guilhermino 2018, Bosker et al. 2019, Colomer et al. 2019, Gerdes et al. 2019, Jaikumar et al. 2019) (Figure 4A,B). Similarly, large-sized fragmented (PE, 102.9–264 μm, 100 mg L^{-1}) and fibre MPs (PET, 60–1400 μm, 12.5–100 mg L^{-1}) had no clear effect on *D. magna* body length (Jemec et al. 2016, Kokalj et al. 2018) (Figure 4C). Body length in these studies was generally reduced by less than 10% compared to the controls, suggesting that none of the three size classes of MPs have a severe impact on *D. magna* body length. However, *Ceriodaphnia dubia* suffered from growth retardation by ~11%–33% after exposure to unknown (1–5 μm; 1 mg L^{-1}), PE MPs (1−4 μm; 0.06–2 mg L^{-1}) and PET fibres (100−400 μm; 0.03–1 mg L^{-1}) (Ziajahromi et al. 2017, Jaikumar et al. 2019). Apart from reduced growth, several abnormalities such as deformed carapaces and abnormal-shape seta were also observed in *C. dubia* (Ziajahromi et al. 2017). The higher sensitivity of *C. dubia* largely contributed to the percentage decreases in body length observed in our analyses (Figure 4A,C). In contrast, body weight appears to be relatively sensitive to MPs (Figure 5A). Studies by Ogonowski et al. (2016) and Tang et al. (2019) found that *D. magna* exposed to unknown type (1–5 μm; 0.0018–1.8 mg L^{-1}) and PS MPs (1.25 μm; 4–8 mg L^{-1}) suffered from growth retardation by ~4%–44% compared to controls. The low percentage change in body weight at >10–100 mg L^{-1} (Figure 5A) is predominantly due to the small number of studies at high concentrations.

As for development time, exposure to both 0.1 and 2 μm PS MPs (0.1–1 mg L^{-1}) did not affect the number of moults (eight) compared to the control (eight) (Rist et al. 2017), suggesting that the development time of *D. magna* was not impacted by MPs. Since the exact development time was not evaluated in this study (Rist et al. 2017), their data were not included in percentage change analysis.

Microplastic-chemical interactions Adding MPs (1–5 μm) to PCB-contaminated *D. magna* (MP + PCB: 0.31 mg; PCB: 0.305 mg) did not significantly affect the organism's dry weight (Gerdes et al. 2019). However, the toxicity level tested in these studies might not have been high enough to induce observable growth effects.

Transgenerational effect MPs have relatively mild transgenerational effects on the growth of the daphnid F_1 generation (Figure 2B). No significant impact on the body length of *D. magna* offspring was observed after exposure to spherical (unknown type, 1–5 μm; PE, 1–4 μm) and irregular-shaped MPs (unknown type, 2.6 ± 1.8 μm; PET, 100–400 μm) (Ogonowski et al. 2016, Ziajahromi et al. 2017). In contrast, Martins & Guilhermino (2018) found that the F_1 generation of *D. magna* suffered from reduced body length by ~7% and even the F_2 and F_3 were still 4% less than the control. Imhof et al. (2017) also found some subtle effects such as reduced body width and increased tail spine length in offspring produced by MP-exposed adults (*D. magna*). These effects are relatively subtle, however, with a mean percentage decrease of less than 5% (Figure 2B). Changes in body size and alterations in tail length of offspring are common anti-predation responses in daphnids. Such defence often occurred when predators were present but was expressed after exposure to MPs (Imhof et al.

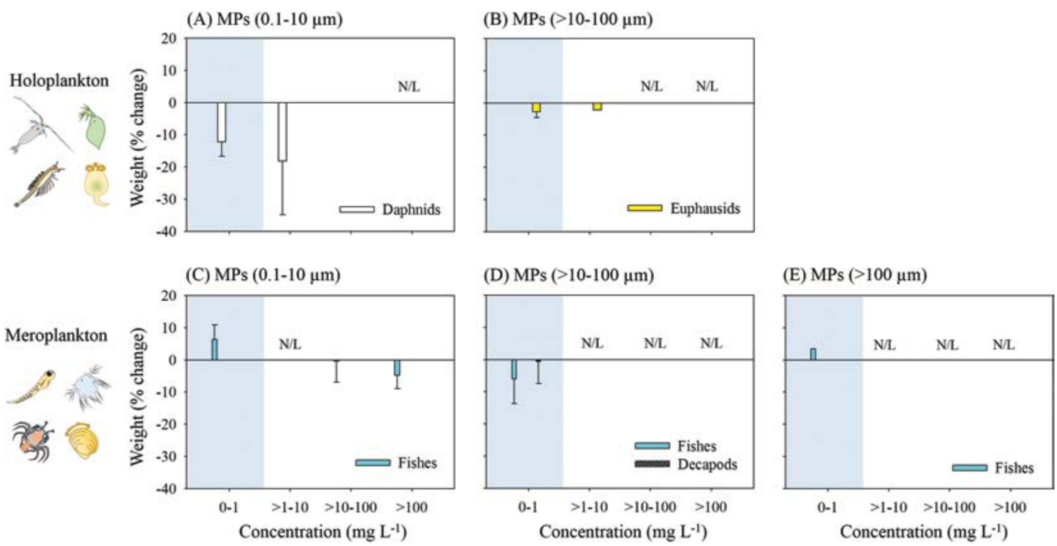

Figure 5 Percentage change in growth (body weight) (mean + 1SD %) of (A, B) holoplankton and (C–E) meroplankton in MP treatments when compared to controls. For literature used for all groups of zooplankton, refer to supplementary Table A5. A negative percentage change means a decreased amount of the value in MP treatment compared to that of the control and vice versa. Note: In figure (A), no data are available for copepods, brine shrimp, euphausids and rotifers at all concentrations, except for daphnids (>10^2 mg L^{-1}). In figure (B), no data are available for copepods, daphnids, brine shrimp and rotifers at all concentrations, except for euphausids (>10 mg L^{-1}). No data are available for holoplankton for >10–100 μm MPs. In figure (C), no data are available for urchins, bivalves, gastropods, barnacles, decapods and ascidians at all concentrations, except for fishes (>1–10 mg L^{-1}). In figure (D), no data are available for urchins, bivalves, gastropods, barnacles and ascidians at all concentrations, except for fishes (>1 mg L^{-1}) and decapods (>1 mg L^{-1}). In figure (E), no data are available for urchins, bivalves, gastropods, barnacles, decapods and ascidians at all concentrations, except for fishes (>1 mg L^{-1}). Note: light blue background indicates the concentration where environmentally relevant, and white background indicates high laboratory concentration, which does not appear in the environment at the moment. N/L = no data available.

2017). This suggests that MPs might have some signals resembling those of predators and thus induce anti-predation responses in daphnids. Nevertheless, it is also possible that these subtle effects are just a natural variation, so further investigation is needed.

Brine shrimp

Larvae Brine shrimp growth was not severely affected by 0.1–10 μm or >100 μm MPs (Figure 4A, C). *Artemia parthenogenetica* body length was not significantly different from the control group after exposure to 10 μm PS MPs (0.00055–5.5 mg L^{-1}) (Wang et al. 2019) (Figure 4A). On the other hand, a small reduction in body length (~12%) was observed in naupliar larvae of *Artemia franciscana* after exposure to MPs >100 μm (PE and PET; 22.8–264 μm; 100 mg L^{-1}) (Kokalj et al. 2018) (Figure 4C). The reduction in growth might relate to the adhesion of MPs on the carapace of naupliar larvae rather than direct ingestion (Kokalj et al. 2018). The development time of brine shrimp larvae was also not impacted by 0.1–10 μm MPs (Figure 3A). The instar development time of PS MPs (10 μm; 0.00055–5.5 mg L^{-1}) treated *A. parthenogenetica* (10 days) did not significantly differ from that of the control (10 days) (Wang et al. 2019).

Adults Similarly, body length of adult *A. franciscana* was not significantly affected by 1–5 μm MPs (0.4–1.6 mg L^{-1}) after 26 days of exposure (Peixoto et al. 2019) (Figure 4A).

Euphausids

Euphausids (*Euphausia superba*) did not suffer from growth retardation after 10 days of exposure to 27–32 μm MPs (PE; 0.2–1.6 mg L^{-1}). Weight loss was less than 10% for all MP treatments and controls (Dawson et al. 2018a), suggesting that PE MPs have no clear effect on *E. superba*'s growth rate (Figure 5B).

Meroplankton

Fishes

Embryos The development time of Japanese rice fish *Oryzias melastigma* embryos was not affected by 4–6 μm PE MPs (1 and 10 mg L^{-1}). No significant difference was observed in mean hatching time between MP treatments (~11–12 days) and the control (~11 days) (Beiras et al. 2018) (Figure 3B).

Larvae Neither body length nor weight of most of the studied fish species were affected by MPs, regardless of the MP's size, polymer type or concentration tested (Figures 4 & 5). Exposure to virgin MPs did not alter growth rate (both body length and weight) in the larvae of zebrafish (*Danio rerio*; PE, <17.6 μm, 0.005–0.5 mg L^{-1}; PS, 45 μm, 1 mg L^{-1}; PE, 10–45 μm, 5 and 20 mg L^{-1}), fathead minnows (*Pimephales promelas*; PE, 180–212 μm, 0.14 and 0.27 mg L^{-1}) and three-spine stickleback (*Gasterosteus aculeatus*; PS, 1 and 9.9 μm, 10.6–1060 mg L^{-1}) (Katzenberger 2015, Chen et al. 2017, Karami et al. 2017, LeMoine et al. 2018, Malinich et al. 2018). The percentage change was generally <10% in these studies, suggesting that MPs do not substantially affect fish larval growth (Figures 4 & 5). As for normal development, all of the sheepshead minnow larvae (*Cyprinodon variegatus*) exposed to 6–350 μm MPs (PE, 50 and 250 mg L^{-1}) still exhibited normal morphology to the control, suggesting that MPs >100 μm did not affect sheepshead minnow larvae development (Choi et al. 2018).

Microplastic-chemical interactions Some studies have reported growth alterations in fish larvae, while others found no significant impacts when MPs and chemicals were co-exposed. For instance, exposure to field-collected HDPE, LDPE and PS MPs (>2 mm; 1 and 10 mg L^{-1}) led to significant increases in pericardial sack size in zebrafish larvae (*D. rerio*) by around 4–6 μm^2 compared to the control, which might be explained by the toxic chemicals associated with the MPs (Ravit et al. 2017). The length of zebrafish larvae exposed to EE2 (17 α-ethynylestradiol) spiked PS MPs (45 μm, 1 mg L^{-1}) shortened by 4.7% and 6.1% after 120 hours of exposure (Chen et al. 2017). The retarded growth was probably related to the synergistic effects of EE2 and MPs. In contrast, ingestion of food (*Artemia* sp.) previously exposed to BPA-spiked MPs (0.5 and 9.9 μm) did not significantly affect length and weight of stickleback larvae (*G. aculeatus*) (Katzenberger 2015). We suggest that the reason there was no effect of growth might be because BPA was not in direct contact with exposed fish larvae but was instead incorporated into the food (*Artemia* sp.) and thus needed to be digested before the BPA was released. As for development time, exposure to BP-3 (20 μg L^{-1}) spiked PE MPs (10 mg L^{-1}) significantly reduced hatching time in *O. melastigma* embryos (Beiras et al. 2018). Moreover, exposure to BaP-loaded PE MPs (1–5 and 10–20 μm; 1 and 4 mg L^{-1}) did not induce any abnormality in zebrafish (*D. rerio*) embryos, despite a prominent BaP signal detected in the embryos (Batel et al. 2018). Similarly, co-exposure to EE2 and PS MPs (45 μm, 1 mg L^{-1}) did not affect the development of zebrafish (Chen et al. 2017). The level of chemicals transferred to fish larvae in these studies might have been too low to induce observable impacts.

Sea urchins

Embryos Both MPs of size 0.1–10 and >10–100 μm induced malformations in sea urchin embryos such as undeveloped and collapsed embryos or abnormal proliferation of the ectodermal membrane

(Figure 6A,B). The percentage of abnormal embryos significantly increased by 8%–15% after exposure to virgin HDPE (0.1–80 μm; 5–5000 mg L^{-1}) and PS MPs (6 μm, 0.12–12 mg L^{-1}) in *Paracentrotus lividus* (Martínez-Gómez et al. 2017).

Larvae MPs (0.1–100 μm) induce several growth alterations in sea urchin larvae, including reduced body length and width and increased arm length (Figure 4D,E). Both 0.1–10 μm PS MPs (10 and 6 μm) and >10–100 μm HDPE MPs (0.1–80 μm) decreased body length by 2%–15% compared to the control in *P. lividus* larvae (Martínez-Gómez et al. 2017, Messinetti et al. 2018). In contrast, arm length significantly increased by 4%–18% upon exposure to 10 μm (PS, 1.25–25 mg L^{-1}) and 10−45 μm MPs (PE, 0.01–3.46 mg L^{-1}) in *P. lividus* and *Tripneustes gratilla* (Kaposi et al. 2014, Messinetti et al. 2018) (note the positive value in Figure 4D,E indicates increased growth). MPs' effects on body width appear to be relatively mild – the percentage changes were generally lower than 10% at all concentrations tested (Figure 4D,E). Exposure to 10 μm PS MPs (1.25–25 mg L^{-1}) and 10−45 μm PE MPs (0.01–3.46 mg L^{-1}) did not affect body width in *P. lividus* or *T. gratilla*, although body width was significantly reduced by ~13% at 300 beads mL^{-1} (Kaposi et al. 2014, Messinetti et al. 2018). Moreover, the larval volume of *P. lividus* decreased by 8%–30% after exposure to PE MPs (5.5 μm; 1 and 10 mg L^{-1}) (Beiras & Tato 2019), but larval volume was not included in the percentage change analysis. Growth may have been altered because MPs limited the amount of food in the environment. Many sea urchin species exhibited phenotypic plasticity, such as increased ciliary band and post-oral arm lengths, to enhance particle capture efficiency under food-limited conditions (Soars et al. 2009). However, there is currently no direct evidence to suggest that MPs affect the feeding capacity of pluteus larvae. Thus, we suggest that future studies evaluate the effects of MPs on filter feeding in urchin larvae to elucidate its underlying mechanisms.

Microplastic-chemical interactions Growth alterations were also observed when MPs and chemicals are co-exposed to sea urchin larvae. Larval volume often changed when *P. lividus* larvae were exposed to 4-n-nonylphenol and PS MPs (0.1 μm; 1 and 10 mg L^{-1}) (Beiras & Tato 2019). Leachates of virgin PS (6 μm; 0.12–12 mg L^{-1}) and HDPE MPs (0.1–80 μm; 5–5000 mg L^{-1})

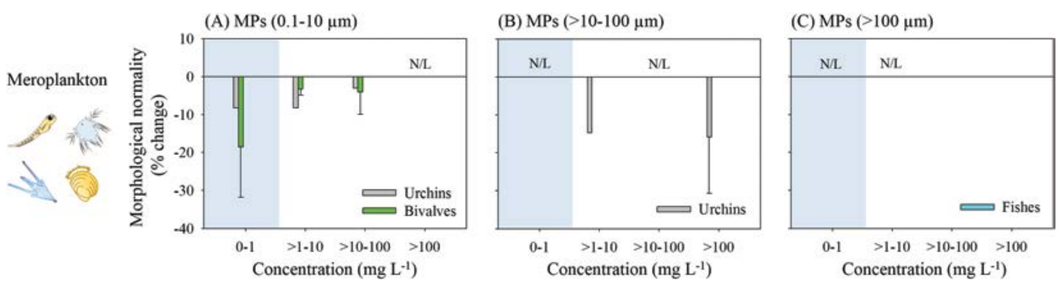

Figure 6 Percentage change in morphological normality (mean + 1SD %) of (a–c) meroplankton in MP treatments when compared to controls. For literature used for all groups of zooplankton, refer to supplementary Table A6. A negative percentage change means a decreased amount of the value in MP treatment compared to that of the control and vice versa. Note: In figure (A), no data are available for fishes, gastropods, barnacles, decapods and ascidians at all concentrations, except for urchins (>10^2 mg L^{-1}) and bivalves (>10^2 mg L^{-1}). In figure (B), no data are available for fishes, bivalves, gastropods, barnacles, decapods and ascidians at all concentrations, except for urchins (0–1, >10–10^2 mg L^{-1}). In figure (C), no data are available for urchins, bivalves, gastropods, barnacles, decapods and ascidians at all concentrations, except for fishes (0–10 mg L^{-1}). No data are available for all groups of holoplankton. Note: light blue background indicates the concentration where environmentally relevant, and white background indicates high laboratory concentration, which does not appear at the environment at present. N/L = no data available.

reduced *P. lividus* body length by ~6%–73%, although the effects were not concentration dependent (Martínez-Gómez et al. 2017). There was, however, a trend towards the toxicity of leachate increasing as MP size decreased (Beiras et al. 2019).

Compared to the relatively slight impacts of virgin MPs, exposure to leachates derived from MPs had relatively large detrimental effects on development of sea urchin embryos. Leachates from both PS and HDPE MPs (6 μm, 0.12–12 mg L^{-1}; 80 μm, 5–5000 mg L^{-1}) significantly decreased the percentage of normal larvae by 8% to 92% in *P. lividus* (Martínez-Gómez et al. 2017). In *Lytechinus variegatus* embryos, the proportion of normal larvae in treatments exposed to leachates of virgin PE MPs (58.1%) and beach-collected pellets (34.6%) were significantly lower than controls (88%) (Nobre et al. 2015). The toxicity of virgin MPs could be explained by plastic additives applied when the MPs are manufactured (Cole et al. 2019). On the other hand, the toxicity of field-collected pellets is based on chemicals adsorbed in the environment and thus largely depends on the chemicals present at the collection site. In a heavily contaminated collection site, beach-collected MPs might be more toxic than virgin MPs.

Bivalves

Larvae MPs (0.1–10 μm) affect the normal development of the bivalve embryos tested, although there was variation (Figure 6A). Neither mussel (*Mytilus galloprovincialis*) nor oyster (*Crassostrea gigas*) embryos exposed to PS (0.5 and 2 mg L^{-1}, 0.1–25 mg L^{-1}; 3 μm, 0.00075–0.15 mg L^{-1}) and PE MPs (4–6 μm; 20–100 mg L^{-1}) decreased the percentage of normal larvae in embryo-larval development (Beiras et al. 2018, Capolupo et al. 2018, Tallec et al. 2018). The proportion of normal larvae in both MP and control groups generally reached over 80% in these studies. In contrast, PS MPs (0.1 and 2 μm; 0.03–0.3 mg L^{-1}) significantly increased the malformation rate of blue mussel larvae (*Mytilus edulis*) by 27%–42% after 11–15 days of exposure (Rist et al. 2019), which might be because of the longer exposure time used in the study. As for growth rate, a study by Cole & Galloway (2015) found no clear effect on oyster larvae (*C. gigas*) exposed to PS MP (1 and 10 μm; 100 beads mL^{-1}). Likewise, exposure to 2 and 0.1 μm PS MPs did not affect growth rate of blue mussel larvae (*M. edulis*) at any concentrations tested (0.0004–0.28 mg L^{-1}) (Rist et al. 2019) (Figure 4D). The lack of influence on growth rate could be explained by the conclusion that MP exposure at these concentrations had no effect on filter feeding of oyster larvae, and thus their growth was not impacted (see 'Feeding rate' in the present review).

Microplastic-chemical interactions Leachate derived from MPs had high toxicity and severely impaired mussel embryo development. The proportion of normal embryos was significantly lower when mussel embryos (*Perna perna*) were exposed to leachate either from beached (0%) or virgin PP MPs (76.5%) compared to the control (90%) (e Silva et al. 2016). Leachate toxicity could derive from chemicals adsorbed onto beached pellets and monomers released from virgin MPs. Similarly, PS-COOH and PS-NH_2 MPs (0.15–0.2 μm; 0.02–2 mg L^{-1}) significantly increased larval malformation rate and decreased developmental rate and growth rate by 220%–449%, 4.78%–7.86% and 0.65%–4.34% in clam *Meretrix meretrix,* respectively (Luan et al. 2019). These studies suggest that early development of bivalve larvae are sensitive to combined effects of MPs and chemicals.

Transgenerational effect A transgenerational effect of MPs on growth was also observed in offspring produced by MP-exposed bivalves (Figure 2B). The offspring larvae produced by PS MP-exposed (2 and 6 μm; 0.023 mg L^{-1}) oysters (*C. gigas*) suffered from an 18.6% growth reduction (shell length) compared to the control oysters (Sussarellu et al. 2016). This growth retardation could be explained by the reduced quality of gametes observed in MP-exposed adults (Sussarellu et al. 2016). This again highlights that MP exposure could have transgenerational impacts and negatively influence the fitness of their offspring.

Gastropods

Larvae Limpet larvae (*Crepidula onyx*) exposed to 2–5 μm PS MPs at concentrations higher than 1.43 mg L^{-1} grew significantly slower [0.12∼ 0.13 mm log ($lday^{-1}$)] than the control [0.16 ± 0.016 mm log ($lday^{-1}$)]. Even though the animals were no longer fed with MPs after the adult stage, the growth rate of juveniles exposed to MPs [∼18 and 17.5 mm log ($lday^{-1}$)] during the larval stage still could not catch up with the control group [20.8 mm log ($lday^{-1}$)] (Lo & Chan 2018). Since their algae consumption did not decrease upon MP exposure (See 'Feeding rate' in the present review), the reduced growth rate could be related to the energy depletion induced by MP ingestion and the toxic chemicals leached from polymers. Because they only reported growth rate, their data were not included in the percentage change analysis.

Decapods

Larvae The weight of grass shrimp larvae (*Palaemonetes pugio*) was not affected by medium-sized PE MPs (38 and 59 μm) (Weinstein 2015) (Figure 5D). The percentage change in weight was lower than 10%. Similarly, larval development time of grass shrimp larvae was not affected by PE MPs (38 and 59 μm), except for those exposed to 38 μm MPs at 1.0 mg L^{-1}, which had a significantly faster development time (20.2 days) than control shrimp (20.8 days) (Weinstein 2015) (Figure 3C).

Ascidians

Embryos Exposure to MPs did not affect the normal development of ascidian embryos (*Ciona robusta*). PS MP-exposed embryos (1 and 10 μm; 0.125–25 mg L^{-1}) still showed the same phenotype as those in the controls (Messinetti et al. 2018, 2019). This study did not, however, quantify the effect of MPs, so no data were included here.

Larvae MPs (0.1–10 μm) severely delay the development time of ascidian larvae. The percentage of ascidian larvae that successfully metamorphosed to stage 4 juvenile was significantly reduced by 30%–40% after 4 days of 1 and 10 μm PS MPs exposure (0.125–25 mg L^{-1}). Moreover, the percentage of stage 3 larvae was higher in the 12.5 and 25 mg L^{-1} treatment groups (∼23%–45%) than the control (∼5%–12%) (Messinetti et al. 2018, 2019). The delayed juvenile development was probably due to the lower amount of food intake caused by MP-induced false satiation. These studies indicate that the development of ascidian larvae is quite sensitive to small-sized MPs (0.1–10 μm). But they did not evaluate the exact development time, so their data were not included in the percentage change analysis.

Comparing the effect of microplastic on growth and development among zooplankton groups under environmentally relevant and high laboratory concentrations

All three sizes of virgin MPs induce growth alterations in most of the zooplankton species examined by either reducing or increasing growth, although no clear concentration trend was observed. However, the percentage change is generally lower than 20% at both environmental (0–1 mg L^{-1}) and high laboratory concentrations (>1 mg L^{-1}) (Figures 4 & 5). Among all the zooplankton groups examined, bivalve larvae and crustaceans, including euphausids, brine shrimp and decapod larvae, appeared to be the most resistant to MPs. In general, the percentage change did not exceed 5% at any of the concentrations tested upon exposure to 0.1–10 μm and >10–100 μm MPs (Figures 4 & 5). Similarly, development (development time and percentage of normal larvae) of most of the zooplankton groups tested is not severely affected by virgin MPs (Figures 3 & 6), except for sea urchins and bivalves, which seemed to be sensitive to the smaller size class of MPs (0.1–10 μm) (Figure 6a). No clear trend can be observed for MPs of size >10–100 μm and >100 μm, predominantly due to the small number of studies (Figures 3C & 6B). Overall, the mean percentage change in growth and development for all the zooplankton groups examined is lower than 20% either at environmental or laboratory concentrations (Figures 3–5). These results suggest that the alterations in growth and development caused by MPs are relatively minor and would not induce detrimental impacts at natural concentrations.

As for the interaction between MPs and chemicals, exposure to leachates derived from MPs reduced the percentage of normal larvae in sea urchins and bivalves. This might be explained by the life stage of the organisms studied. Early developmental stages such as gamete and embryo were used as models in these studies. Thus, the high sensitivity of early stages might contribute to the high percentage decrease observed (Fernández & Beiras 2001), but the underlying mechanisms still needs further investigation. On the other hand, both growth and development of copepods, daphnids and larvae of fishes, sea urchins and molluscs, are not severely affected by co-exposure to chemicals and MPs, but the toxicity depends on properties of chemicals and MPs. In addition, MPs might reduce growth and delay development of the offspring produced by MP-exposed bivalves, copepods and daphnids (Figure 2B,C). But the transgenerational effects are still poorly studied and further investigation are certainly needed to draw a comprehensive conclusion.

Feeding rate

Holoplankton

Copepods

Juveniles and adults MPs of size 0.1–10 μm have detrimental impacts on the feeding rate of copepods. There was a clear trend between increased concentration and decreased feeding rate; the percentage decrease reached over 75% at >1–10 mg L^{-1} for smaller-sized MPs (0.1–10 μm) (Figure 7A). The effects of MPs on feeding rate were mainly studied in calanoid copepods – including *Centropages typicus*, *Calanus helgolandicus, Calanus finmarchicus* and *Acartia tonsa* – all of which showed reduced feeding rates after being exposed to MPs. In *C. typicus*, exposure to natural assemblages of algae and 7.3 μm PS MPs (0.86–5.39 mg L^{-1}) for 24 hours significantly reduced algal consumption by 45%–88% compared to copepods that did not eat MPs (Cole et al. 2013). In *C. finmarchicus*, the average algae removal decreased by 32% and 27% after being exposed to 10 μm PS MPs for 24 and 48 hours, respectively, although these results were not significant (Halland 2017). MPs of size >10–100 μm impaired copepod feeding rate as well (Figure 7B). *C. helgolandicus*'s filter feeding rate decreased by 11% after exposure to 20.0 μm PS MPs (0.33 mg L^{-1}) for 6 days (Cole et al. 2015). Carbon uptake decreased by 54 and 43.5%, respectively, in *A. tonsa* and *C. helgolandicus* exposed to a mixture of 10 and 20 μm PS MPs (0.25 mg L^{-1}) (Dedman 2014). Exposure to nylon fibres (10 × 30 μm, 0.14 mg L^{-1}; 10 × 40 μm, 0.36 mg L^{-1}) caused an overall decrease in total algal ingestion rates and clearance rates in *C. finmarchicus* and *C. helgolandicus*. Exposure to nylon fragments (20 μm, 0.48 mg L^{-1}) significantly decreased the ingestion of algae that had similar size and shape to the fragments in *C. helgolandicus*, although it did not significantly alter the total algal consumption (Coppock et al. 2019).

The impaired feeding rate could be explained by prey selection widely reported in calanoid copepods (Frost 1972, Irigoien et al. 2000, Dedman 2014). Chemoreceptors on the mouthparts of copepods can sense particles and actively capture or reject them (Friedman & Strickler 1975). Previous studies have documented that calanoid copepods shift their preference to avoid ingestion of algae that have similar size to MPs. For example, Cole et al. (2015) found that copepods selectively fed on smaller-sized algal prey (11.6–14.8 μm) to avoid ingesting larger 20 μm MPs, thus decreasing their filtering rate. Cole et al. (2019) and Coppock et al. (2019) observed that copepods avoided food of a similar size or shape to the microfibres. This mechanism might avoid directly ingesting non-nutritious MPs, but at the same time, it impairs their algae consumption rate, reducing the carbon biomass acquired and causing energy depletion. Moreover, the Calanoida are an important food source for many marine organisms. Therefore, energy depletion in copepods might adversely impact the energy transfer from lower to higher trophic levels.

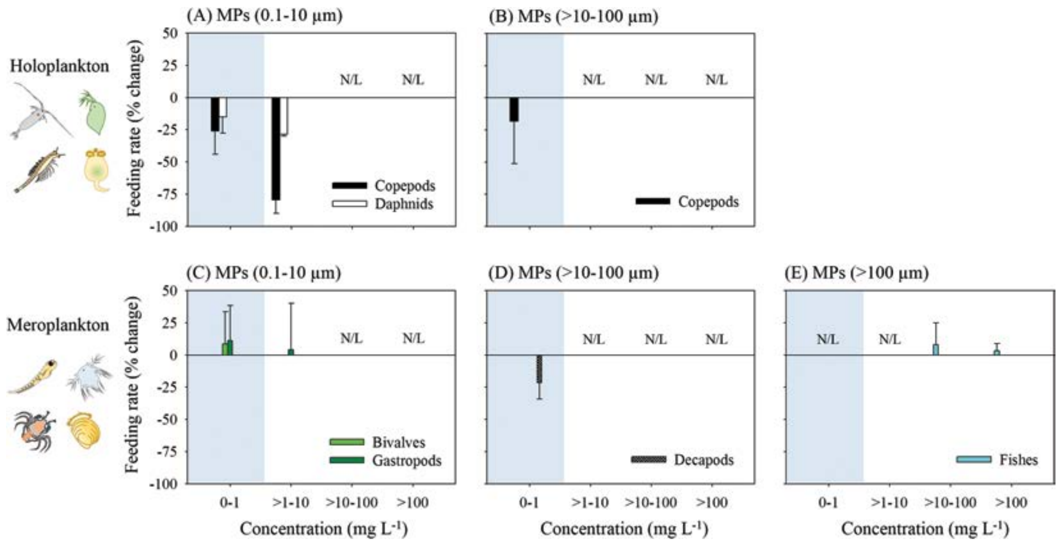

Figure 7 Percentage change in feeding rate (mean + 1SD %) of (a, b) holoplankton and (c–e) meroplankton in MP treatments when compared to controls. For literature used for all groups of zooplankton, refer to supplementary Table A7. A negative percentage change means a decreased amount of the value in MP treatment compared to that of the control and vice versa. Note: In figure (A), no data are available for brine shrimp, euphausids and rotifers at all concentrations, except for copepods (>10 mg L^{-1}) and daphnids (>10 mg L^{-1}). In figure (B), no data are available for daphnids, brine shrimp, euphausids and rotifers at all concentrations, except for copepod (>1 mg L^{-1}). No data are available for holoplankton for MPs >100 μm. In figure (C), no data are available for fishes, urchins, gastropods, barnacles, decapods and ascidians at all concentrations, except for bivalves (>1 mg L^{-1}) and gastropods (>10 mg L^{-1}). In figure (D), no data are available for fishes, urchins, gastropods, barnacles and ascidians, except for decapods (>1 mg L^{-1}). In figure (E), no data are available for urchins, bivalves, gastropods, barnacles, decapods and ascidians at all concentrations, except for fishes (0–10 mg L^{-1}). Note: light blue background indicates the concentration where environmentally relevant, and white background indicates high laboratory concentration, which does not appear at the environment at present. N/L = no data available.

In contrast, exposure to 20 μm PS MPs (0.33 mg L^{-1}) had no significant effect on algae consumption in the cyclopoid copepod *Oithona similis* (Dedman 2014). The carbon uptake of MP-exposed animals (1.72 μg C cop^{-1} d^{-1}) did not significantly differ from controls (1.1 μg C cop^{-1} d^{-1}). This is probably because *O. similis* possesses a different feeding mode from calanoid copepods. *O. similis* is an ambush feeder that relies on detecting disturbance in the water column to capture motile prey such as ciliates. The species is unlikely to detect non-motile particles such as MPs, and thus no significant impacts on total ingestion rate and carbon biomass uptake can be observed (Dedman 2014). This suggests that cyclopoid copepods might be more tolerant to MP pollution than calanoid copepods.

Daphnids

MPs (0.1–10 μm) reduce the feeding rate of daphnids in a concentration-dependent manner (Figure 7A), although there is variation among studies. Both adverse impact and no clear effect have been documented in daphnids. Reduction in total algae consumption by 29% and 28% were observed in both spherical (1–5 μm; 4.13 mg L^{-1}) and irregular-shaped (2.6 ± 1.8 μm; 2.69 mg L^{-1}) MP-exposed *Daphnia magna*, respectively (Ogonowski et al. 2016). *D. magna*'s feeding rate also decreased by 30% and 21% after exposure to 1–5 μm (0.65 mg L^{-1}) and 0.1 μm (1 mg L^{-1}) PS MPs, respectively (Puranen Vasilakis 2017, Rist et al. 2017). In contrast, no significant effect was found on the feeding rate in PLA (1–4 μm; 0.93 mg L^{-1}) and PS MPs (2 μm; 1 mg L^{-1}) exposed *D. magna* (Puranen Vasilakis 2017, Rist et al. 2017). The percentage decreases in these studies were lower than 10%. Since the size and concentration of MPs used in these studies were similar, the underlying mechanism is still unclear.

Transgenerational effect No significant effect on the filter feeding rate was observed in the F_1 offspring produced by both spherical (1–5 μm) and irregular-shaped MP (2.6 ± 1.8 μm) exposed animals (*D. magna*) (Ogonowski et al. 2016), although the feeding rate was reduced by 29% and 28% in the F_0 generation, respectively. This result suggests that MP has no adverse transgenerational effect on feeding rate, but the raw data were not reported in this study, and thus the percentage change cannot be calculated.

Meroplankton

Fishes

Larvae The percentage change in the feeding rate of fish larvae was generally lower than 10% upon exposure to MPs >100 μm at any concentrations tested (Figure 7E). The presence of PE MPs (mixture of 425–500 μm and 180–212 μm) did not affect the number of *Artemia* nauplii consumed by fathead minnow larvae (*Pimephales promelas*). No significant difference was found between MP treatments (7.4–9.21) and the control (6.9–8.69) (Malinich et al. 2018). A possible explanation is that the larvae were able to distinguish between MPs and *Artemia* nauplii and actively avoid ingesting MPs during feeding. Similarly, foraging activity (number of bites) of the surgeon fish *Acanthurus triostegus* was not significantly affected after exposure to PS MPs (90 μm; 2.02 mg L^{-1}) (Jacob et al. 2019).

Bivalves

Larvae In general, bivalve larvae do not suffer from reduced feeding rate upon exposure to 0.1–10 μm MPs (Figure 7C), although there is some variation among studies. Exposure to MPs smaller than 1 μm at 1000 beads mL^{-1} (0.00055 mg L^{-1}) significantly reduced carbon uptake by 75% compared to control larvae of oysters (*Crassostrea gigas*) (Cole & Galloway 2015). In contrast, presence of PS MPs >2 μm did not affect the filter feeding rates of the mussels *Mytilus galloprovincialis* (3 μm; 0.03 mg L^{-1}) and *M. edulis* (2 μm; 0.003 mg L^{-1}) or the oyster *C. gigas* (10 μm; 0.00055–0.55 mg L^{-1}) at any concentrations tested (Cole & Galloway 2015, Capolupo et al. 2018, Rist et al. 2019). The percentage decrease in these sizes of MPs were generally lower than 10%. It has been shown that mussel D-veligers express food preferences by actively selecting relatively high nutritional particles with the cilia of the velum (Sprung 1984). These results suggest that the ability of bivalve larvae to select food particles might be influenced by MP size. MPs smaller than 1 μm significantly reduced the filter feeding rate of oyster larvae, but MPs >2 μm did not. The causal mechanisms require further investigation. Due to the variation between studies, the mean percentage decrease on feeding rate of bivalve larvae is lower than 10% (Figure 7C).

Gastropods

Larvae The feeding rate of gastropod larvae is not severely affected by 0.1–10 μm MPs (Figure 7C). The algal consumption rate of *Crepidula onyx* larvae was not significantly affected after 14 days of exposure to high concentrations of MPs (2–5 μm; 0.00024–3.33 mg L^{-1}). All MP-exposed and control individuals had similar algal consumption rates. Although an increased total clearance rate (algae + MP) was observed in the larvae fed with MPs, their algal consumption did not increase (Lo & Chan 2018). This result suggests that *C. onyx* larvae do not selectively feed on algal particles, even though MP exposure increases their clearance rate. It is possible that the absence of effects also related to the size of MP, but the mechanisms are still unclear.

Decapods

Larvae A reduced feeding rate was documented in decapod larvae exposed to >10–100 μm MPs (Figure 7D). Porcellanid larvae suffered from a decreased feeding rate after exposure to PS MPs (10 + 20 μm; 0.25 mg L^{-1}). The ingestion rate and carbon uptake of MP-exposed larvae were approximately 30% and 23% lower, respectively, than those of the control group, although these results were not significant (Dedman 2014).

Comparing the effect of microplastic on feeding rates among zooplankton groups under environmentally relevant and high laboratory concentrations

Reduction in feeding rate is widely documented in copepods, daphnids and decapod larvae (Figure 7). Among all zooplankton groups tested, crustacean zooplankton – including copepods, daphnids and decapod larvae – seem to be the most vulnerable to MPs. Copepods are the most sensitive group; their mean percentage decrease in feeding rate reached 26% at environmental concentrations (0–1 mg L^{-1}) and exceeded 75% at >1–10 mg L^{-1} upon exposure to 0.1–10 μm MPs (Figure 7A). MPs of size >10–100 μm also influence copepods' feeding rates (Figure 7B). Daphnids and decapod larvae are quite sensitive to MPs as well, decreasing feeding rate by 15%–22% at environmental concentrations (0–1 mg L^{-1}) (Figure 7A,D); the least sensitive groups are molluscs (including bivalves and gastropods) and fishes. An increase in feeding rate was reported in these groups upon MP exposure at all the concentrations tested (Figure 7C,E). These results indicate that the feeding rate of crustacean zooplankton would be adversely affected by MPs at environmental concentrations, and the effects would be exacerbated further at sites heavily contaminated by MPs.

Drastic decreases in feeding rate might be explained by the strong selectivity observed in crustacean zooplankton. They selectively feed on phytoplankton and are able to avoid MPs; thus, they might be less efficient at feeding when MPs are present. Nevertheless, this does not mean that unselective feeders will be the 'winners' under MP pollution. If the MPs were heavily contaminated with chemicals, undiscriminating ingestion might have detrimental impacts due to the transfer of toxic chemicals absorbed from MPs after ingestion, while selective feeding might help prevent animals from ingesting toxic MPs, even if it reduces their feeding efficiency.

Swimming speed

Holoplankton

Brine shrimp

MPs (0.1–10 μm) reduce swimming speed in brine shrimp naupliar larvae (*Artemia franciscana*) by 10% after 24 hours of exposure to PS MP (0.1 μm) at 10 mg L^{-1}. However, the speed was significantly accelerated by 10%–18% at high MP concentrations (1 and 10 mg L^{-1}) after 48 hours of exposure (Gambardella et al. 2017) (Figure 8A).

Rotifers

MP (0.1–10 μm) exposure significantly impairs adult rotifer swimming speed (Figure 8A). At a low concentration (0.001 mg L^{-1}), *Brachionus plicatilis* swimming speed first accelerated and then gradually decreased (18%–30%) from 0.1 mg L^{-1} upwards (Gambardella et al. 2018).

Meroplankton

Fishes

Larvae PS MPs (45 μm, 1 mg L^{-1}) do not significantly affect zebrafish (*Danio rerio*) locomotion (Figure 8C). The total swimming distance of the MP-exposed larvae (~950 cm/10 min) was similar

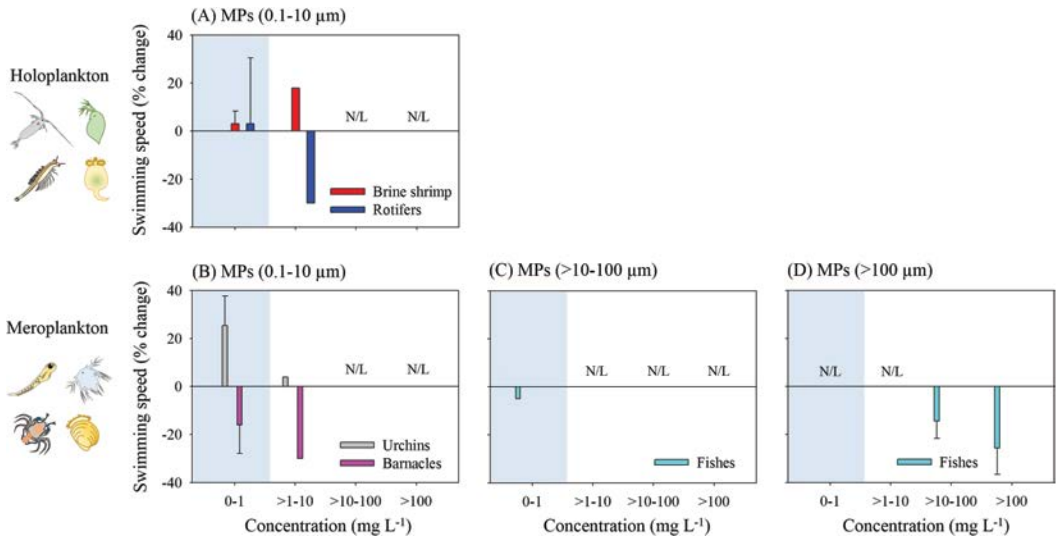

Figure 8 Percentage change in swimming speed (mean + 1SD %) of (a) holoplankton and (b, c) meroplankton in MP treatments when compared to controls. For literature used for all groups of zooplankton, refer to supplementary Table A8. A negative percentage change means a decreased amount of the value in MP treatment compared to that of the control and vice versa. Note: In figure (A), no data are available for copepods, daphnids and euphausids at all concentrations, except for brine shrimp (>10 mg L^{-1}) and rotifers (>10 mg L^{-1}). No data are available for holoplankton for MPs of size >10–100 μm and >100 μm. In figure (B), no data are available for fishes, bivalves, gastropods, decapods and ascidians at all concentrations, except for sea urchins (>10 mg L^{-1}) and barnacles (>10 mg L^{-1}). In figure (C), no data are available for urchins, bivalves, gastropods, barnacles, decapods and ascidians at all concentrations, except for fishes (>1 mg L^{-1}). In figure (D), no data are available for urchins, bivalves, gastropods, barnacles, decapods and ascidians at all concentrations, except for fishes (0–10 mg L^{-1}). Note: light blue background indicates the concentration where environmentally relevant, and white background indicates high laboratory concentration, which does not appear at the environment at present. N/L = no data available.

to that of the control (~1000 cm/10 min) after 120 hours of exposure (Chen et al. 2017). In contrast, exposure to 6–350 μm PE MPs (250 mg L^{-1}) for 96 hours decreased distance travelled and swimming velocity by 17%–25% and 14%–46%, respectively, in minnow larvae (*Cyprinodon variegatus*) (Choi et al. 2018) (Figure 8D).

Microplastic-chemical interactions A mixture of PS MPs (45 μm; 1 mg L^{-1}) and 2 μg L^{-1} EE2 did not have a clear effect on the swimming activity of zebrafish larvae (*D. rerio*). This might be because the MP absorbs EE2 and thus reduces the amount of dissolved EE2 in solution. In contrast, co-exposure to higher-concentration EE2 (20 μg L^{-1}) and PS MPs (45 μm; 1 mg L^{-1}) significantly suppressed locomotion of fish larvae by 23%–34% (Chen et al. 2017). Swimming activity of fish larvae is closely related to energy requirements and predator avoidance. An inhibited ability to swim might largely affect fish larvae's ability to avoid predators and thus reduce their fitness when exposed to MP.

Sea urchins

Larvae The swimming ability of sea urchin larvae is significantly altered by PS MPs. Larval swimming speed of *Paracentrotus lividus* significantly increased by 22%–38% at low MP concentrations (0.001–0.1 mg L^{-1}), although no significant effect was found on those exposed to higher concentrations (1–10 mg L^{-1}) (Gambardella et al. 2018) (Figure 8B). This might be related to an overcompensation response, which indicates apparent stimulations at low levels of toxicity. Such responses have been observed in marine organisms exposed to pesticides and other environmental toxins at low-dose concentrations (Costa et al. 2016).

Bivalves

Gametes Spermatozoa motility (velocity) of the oyster *Crassostrea gigas* was not affected by five-hour exposure to PS-COOH or PS-NH_2 MPs exposure (0.1 μm; 0.1–10 mg L^{-1}). The absence of effect might be because of the short exposure time used in this study (González-Fernández et al. 2018). Because the measured values were not reported, the percentage change was not calculated.

Barnacles

Larvae Exposure to virgin PS MPs (0.1 μm) caused mechanical disturbance and significantly inhibited the swimming speed of barnacle nauplius larvae (*Amphibalanus amphitrite*) by ~30% compared to the control at concentrations of 1 and 10 mg L^{-1} (Gambardella et al. 2017) (Figure 8B). These results indicate that barnacle larval locomotion might be altered when MPs are present in the seawater.

Comparing the effect of microplastic on swimming speed among zooplankton groups under environmentally relevant and high laboratory concentrations

Small-sized MPs (0.1–10 μm) significantly alter the swimming speed of several zooplankton groups, including brine shrimp, rotifers and larvae of sea urchins and barnacles at both environmentally relevant (0–1 mg L^{-1}) and high laboratory concentrations (>1 mg L^{-1}) (Figure 8A,B). In addition, MPs of size >10–100 μm and >100 μm reduced swimming speed of fishes as well (Figure 8C,D). However, due to the relatively small number of studies, it is currently difficult to identify which zooplankton group may be more sensitive to MP exposure. These results suggest that swimming speed is a sensitive endpoint which might be useful for detecting MPs at non-lethal concentration levels. Moreover, co-exposure to MPs and chemicals can potentially enhance the inhibition effects of toxic chemicals, but further investigation is needed to draw a comprehensive conclusion. Nevertheless, these results suggest that MPs cause some mechanical disturbance and change the swimming speed of the exposed organisms.

Reproduction

The reproduction traits mentioned here include egg production rate, number of aborted eggs, number of total offspring produced, number of offspring per brood, number of mobile/immobile juveniles, number of broods, time it takes to produce the first brood of offspring and time between broods. To facilitate comparisons, only reproductive traits related to fecundity – egg production rate, number of total offspring produced and number of offspring per brood – were used to calculate percentage change.

Holoplankton

Copepods

MPs (0.1–10 μm) significantly reduce the number of offspring produced in calanoid and harpacticoid copepods (Figure 9A). For instance, *Paracyclopina nana* (Calanoida) exposed to doses of 0.5 μm PS MPs (0.1–20 mg L^{-1}) showed a 12%–24% decrease in nauplii offspring produced, while 6 μm MPs had no significant effect (Jeong et al. 2017). The harpacticoid copepod *Tigriopus japonicus* produced significantly fewer nauplii (56%–72% compared to the control) when exposed to PS MPs (0.5 and 6 μm; 0.1–25 mg L^{-1}) (Lee et al. 2013). *Parvocalanus crassirostris* exposed to PS MPs (<11 μm;

57.78 mg L⁻¹) decreased egg production by 88% (Heindler et al. 2017). The percentage decrease reached nearly 50% at concentrations of >10–100 mg L⁻¹ (Figure 9A), suggesting that MPs of size 0.1–10 μm can severely reduce copepod fitness. In contrast, >10–100 μm MPs did not severely affect egg production and hatching success in *Calanus helgolandicus* after exposure to PS MPs (20.0 μm; 0.33 mg L⁻¹). Even a slight increase in egg production was observed, but this was not significant, predominantly due to the high variation (Cole et al. 2015) (Figure 9B).

Transgenerational effect The adverse impact of MPs on fitness can affect the next generation's reproduction. The number of offspring produced by *T. japonicus* was significantly reduced by 49%–87% after exposure to PS MPs (0.5 and 6 μm; 0.1–25 mg L⁻¹) (Lee et al. 2013) (Figure 2D). If fecundity was negatively impacted by MP exposure, then long-term exposure could have a detrimental influence on both calanoid and harpacticoid copepod populations, as supported by Heindler et al. (2017), who found that exposure to PET MPs (<11 μm; 14.44 mg L⁻¹) for 24 days significantly depleted population size by 40% in the calanoid copepod *P. crassirostris*.

Daphnids

Several studies have evaluated the effects of MP toxicity on daphnid reproductive traits (e.g. number of offspring produced, number of broods and the time to first offspring). This section will be subdivided into three parts discussing the effects of MPs on different reproductive traits:

1. *Number of offspring*: The number of offspring produced by daphnids is significantly reduced upon exposure to 0.1–10 μm MPs (Figure 9A). *Daphnia magna, D. pulex* and *Ceriodaphnia dubia* suffer decreased offspring numbers when exposure to spherical and irregular MPs. Some studies found that the offspring number produced by MP-exposed females was significantly decreased by 9%–94%, 26%–46% and 24%–65% in *D. magna, D. pulex* and *C. dubia*, respectively (Pacheco et al. 2018) (1–5 μm; 0.02 and 0.2 mg L⁻¹), Martins & Guilhermino (2018) (1–5 μm; 0.1 mg L⁻¹), Puranen Vasilakis (2017) (PS and PLA, 1–5 μm; 0.65–0.93 mg L⁻¹), Ziajahromi et al. (2017) (PE and PET, 1–100 μm; 0.03–5 mg L⁻¹),

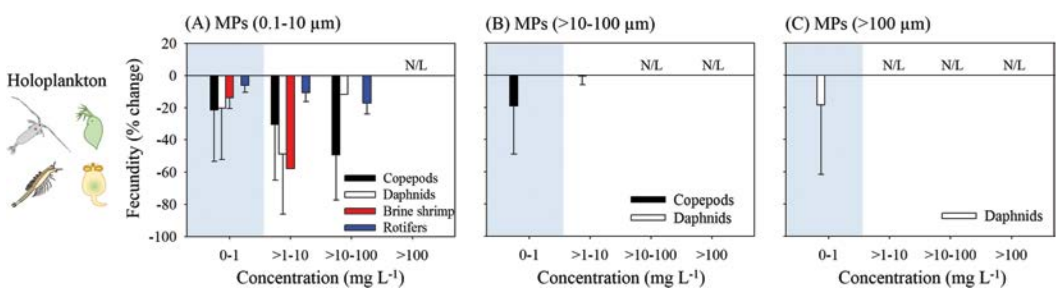

Figure 9 Percentage change in fecundity (mean + 1SD %) of (a–c) holoplankton in MP treatments when compared to controls. For literature used for all groups of zooplankton, refer to supplementary Table A9. A negative percentage change means a decreased amount of the value in MP treatment compared to that of the control and vice versa. Note: In figure (A), no data are available for copepods (>10² mg L⁻¹), daphnids (>10² mg L⁻¹), brine shrimp (>10 mg L⁻¹), euphausids (all concentrations) and rotifers (>10² mg L⁻¹). In figure (B), no data are available for brine shrimp, euphausids and rotifers at all concentrations, except for copepods (>1 mg L⁻¹) and daphnids (0–1, >10 mg L⁻¹). In figure (C), no data are available for copepods, brine shrimp, euphausids and rotifers at all concentrations, except for daphnids (>1 mg L⁻¹). No data are included in meroplankton, since adults of fishes, urchins, bivalves, gastropods, barnacles, decapods and ascidians are not zooplankton. Note: light blue background indicates the concentration where environmentally relevant, and white background indicates high laboratory concentration, which does not appear at the environment at present. N/L = no data available.

Jaikumar et al. (2019) (PE, 1–10 μm). Similarly, exposure to fragmented MPs (2.6 ± 1.8 μm) significantly decreased the number of total offspring released by 76% (*D. magna*) (Ogonowski et al. 2016). However, a small number of studies reported no clear effects on fecundity in *D. magna* after exposure to MPs (Ogonowski et al. 2016) (1–5 μm, 0.0018–1.8 mg L^{-1}), Rist et al. (2017) (PS, 0.1 and 2 μm, 0.1–1 mg L^{-1}), Aljaibachi & Callaghan (2018) (PS, 2 μm, 1.39×10^{-3}–1.11×10^{-2} mg L^{-1}) and Gerdes et al. (2019) (1–5 μm, 12.86 mg L^{-1}). Overall, MPs of 0.1–10 μm decreased daphnid fecundity over 40% at >1–10 mg L^{-1}. But the percentage decrease reduced after this concentration, predominantly because only a small number of studies investigated high concentrations (Figure 9A).

Similarly, 100−400 μm PET MPs significantly decreased the number of offspring produced by 20%−80% in *C. dubia* (Ziajahromi et al. 2017) (Figure 9C). In contrast, offspring number was not significantly affected after exposure to 40 μm MPs (PA + PC + PET + PVC, ABS + PVC + POM + SAN, 3.24–4.89 mg L^{-1}) in *D. magna* (Imhof et al. 2017) (Figure 9B). But the effects of these size classes of MPs are still poorly studied, and thus further investigations are needed.

2. *Number of broods and time to first offspring*: The number of broods produced and the time to first offspring were not affected by MPs in most studies. *D. magna* exposed to PS (2 μm; 0.1–1 mg L^{-1}), PE (1–10 μm) and unknown type MPs (1–5 μm; 0.1 mg L^{-1}) did not significantly change number of broods and their time to first brood (Ogonowski et al. 2016, Rist et al. 2017, Martins & Guilhermino 2018, Jaikumar et al. 2019). No significant difference was found in PE bead (10^{-1} μm) or PET fibre (100–400 μm) exposed *C. dubia*, except at high concentration (1 mg L^{-1}) (Ziajahromi et al. 2017, Jaikumar et al. 2019). However, a study by Pacheco et al. (2018) showed that MP-exposed *D. magna* (1–5 μm; 0.2 mg L^{-1}) decreased brood numbers produced and delayed their reproduction time by 71% and 49% compared to those of the controls. Both MP size and concentration are similar in these studies, and thus further investigations are needed to explain the discrepancies.
3. *Production of dead juveniles and time between broods*: As for other reproductive traits, MP-exposed *D. magna* (1–5 μm, 0.02–0.2 mg L^{-1}) produced dead juveniles (~6–15 animals) (Martins & Guilhermino 2018, Pacheco et al. 2018); MPs (1–5 μm, 0.0018–1.8 mg L^{-1}) did not, however, impact their time between broods (Ogonowski et al. 2016). These reproductive traits are not intensively studied, and thus further research is needed.

Microplastic-chemical interactions It has been commonly reported that MP can be a vector for pollutants. However, it is also possible that the reverse transport of pollutants from biota to MPs can occur if the organisms have higher concentrations of contaminants than that on the ingested MPs. For example, Gerdes et al. (2019) found that clean MPs (1–5 μm; 12.86 mg L^{-1}) eliminated some PCB in heavily contaminated *D. magna*, resulting in the PCB209 body burden of the MP-treated group (0.13 μg g *Daphnia*$^{-1}$) being lower than that of the non-treated group (0.37 μg g *Daphnia*$^{-1}$). Adding MPs even increased fecundity (the number of eggs) by ~35%, suggesting that ingesting MPs might have the positive effects of eliminating toxicity and increasing fitness in exposed organisms.

Transgenerational effect The number of offspring that F_1 neonates yielded also significantly decreased upon exposure to MPs, suggesting that there is a transgenerational effect on daphnid fecundity (Figure 2D). The number of offspring produced by the F_1 generation was 29%–75% less than the control after exposure to PS (2 μm, 1.11×10^{-2} mg L^{-1}) and unknown type MPs (1–5 μm, 0.1 mg L^{-1}) (Aljaibachi & Callaghan 2018, Martins & Guilhermino 2018). In addition, a transgenerational effect was also observed in other reproductive traits. The number of broods and living juveniles released were still ~16%–40% less than the control in F_1 offspring and the following generations (*D. magna*) (unknown type, 1–5 μm, 0.1 mg L^{-1}), although some reproductive traits such as time to first brood had already recovered (Martins & Guilhermino 2018).

Brine shrimp

Adults The total number of offspring significantly decreased by 9%–58% in *Artemia franciscana* after being exposed to 1–5 μm MPs (0.4–1.6 mg L^{-1}) (Figure 9A), suggesting that MPs can negatively affect brine shrimp population size in the long term (Peixoto et al. 2019).

Rotifers

Exposure to 0.5 μm PS MPs (20 mg L^{-1}) significantly reduced the number of offspring produced by 7%–21% in the rotifer *Brachionus koreanus*. On the other hand, 6 μm MPs (0.1–20 mg L^{-1}; 12 days) had relatively mild impacts, with the number of offspring being only 0%–12% less than the control (Jeong et al. 2016) (Figure 9A). However, other reproductive traits in rotifers have different responses toward MPs. The time needed from hatching to maturation did not significantly differ from the control (25.41 hours) after exposure to both 0.5 and 6 μm PS MPs (10 mg L^{-1}) (26.15 and 25.13 hours, respectively) (*B. koreanus*) (Jeong et al. 2016).

Comparing the effect of microplastic on reproduction among zooplankton groups under environmentally relevant and high laboratory concentrations

MP significantly reduces the number of offspring in copepods, daphnids, brine shrimp and rotifers (Figure 9). At environmentally relevant concentrations (0–1 mg L^{-1}), fecundity of zooplankton decreased by 6%–21% upon exposure to 0.1–10 μm MPs (Figure 9A). The percentage change decreased with increasing MP concentrations. At high laboratory concentrations (>1 mg L^{-1}), the percentage decrease can reach 30%–57% for crustacean zooplankton (daphnids, copepods and brine shrimp) (Figure 9A). One exception was in daphnids, for which the percentage decrease markedly lowered at >10–100 mg L^{-1}, probably due to the small number of studies (Figure 9A). Of all the zooplankton groups analysed, daphnids, copepods and brine shrimp appear to be most susceptible to MPs, followed by rotifers (Figure 9A). Moreover, MPs of size >10–100 μm and >100 μm also affect fecundity of copepods and daphnids (Figure 9B,C); however, these size classes are still poorly studied, and further investigations are still needed. These results suggest that MP exposure decreases zooplankton fecundity at environmentally relevant concentrations (0–1 mg L^{-1}). The negative effects might be more prominent under extreme conditions where high MP concentrations occur (>1 mg L^{-1}). Of note is that crustacean zooplankton are most sensitive to MPs than others. One possible reason is that a reduction in feeding rate observed in crustacean zooplankton (See 'Feeding rate' in the present review) leads to less energy available for reproduction. But further investigations are needed to elucidate the underlying mechanisms.

The current studies reviewed here show that the combined MPs and chemicals tested do not enhance the toxicity of chemicals on zooplankton reproduction. But the study numbers are still small, so future research on chemicals is strongly suggested. In addition, MPs have prominent transgenerational effects on copepod and daphnid reproduction, which drastically decrease the fecundity of the F_1 offspring (Figure 2D). This suggests that zooplankton population size is likely to significantly decrease across generations upon continuous MP exposure.

Organ damage

Holoplankton

Brine shrimp

Larvae Several ultrastructural changes have been found in the epithelial cells of the digestive tract in PS MP-exposed brine shrimp larvae (*Artemia parthenogenetica*). The number of microvilli decreased, the number of mitochondrion increased and the autophagosome was present in epithelial cells after 24 hours of MP exposure (10 μm; 0.00055–5.54 mg L^{-1}; 24 hours)

(Wang et al. 2019). These damages to cells in the digestive gut might have negative effects like accelerating energy consumption and disrupting nutrient absorption which could lead to starvation in the long term.

Meroplankton

Fishes

Larvae Most studies suggest that MP causes only negligible damage to fish organs at the larval stage. No cellular structure damages or inflammatory changes to gills, liver, brain, kidneys or intestine were observed in either MP-treated (LDPE, 0.5 mg L^{-1}) or control zebrafish larval groups (*Danio rerio*) (Karami et al. 2017). In silver barbs (*Barbodes gonionotus*), no damage was found to internal organs or gills after exposure to PVC fragments (40–300 μm; 0.2–1.0 mg L^{-1}), although the intestinal lining thickened by 29%–73% (Romano et al. 2018). One exception was minnow larvae (*Cyprinodon variegatus*), which showed intestinal distention, probably due to the excessive ingestion of bead and fragmented MPs (PE, 6–350 μm; 250 mg L^{-1}) (Choi et al. 2018). One reason these conditions were found to be harmless may be that the zooplankton are highly efficient at eliminating MPs. Polyethylene MPs (45 μm) were totally egested out of the European sea bass after 48 hours (Mazurais et al. 2015). This high potential for MP egestion may explain why there was no intestinal damage in fish larvae.

Gene expression

Biomarkers are sensitive to environmental stimulus and thus could reflect the real-time stresses that animals face under MP exposure. Several alterations in gene expression have been widely reported in MP-exposed zooplankton groups. Table 2 lists some commonly used biomarkers and their functions.

Holoplankton

Copepods

Production of cellular reactive oxygen species (ROS) in the calanoid copepod *Paracyclopina nana* increased and its antioxidant enzymatic activities – including GPx, GST and SOD – changed when exposed to PS MPs (0.5 and 6 μm; 20 mg L^{-1}) (Jeong et al. 2017), suggesting that oxidative stress was induced after exposure to MPs. In contrast, no stress response was observed in PET MP (14.44 mg L^{-1}) exposed *Parvocalanus crassirostris* (Calanoida), as indicated by no alteration

Table 2 Common gene biomarkers and their functions

Gene	Process
Cyp1a1	Detoxification
IL-1β, LYS, MYTC, MYTLB, Cxcr5	Immune response
Casp3, tp53	Apoptosis
Sod1, GPx, CAT, GST, GSH, Sod3, CAT, Dm-TRxR	Oxidative stress
AChE, GFAP, α1-tubulin, PChE	Neurotoxicity
HEX, GUSB, CTSL	Inflammatory response
CA, EP, CS, MT10, MT20	Shell biogenesis
HSP60, HSP70	General stress
AK	Energy production
Permeases, p-gp, MRP	Membrane transportation
SERCA	Anti-predation response

in *Hsp70*-like expression after 6 days of exposure. Although expression of the Histone 3 (*H3*) protein, which is related to tumourigenesis in humans (Zhao et al. 2002), was first downregulated after 6 days of exposure, it was not different from the control after 18 days of recovery (Heindler et al. 2017).

Daphnids

PS MPs (1–10 μm; 0.1–8 mg L^{-1}) induced oxidative stress in *Daphnia magna*, as indicated by alterations in CAT, GPx, MDA, GST and Dm-TRxR transcript levels (Tang et al. 2019, Zhang et al. 2019). Enzymes related to energy production and extracellular transportation, AK and permeases, were upregulated in the presence of PS MPs (1.25 μm; 2–8 mg L^{-1}) as well (Tang et al. 2019). Moreover, a batch of genes, including HSP 60, HSP 70 (general stress genes), GST and housekeeping genes (GAPDH, Stx16, aTub, Act and SERCA), were differentially expressed in *D. magna* exposed to plastic mixtures, suggesting that MPs interfered with oxidative pathways and activated protection mechanisms (Imhof et al. 2017). The different expression levels of the gene SERCA upon exposure indicated that there was an interference in the signalling pathway of anti-predation responses. However, it is noteworthy that there was variation between clones. Genetic alterations were only found in clones BL2.2 and Max4, but not clone K34J, suggesting that interclonal variation was high. Since *Daphnia* have the ability to rapidly evolve, potentially acquiring resistance to toxicants, the observed variation between clones might stem from their adaptation to MPs in their collection sites.

Microplastic-chemical interactions Adding PS MPs (1 and 10 μm; 0.1 mg L^{-1}) to roxithromycin (0.01 mg L^{-1}) exposed *D. magna* significantly decreased the responses of MDA, GPx and GST than roxithromycin alone. Moreover, integrated biomarker response analysis revealed that combined effect of PS MPs and roxithromycin induce more serious oxidative damages in *D. magna* than roxithromycin alone, suggesting that MPs enhanced the toxicity of roxithromycin (Zhang et al. 2019).

Brine shrimp

Larvae PS MP (0.1 μm) significantly affected biochemical responses in brine shrimp larvae (*Artemia franciscana*). Inhibition of AChE activity was observed in MP-exposed larvae at 0.001 and 0.01 mg L^{-1}, while PChE activity significantly increased at 0.01 and 0.1 mg L^{-1}, although not in a dose-dependent manner. Catalase activity also increased in MP-exposed larvae at all the tested concentrations (0.001–1 mg L^{-1}) (Gambardella et al. 2017). Cholinesterases (AChE and PChE) and catalase are biomarkers for neurotoxicity and oxidative stress in marine invertebrates. The significant inhibition of cholinesterases, and increase in catalase activity, indicate that neurotoxicity and oxidative stress were induced in brine shrimp larvae after MP exposure.

Rotifers

Several alterations in gene expression were observed in MP-exposed rotifers. Intracellular ROS levels in rotifers (*Brachionus koreanus*) increased significantly after exposure to both 0.5 and 6 μm PS MPs (10 mg L^{-1}). The activity of antioxidant-related enzymes including SOD, GST, GR and GPx increased significantly in MP-exposed rotifers compared to the control (Jeong et al. 2016). The induction of ROS and activation of antioxidant-related enzymes suggest that MPs induce oxidative stress in exposed rotifers. Furthermore, P-gp and MRP activities decreased in a size-dependent manner after exposure to PS MPs (0.5 and 6 μm). P-glycoprotein (P-gp) and multidrug resistance protein (MRP) played an important role in aquatic invertebrates' defence systems that pump many xenobiotics out of cells. They were the first line of defence against xenobiotic pollutants (Jeong et al. 2018). P-gp and MRP inhibition suggests that MP might affect rotifer defence mechanisms by making them more vulnerable to toxicants when MPs are presented in the environment.

Meroplankton

Fishes

Embryos Strong genetic responses have been observed in MP-exposed fish embryos. A transcriptome analysis showed that injecting PS MPs (0.7 μm) into embryos causes significant changes in zebrafish (*Danio rerio*) transcriptomic profiles, with 26 genes differentially expressed when MPs were injected into the yolk of the embryos compared to the non-injected controls. These differentially expressed genes were related to various functions, including lipid metabolism, oxidative stress, complement system and immune responses, suggesting that MP-exposed embryos had a broad response to MPs (Veneman et al. 2017).

Larvae Signs of oxidative stress, chemical toxicity, immune response and apoptosis have been observed in many fish species under MP exposure. In the European sea bass (*Dicentrarchus labrax*), exposure to PE MPs (45 μm) significantly increased cytochrome-P450-1A1 (*cyp1a1*) expression levels (12 mg per gram of diet), suggesting that MP exposure induced chemical toxicity (Mazurais et al. 2015). Exposure to both microbeads and fragmented PE MPs altered gene expressions of *Casp3*, *tp53* and *Cxcr5* in sheepshead minnow larvae (*Cyprinodon variegatus*), indicating apoptosis and immune response were elicited in exposed larvae (Choi et al. 2018). The transcriptions of a visual gene (*zfrho*) significantly increased by 6.4-fold compared to the control in MP-exposed zebrafish larvae (*D. rerio*) (45 μm, 1 mg L^{-1}), indicating an enhanced sensitivity to the light (Chen et al. 2017). AChE activity was inhibited in MP-exposed zebrafish larvae (45 μm, 1 mg L^{-1}), indicating that something was interfering with how the nervous system was functioning (Chen et al. 2017). An upregulation in CYP1A expression suggested that the detoxification processes was upregulated in three-spined stickleback larvae (*Gasterosteus aculeatus*) after seven days of exposure to PS MPs (1 μm, 10.6–1060 mg L^{-1}) (Katzenberger 2015). Furthermore, oxidative stress was induced in PE microfragment (6–350 μm) exposed minnow larvae (Choi et al. 2018) and PS MPs (5 and 50 μm) exposed zebrafish larvae (Wan et al. 2019). On a broader scale, whole animal transcriptomics and gene transcription analysis in zebrafish larvae show a transient and extensive change in gene expression. The majority of the differentially expressed genes were related to the nervous system, immune response and energy metabolism, suggesting that MPs are recognised by the immune system and impair neurodevelopment and metabolic pathways in zebrafish larvae (Veneman et al. 2017) (PS, 0.7 μm, 5 mg mL^{-1}), LeMoine et al. (2018) (PE, 10–45 μm, 5 and 20 mg L^{-1}), Wan et al. (2019) (PS, 5 and 50 μm, 0.1 and 1 mg L^{-1}).

In contrast, zebrafish larvae exposed to LDPE MPs (0–18 μm; 0.5 mg L^{-1}) displayed no change in anti-apoptotic, oxidative and neurotoxic genes (Karami et al. 2017). Similarly, expression of nervous-related genes (*gfap* and *α1-tubulin*) and CAT and GPx levels were both unchanged compared to those of the control after MP exposure (45 μm, 1 mg L^{-1}) (Chen et al. 2017). Moreover, vitellogenin B (VTG B) expression did not change after exposure to PS MPs (1 μm, 10.6–1060 mg L^{-1}) in three-spined stickleback larvae, suggesting that no oestrogenic chemicals were released from MPs (Katzenberger 2015). These discrepancies could be explained by the difference in genetic markers and polymer types used.

Microplastic-chemical interactions Co-exposure to MPs and chemicals might have an even higher impact than each individually. The combined effects of PS MPs (45 μm, 1 mg L^{-1}) and EE2 (2 and 20 μg L^{-1}) upregulated a batch of biomarkers, including nervous-related genes (*gfap* and *α1-tubulin*), visual-related genes (*zfrho* and *zfblue*) and the activities of GPx, CAT, GST (oxidative damage) and AChE (related to neurodevelopment) enzymes in zebrafish larvae, suggesting that the co-exposure induced neurotoxicity and oxidative stress (Chen et al. 2017). Moreover, BaP-spiked PE MPs (1–5 μm, 10–20 μm, 1 and 4 mg L^{-1}) induced chemical toxicity in zebrafish, as indicated by CYP 1A induction (Batel et al. 2018).

Bivalves

Embryos Significant alterations in gene expression have been found in virgin MP-exposed mussel embryos. Down-regulation of lysosomal enzyme activities – including hexosaminidase (*HEX*), b-glucorinidase (*GUSB*) and cathepsin-L (*CTSL*) – were observed in PS MP (3 μm; 0.0007–0.007 mg L^{-1}) exposed embryos, as indicated by inflammatory responses in mussels (*Mytilus galloprovincialis*). Exposure to MP also significantly impacted the expression of immune-related genes (*LYS*, *MYTC* and *MYTLB*), shell biogenesis genes (carbonic anhydrase [*CA*], extrapallial protein [*EP*] and chitin synthase [*CS*]) and methallotionein genes (*MT10* and *MT20*) (Capolupo et al. 2018). Total multixenobiotic resistance (MXR)efflux activity was reduced and Mrp and P-gp transcripts were down-regulated in PS MP-exposed (3 μm; 0.0007 and 0.007 mg L^{-1}) embryos (*M. galloprovincialis*), suggesting that cytoprotective mechanism was impaired (Franzellitti et al. 2019). These studies suggest that MP can induce a range of responses in MP-exposed embryos, including oxidative stress, immune response and neuroendocrine interference, and impaired their defence system toward environmental stresses.

Microplastic-chemical interactions PS-COOH MPs (0.1 μm; 10 and 100 mg L^{-1}) significantly increased ROS production by 30%–70% in oyster spermatozoa (*Crassostrea gigas*) after five hours of exposure. In contrast, PS-NH_2 MPs (0.1 μm; 0.1–100 mg L^{-1}) did not affect ROS production in spermatozoa or oocytes (González-Fernández et al. 2018). The differential effects of MPs could be explained by the surface functionalisation coated on MPs or membrane characteristics of the exposed cells.

Barnacles

Larvae Oxidative stress and neurotoxicity have been observed in MP-exposed *Amphibalanus amphitrite* larvae. The activity of the oxidative stress related enzyme catalase was inhibited at low concentrations of PS MPs (0.1 μm; 0.001–0.1 mg L^{-1}), indicating that oxidative stress was induced upon MP exposure. Significant increases in cholinesterases expression (both AChE and PChE activity) were also observed in MP-exposed larvae (stage II nauplii) (Gambardella et al. 2017), suggesting that PS MPs impair neurofunction in exposed nauplius larvae (*A. amphitrite*).

Comparing the effect of microplastic on gene expression among zooplankton groups under environmentally relevant and high laboratory concentrations

MPs elicit various genetic alterations at the molecular level in all the zooplankton groups tested at both environmentally relevant and high laboratory concentrations. Oxidative stress, immune response and neurotoxicity are the most commonly reported responses to MPs. Besides, alterations in genes related to inflammatory response, chemical toxicity and membrane transportation are also widely documented. Due to the variation in biomarkers used in different studies, it is difficult to compare which zooplankton group is more sensitive to MPs at the present stage. But the genes whose expressions are influenced are usually related to important life functions. Hence, these studies emphasise that MPs might disrupt normal cell functions and damage many zooplankton organisms in the long term.

Knowledge gaps and recommendations for future studies

1. There is a growing number of studies exploring the effects of MPs on zooplankton. However, the effects of MPs on early stages such as gametes and embryos are still under-represented. With a well-developed *in vitro* fertilisation technique, gametes and embryos of sea urchins and bivalves might be suitable models for evaluating the impacts of MPs on early developmental stages. More studies evaluating the effects of MPs on early developmental stages are needed.

2. Several sublethal impacts of MPs, including alteration in growth, decreases in feeding rate and fecundity, are being extensively studied in zooplankton groups. Where adverse effects have been observed, the causal mechanisms are often poorly elucidated. For example, MP-induced changes to growth in sea urchins might relate to decreased food intake; however, no study has evaluated the impact of MPs on sea urchin feeding rate. Further investigations to elucidate the underlying causes of the observed effects are needed.
3. The impacts of MPs on swimming speed of zooplankton mainly focus on smaller-sized MPs (0.1–10 μm). However, MPs of larger size classes (>10 μm) can cause a physical disturbance to zooplankton, although they might not be directly ingested. Hence, we recommend future studies use high-speed high-resolution cameras to record how the MPs interfere with the appendage movements and swimming patterns of zooplankton (see Chan et al. 2013). Moreover, the underlying cause of altered swimming speed (and indeed behaviour) requires further study, particularly for zooplankton other than fish larvae.
4. MP can have prominent impacts on zooplankton fecundity and affect the quality of their offspring. Recent studies have suggested that MP can even reduce the number of offspring produced by their F_1 generation, suggesting a transgenerational effect. This can have long-term detrimental effects on zooplankton populations. However, current studies assessing MPs' effects largely focus on the organismal or suborganismal level. To evaluate the potential effects of MPs on zooplankton populations, studies on higher organisational levels such as population or community are strongly recommended.
5. Organ damage caused by MPs is not well studied in zooplankton groups, except for fish larvae and brine shrimp. Moreover, irregular MPs appear to cause more severe damage to internal organs than spherical particles, but their effects are still poorly studied. More histopathological analyses on effects of the microfibres and fragmented MPs are suggested in future studies.
6. Transcriptomic studies on gene expression in the presence of MP largely focus on fish larvae, whereas only a few genetic markers have been studied in other zooplankton groups. In addition, compared to the studies on the larval and adult stages, transcriptomic studies on the embryonic stage are relatively rare and should receive further attention.
7. Different feeding types might affect the amount of MP ingested and hence affect the impacts of MPs (Setälä et al. 2016, Scherer et al. 2017). Salps are a particularly interesting group, as they exhibit a different feeding mode from other zooplanktons. They feed by secreting mucus to form a net and unselectively filter particles (Harbison & McAlister 1979). MP ingestion has been documented in several salp species (Chan & Witting 2012, Wieczorek et al. 2019), but there are currently no MP toxicity studies. Zooplankton are a diverse group of organisms. To assess MP impacts on zooplankton communities more fully, toxicity studies on zooplankton groups exhibiting different feeding strategies such as salps (holoplankton) and larvae of polychaete and cnidarian species (meroplankton) are strongly recommended.
8. The interactions between MPs and chemicals are still rarely studied in zooplankton. Polycyclic aromatic hydrocarbons (PAHs), phenanthrene (Phe), pyrene, 17 α-ethynylestradiol (EE2), Benzo[a]pyrene and Bisphenol A are commonly reported pollutants that adhere to MPs in aquatic environments (Teuten et al. 2007). Despite the presence of chemicals on MPs, their interactive effects with different sizes and types MPs are still poorly investigated. Further studies should evaluate the combined effects of MPs and chemicals on biological endpoints including feeding rate, swimming speed and reproduction.
9. The characteristics of MP (e.g. size, shape and polymer type) might affect its impacts on organisms. Spherical MPs are currently the most commonly studied shape in MP toxicity studies because they are commercially available and often used in experiments. But fibres and fragments are the most commonly detected types of MPs in aquatic environments. The

use of spherical MP might not be a good representative of all shapes of MPs present in real environments. Thus, more research using irregular-shaped MPs are needed. In addition, current MP toxicity studies mainly focus on the effects of single-sized or single type and shaped MPs. In natural environments, however, zooplankton would encounter mixed MPs from various sizes, shapes and types. We recommend that future studies include a range of sizes, shapes and polymer types of MPs to identify the variety of effects on zooplankton.

10. We note that the MP concentrations used in most of the MP toxicity studies exceed those currently documented in the aquatic environments. These unrealistically high concentrations could potentially overestimate the impacts of MPs. Instead of acute toxicity assessment using high MP concentrations under laboratory conditions, experiments with environmentally relevant concentrations and longer exposure times are recommended. Further, signs of transgenerational MP effects have been observed in some studies. Hence, experiments over several generations are strongly recommended.
11. Compared to marine zooplankton, the effect of MP on freshwater zooplankton is poorly studied. Daphnids and fish larvae are the only freshwater zooplankton that have been investigated to date. Other common zooplankton groups such as freshwater copepods, rotifers and decapod larvae are still understudied and need more attention.
12. The relative impacts between natural microparticles such as silt and clay and MPs have been less studied so far. Small, naturally occurring microparticles are commonly found in aquatic environments. These particles are similar to MPs in that both of them are non-digestible and non-nutritious and are potential vectors for hydrophobic organic contaminants (HOCs) (Teuten et al. 2007). Ingestion of all these microparticles may be detrimental to zooplankton. Future studies should consider the relative abundance of MPs compared to natural microparticles in the natural environment and make an attempt to study the combined effects of MP and natural microparticles in laboratory assays.
13. There is a lack of studies on MPs with different surface characteristics and the impact that this has on zooplankton. MPs present in the environment are usually soaked in seawater for a long periods and are often coated with biofilm made up of microbes or carry compounds produced by phytoplankton (e.g. dimethyl sulphide [DMS]). It has been shown that DMS infused MPs increase grazing rates of calanoid copepod *Calanus helgolandicus* (Procter et al. 2019), suggesting that this compound could be an olfactory stimuli to enhance MP foraging response. Presences of these coatings might affect the fate and bioavailability of MPs, potentially enhancing ingestion of MPs by zooplankton. Thus, the surface characteristics of MPs should be considered in future studies.
14. The interactive effects of MPs and other anthropogenic stressors are still poorly studied. Temperature rise, acidification and hypoxia are likely to occur simultaneously with MP pollution, especially in estuaries and coastal ecosystems which are highly anthropogenic impacted regions. The combined effects of these stresses may be synergistic or antagonistic due to the complex interaction among these stresses (Wen et al. 2018). For example, elevated temperature can possibly enhance the food consumption and feeding activities of fish. Presences of MP in such conditions can at the same time reduce fish feeding activities. Digestive enzyme activities and energy metabolism of fish can be affected by elevated temperature of lowered environmental pH. MP can also affect the enzyme activities and energy metabolism of fish when ingested. Therefore, the synergistic effect of MPs with other anthropogenic stressors should be a direction for further studies.

Conclusion

MPs rarely cause direct mortality but can induce sublethal effects on zooplankton which may alter individual- to population-level dynamics. Feeding rate, swimming speed, reproduction and gene expression are affected at both environmentally relevant and unrealistically high laboratory MP concentrations, suggesting that these endpoints are sensitive and potentially can act as a bioindicator to detect MP levels in environments. Survival, growth, development and organ damage are less sensitive endpoints. Survival and organ damage are not influenced at environmental concentrations, but negative effects can be observed at high laboratory concentrations, while no severe impacts on growth and development were found at any concentrations tested. Among the zooplankton groups studied, daphnids are the most sensitive; their survival, feeding rate and fecundity significantly decreased after being exposed to virgin MPs. Moreover, daphnid survival is heavily affected by feeding condition of the animal and exposure time, with unfed daphnids and longer exposure time inducing the most severe impacts. Copepods suffered from reduced feeding rate and fecundity upon MP exposure, which might adversely affect copepod populations in the long term. In contrast to daphnids and copepods, larvae of molluscs and barnacles, brine shrimp and euphausids appear to be relatively tolerant to MPs, suggesting that these groups would be more dominant when faced with prolonged MP pollution.

Leachates derived from MPs have severe impacts on zooplankton, including abnormal development in bivalve and sea urchin embryos. However, their effect on other zooplankton groups are still not well understood owing to the small number of studies. More studies are needed before any conclusion can be drawn. In addition, MPs have been shown to cause prominent effects on the survival and fecundity of F_1 offspring in bivalves, copepods and daphnids, indicating that MP might have transgenerational effects and can drastically affect zooplankton populations in the long term. This is probably owing to the chronic exposure to small amounts of additives and monomers leached from virgin MPs, suggesting that the effects of virgin MPs are not just related to the physical characteristics of the particle itself. We have noted that the causal mechanisms are often poorly demonstrated within MP studies, and the elucidation of the physio-chemical triggers for stress and adverse health in zooplankton and other biota should be considered a key priority for future research.

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