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Short communication

Evidence-based meta-analysis of the genotoxicity induced by microplastics in aquatic organisms at environmentally relevant concentrations

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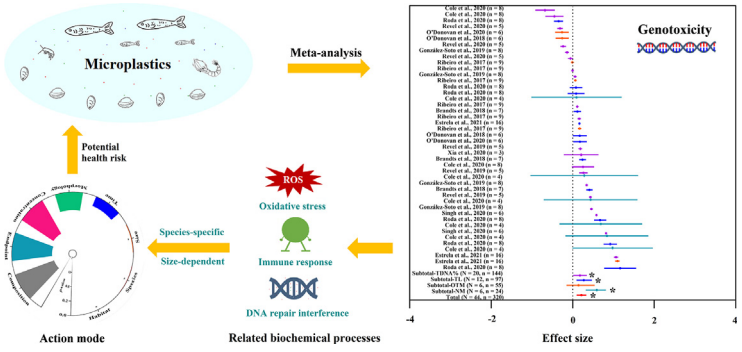
HIGHLIGHTS

- Genotoxicity of realistic MP concentrations to aquatic organisms was meta-analyzed.
- The genotoxic damage in MPs-treated group increased by 24% compared to the control.
- This estimation was robust with high statistical power and no obvious publication bias.
- The genotoxic effect of MPs to aquatic organisms was size-dependent.
- Species-specific genotoxicity response to MPs stress was observed.

GRAPHICAL ABSTRACT



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ABSTRACT

Microplastics (MPs) attract global concern due to their ubiquitous existence in aquatic environments. However, the genotoxic effect of MPs on aquatic organisms in the natural environment remains controversial. Therefore, this meta-analysis was conducted by recompiling 44 individual studies from 12 publications to determine whether MPs could induce genotoxicity in aquatic organisms at environmentally relevant concentrations (≤1 mg/L, median = 0.5 mg/L). Multiple genotoxic endpoints were involved, including the percentage of DNA in tail (TDNA%), tail length (TL), olive tail moment (OTM), and the number of micronuclei (NM), and their increases represented the biologically adverse effects (i.e. genotoxicity). The results showed that all included endpoints tended to increase after exposure to MPs, among which TDNA%, TL and NM were significantly increased by 20%, 32% and 81% compared with the control group, respectively. The overall estimate of all endpoints in the MPs-treated groups was remarkably increased by 24%, with high statistical power and no obvious publication bias, suggesting the evident genotoxicity caused by MPs. In addition, the magnitudes of MPs-induced genotoxicity were independent of selected endpoint, MP composition, morphology, exposure concentration and duration, but closely correlated with particle size, living habitat and tested species. Overall, this work provided a reference for the health risk assessment of MPs in the natural environment, contributing to our understanding the action mode of MPs at environmentally relevant concentrations.

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1. Introduction

As emerging contaminants, microplastics (MPs, <5 mm in size) have been observed in diverse marine and freshwater matrices worldwide (Cole et al., 2011; Eerkes-Medrano et al., 2015; Barceló and Picó, 2019; Du et al., 2020). With the small size and resemblance to natural food, MPs are bioavailable to various aquatic organisms, posing a potential threat to aquatic life (Botterell et al., 2019; Alomar et al., 2020). Therefore, the toxicity of MPs to aquatic organisms has become the research focus and aroused extensive attention in the scientific community (Akdogan and Guven, 2019; Ma et al., 2020; Wang et al., 2020). However, many adverse effects of MPs were achieved based on environmentally unrealistic exposure (Lenz et al., 2016; Burns and Boxall, 2018). Bucci et al. (2020) reported that only 17% of exposure concentrations used in experimental studies could be found in the natural environment (i.e. environmentally realistic exposure), while 83% of exposure concentrations were inconsistent with those in the nature (i.e. environmentally unrealistic exposure). Therefore, the biological effects of MPs at environmentally relevant concentrations are still debatable (Besseling et al., 2017; Ziajahromi et al., 2018).

Genotoxicity, as a vital component of a comprehensive toxicological profile, has been affirmed to be mechanistically connected with numerous adverse health consequences, such as neurodegenerative disorders and birth defects (Turkez et al., 2017; Hsieh et al., 2019). Recently, many researchers have performed the genotoxicity assays of MPs on aquatic organisms at environmentally relevant concentrations (Brandts et al., 2018; González-Soto et al., 2019; Revel et al., 2019; Revel et al., 2020; Estrela et al., 2021). However, there were divergent findings documented. For example, Revel et al. (2019) reported that after exposure to MP mixture of 287 μm polyethylene (PE) and 204 μm polypropylene (PP) at concentrations of 10 and 100 μg/L for 10 days, the percentage of DNA in the comet tail of *Mytilus* spp. was significantly increased. Conversely, Revel et al. (2020) stated that after *Crassostrea gigas* were exposed to 300 μm PE and 200 μm PP mixture at concentrations of 10 and 100 μg/L for 10 days, genotoxicity did not occur, but presented a potential positive effect of repairing DNA damage, manifesting as the decreased tailed DNA in MPs-treated groups. Due to the conflicting findings and inconsistent experimental designs (e.g. tested species, MP composition, and exposure time), it remains difficult to determine whether MPs at environmental levels could induce genotoxicity in aquatic organisms. Therefore, a proper analysis is required for logical assessment.

Meta-analysis provides a statistical framework and quantitative tool for rigorously comparing and synthesizing the outcomes of studies with the same topic, which allows us to measure the variation between studies and further explain the variability with defined moderators (Harrison, 2011; Gurevitch et al., 2018; Higgins et al., 2020). Recently, several meta-analyses on MPs have been reported. Foley et al. (2018) pooled the existing consistent/inconsistent evidence, indicating that MP exposure had significantly negative influence on the survival, growth, reproduction and consumption of aquatic organisms. Everaert et al. (2018) meta-analyzed the ecotoxicity data concerning the effect of MPs on marine organisms, suggesting that the adverse effect of buoyant MPs in the marine environment might occur at concentrations above 6650 particles/m^3. Burns and Boxall (2018) reviewed the literature reporting the abundance of MPs in the natural environment and the impacts of MPs on aquatic organisms in laboratory studies, identifying weak evidence of environmental harm caused by MPs, and a mismatch between MPs used in experiments and those measured in the natural environment. Based on systematic review and meta-analysis, Bucci et al. (2020) further confirmed this mismatch, suggesting that 83% of concentrations and 80% of sizes used in exposure experiments were environmentally unrealistic. These meta-analyses provided critical information for assessing the environmental/ecological/health risk of MPs in the natural environment from different perspectives. However, there is still a lack of meta-analysis-based evaluation on the potential genotoxicity induced by MPs in aquatic organisms at environmentally relevant concentration. Based on the powerful statistical and analytic capabilities of meta-analysis, this study was conducted to (i) quantitatively determine whether MPs could induce genotoxicity in aquatic organisms at environmentally relevant concentrations; (ii) identity potential factors influencing the genotoxicity of MPs; (iii) discuss possible biochemical processes and consequences of MPs-induced genotoxicity.

2. Materials and methods

2.1. Exposure concentration threshold of MPs

In order to realistically evaluate the potential genotoxicity of MPs to aquatic organisms, the determination of exposure concentration threshold (i.e. maximum exposure concentration) of MPs was a crucial issue prior to screening studies. In this study, the concentration of 1 mg/L was used as the threshold concentration of MPs (*see* also the review of Yu et al. (2020)), based on two reasons: (i) in some laboratory studies focusing on the toxicity of MPs at environmentally relevant concentrations, 1 mg/L was generally used as the exposure concentration threshold of MPs (O'Donovan et al., 2018; Chen et al., 2020a; Teng et al., 2021); (ii) the concentration of 1 mg/L was in the same order of magnitude as the maximum concentrations of MPs reported in multiple field investigations (estimated using an average weight of 5 μg/particle and trawling depth of 0.1 m proposed by Besseling et al. (2019) where needed), such as 0.94 mg/L in Amsterdam canals, the Netherlands (Leslie et al., 2017), 6.96 mg/L in Yellow River estuary, China (Han et al., 2020), 0.62 mg/L in Colombian Caribbean, Colombia (Garcés-Ordóñez et al., 2020), 0.69 mg/L in Sanggou Bay, China (Wang et al., 2019a), 0.63 mg/L in the Gulf of Mannar, India (Patterson et al., 2020), 1.01 mg/L in Geoje Island, South Korea (Song et al., 2014), and 1.26 mg/L in Saigon River, Vietnam (Strady et al., 2020). It should be noted that the threshold concentration (i.e. 1 mg/L) used in this study did not mean that it was the highest concentration of MPs observed in the natural environment, but it could cover/overlap/be at the same order of magnitude as concentrations used in the exposure experiment and measured in the natural environment.

2.2. Literature retrieval

Three databases, including *Web of Science Core Collection*, *the Cochrane Library* and *Medline* were searched according to the Cochrane Handbook v.6.1 (Higgins et al., 2020), using the terms and modifiers: **plastic**, *debris*, *genotoxic**, *mutagenic**, *DNA damage*, *DNA integrity*, *DNA strand break*, *comet assay*, *micronucleus assay*, *the percentage of DNA in tail*, *tailed DNA*, *tail length*, *olive tail moment*, *number of micronuclei*, *aquatic biota*, *aquatic organism*, *aquatic life*, *marine*, *sea*, *ocean*, *freshwater*. Grey literature (e.g. conference proceedings and dissertations) were also supplemented by *Index to Scientific and Technical Proceedings* and *Baidu Scholar*.

2.3. Inclusion criteria

The inclusion criteria were as follows: (i) peer-reviewed research paper; (ii) there was a control group that had not received MP treatment; (iii) reported the biological effects of MPs on the percentage of DNA in tail (TDNA%), tail length (TL), olive tail moment (OTM), and/or the number of micronuclei (NM) of aquatic organisms at environmentally relevant concentrations (≤1 mg/L); (iv) the effects were only induced by MPs (i.e. not combined exposure with trace metals/organic pollutants/antibiotics); (v) documented the mean, standard deviation (*SD*)/standard error (*SE*), and sample size (*n*) of the control and MPs-treated groups.

2.4. Data extraction

Out of 415 retrieved records, 12 publications yielded 44 individual studies met the criteria and were included in this meta-analysis

(Table S1). The following data were extracted: (i) the publication information (e.g. the first author and publication year); (ii) tested species; (iii) living habitat (i.e. freshwater or marine environment); (iv) exposure time and concentration; (v) the characteristics of MPs (composition, morphology and size); (vi) the endpoint with mean, *SD*/*SE* and *n*. If the data were represented graphically, the Getdata Graph Digitizer v.2.24 software (http://getdata-graph-digitizer.com/) was utilized to obtain the values (Yang et al., 2020). Two researchers cross-checked the extracted data, with any discrepancies resolved through discussion or consultation with a third researcher.

2.5. Data analysis

The response ratio (*RR*) was adopted to quantify the effect size (*ES*) of MPs on the genotoxicity of aquatic organisms (Hedges et al., 1999). Eqs. (1) and (2) were used to calculate the *ES* and its variance (*v*), respectively.

$$ES = RR = ln\left(\frac{x_t}{x_c}\right) = ln(x_t) - ln(x_c) \quad (1)$$

$$v = \frac{(SD_t)^2}{n_t x_t^2} + \frac{(SD_c)^2}{n_c x_c^2} \quad (2)$$

where x_c, SD_c and n_c represented the arithmetic mean of the genotoxic endpoints (i.e. TDNA%, TL, OTM and NM), *SD* of the mean and the number of replicate measurements, of the control group, respectively. And x_t, SD_t and n_t were the arithmetic mean, *SD* of the mean and replicate number, of the MPs-treated group, respectively. *SD* could be estimated by *SE* and *n*, using the Eq. (3) (Higgins et al., 2020).

$$SD = SE \times \sqrt{n} \quad (3)$$

The overall *ES* ($\overline{ES^*}$) and its *SE* ($\overline{SE^*}$) were then computed by Eqs. (4) and (5), respectively.

$$\overline{ES^*} = \left(\sum_{i=1}^{k} w_i^* ES_i\right) / \left(\sum_{i=1}^{k} w_i^*\right) \quad (4)$$

$$\overline{SE^*} = 1 / \left(\sum_{i=1}^{k} w_i^*\right)^{\frac{1}{2}} \quad (5)$$

where *k* and *i* represented the number of included endpoints and the *i*th endpoint, respectively. And w_i^* denoted the reciprocal of the total variance of *ES*, which was calculated by the Eq. (6).

$$w_i^* = 1 / \left\{ v_i + \left[\sum_{i=1}^{k} \left(ES_i^2 / v_i\right) - \left(\sum_{i=1}^{k} (ES_i / v_i)\right)^2 / \sum_{i=1}^{k} (1/v_i) - (k-1) \right] \div \left[\sum_{i=1}^{k} (1/v_i) - \sum_{i=1}^{k} (1/v_i)^2 / \sum_{i=1}^{k} (1/v_i) \right] \right\} \quad (6)$$

If the bootstrapped (64,999 iterations) 95% confidence interval (CI) of ($\overline{ES^*}$) did not overlap with zero, a significant induction of MPs on genotoxicity of aquatic organisms at environmentally relevant concentrations was determined (Chen et al., 2017a). The publication bias was examined by Egger's test (Egger et al., 1997) using Stata v.12.0 software (StataCorp, College Station, USA). Group comparison was adopted to identify potential factors influencing the biological effects of MPs. The tested species could be divided into freshwater and marine species according to their living habitat. The composition of MPs was classified as polyamide (PA), PE, PE-PP, and polystyrene (PS), and the morphology was categorized as regular and irregular. As for exposure concentration, according to data distribution, the concentrations of 20, 200, 800 and 1000 μg/L (i.e. $C \le 20$, $20 < C \le 200$, $200 < C \le 800$, $800 < C \le 1000$) were used as the limits of grouping, which could ensure that each group included 8 or more effect sizes. Regarding the exposure duration, a total of 9 different exposure periods were included, which could be divided into three groups (each group involving 9 or more effect sizes), including groups of acute exposure (1, 3 and 4 days), sub-acute exposure (5, 7 and 10 days) and sub-chronic (14, 15 and 26 days) exposure (Bao et al., 2020). In terms of the particle size, the data could be divided into two groups (each group included more than 10 effect sizes), and the grouping limit was set at 1 μm that was also the size limit between nanoplastics (NPs) and large MPs (LMPs) (Gigault et al., 2018). In addition, to test whether the sample size in this meta-analysis was powerful or not, the post-hoc power analysis (via *t*-test) was performed using G*Power v.3.1.9.2 software (Universität Kiel, Kiel, Germany). The conventional statistical power was set at 0.8 (Cohen, 1988). The statistical significance was set at $\alpha = 0.05$ (Higgins et al., 2020).

3. Results and discussion

3.1. MPs-induced genotoxicity at environmentally relevant concentrations

In this meta-analysis, the exposure of aquatic organisms to MPs at environmentally relevant concentrations (≤1 mg/L, median = 0.5 mg/L) induced obvious genotoxicity (*ES* = 0.22; 95% CIs, 0.10 to 0.34; $p < 0.05$), which was reflected by the increased TDNA% (*ES* = 0.18; 95% CIs, 0.02 to 0.35; $p < 0.05$), TL (*ES* = 0.27; 95% CIs, 0.10 to 0.47; $p < 0.05$), OTM (*ES* = 0.15; 95% CIs, −0.15 to 0.54; $p > 0.05$) and NM (*ES* = 0.59; 95% CIs, 0.32 to 0.82; $p < 0.05$). The overall genotoxic damage of aquatic organisms in MPs-treated groups was significantly increased by 24% compared to the control group ($p < 0.05$) (Fig. 1 and Table 1). The post-hoc power analysis showed high statistical

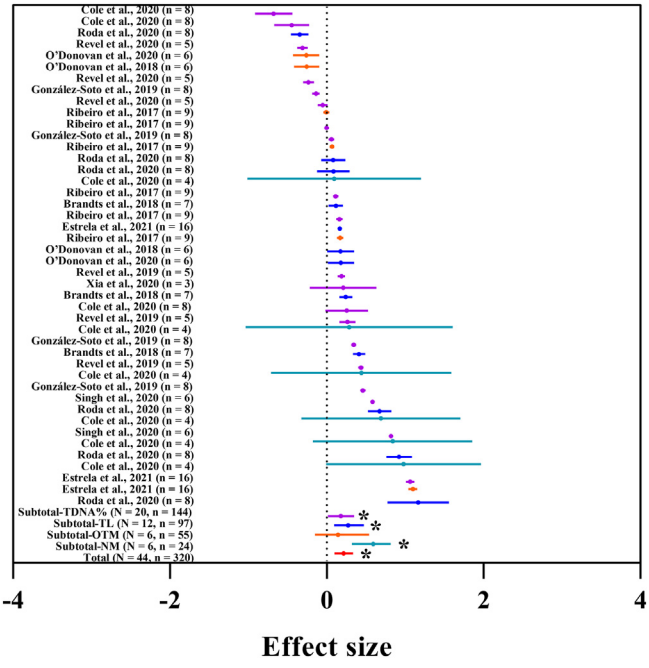

Fig. 1. Forest plot of included individual studies. Detailed information of each study was displayed in Table S1. The asterisk (*) indicated significant alternation of this genotoxic endpoint induced by MP exposure ($p < 0.05$). The capital letter *N* represented the number of included studies, while the lowercase letter *n* denoted the sample size. The 95% confidence interval (CI) of a single study was calculated by *x* standard errors wide, and the factor *x* was from the *t* distribution (*see* Cochrane Handbook, Chapter 6.5.2.2). It should be noted that for a single study, it was not appropriate to determine the significant difference through this figure, as the 95% CI was obtained by estimation rather than given in the original paper, and the bootstrap method was not applicable to a single value. Data were represented by mean with 95% CIs. Abbreviations: NM, the number of micronuclei; OTM, olive tail moment; TDNA%, the percentage of DNA in tail; TL, tail length.

power of the overall effect, reaching 0.99 for one-tailed test, suggesting that the sample size in this meta-analysis ($n = 320$) was of sufficient power to determine the genotoxicity of MPs (Table 1). Specifically, a relatively high statistical power was observed in TL and NM, between 0.76 and 0.88, which was close to or higher than the conventional threshold (Cohen, 1988). TDNA% exhibited a moderate statistical power, with one-tailed test of 0.70 and two-tailed test of 0.58, indicating that at least 191 and 243 samples were needed to reach the power of 0.8. However, very low statistical power was found in OTM (less than 0.3) with high probability of the type II error (above 0.7), suggesting that the current sample size of OTM was insufficient to robustly determine the effect of MPs on OTM. Overall, with high statistical power and no obvious publication bias ($p > 0.05$) (Fig. 2), we can draw a robust conclusion that MPs at environmentally relevant concentrations induced obvious genotoxicity to aquatic organisms. However, the neutral effect of MPs to OTM ($p > 0.05$) identified in this meta-analysis should be interpreted carefully due to the limited statistical power.

In addition, it should be noted that in some cases, a potential positive effect of MPs might occur, reflected in lower genotoxic damage in MPs-treated group, highlighting the complex effects of MPs on aquatic organisms. After comparing the exposure variables in positive and negative effect studies, the beneficial effect of MPs might be explained as: (i) smaller MPs could cause stronger genotoxicity, while larger MPs were more likely to produce positive effect; (ii) lower concentration of MPs was easier to induce hormesis that could repair the initial damage and strengthen the biological performance (Note that the strengthened biological performance might lead to unknown ecological risks) (Agathokleous and Calabrese, 2020; Calabrese and Agathokleous, 2020); (iii) relatively longer exposure duration could ensure aquatic organisms have time to produce adaptive response to enhance the biological resistance (Jakovljević et al., 2014); (iv) the positive effect induced by MPs was species-specific.

3.2. Potentially genotoxicity-related biochemical processes

Recently, several biochemical processes in aquatic organisms after environmentally realistic exposure to MPs have been reported, including oxidative stress, immune response and DNA repair interference, which provide insights for understanding the genotoxicity of MPs at environmentally relevant concentrations (Imhof et al., 2017; Brandts et al., 2018; Qiao et al., 2019; Revel et al., 2019; Jakubowska et al., 2020; Singh et al., 2020; Estrela et al., 2021).

As for oxidative stress, Jakubowska et al. (2020) proposed that strand breaks, as the major form of DNA damage, was caused by the increased formation of reactive oxygen species (ROS) in response to MP stress. Consistently, Revel et al. (2019) investigated the effect of a 10-day exposure to PE-PP MPs on blue mussels (*Mytilus* spp.) at environmentally relevant concentrations, showing that the increase in TDNA% was accompanied by the production of ROS. Moreover, enhanced lipid peroxidation (LPO) was also observed. Singh et al. (2020) showed that after zebrafish (*Danio rerio*) were exposed to two sizes (0.055 and 0.1 μm) of MPs at 1 mg/L for 5 days, the TDNA% increased, accompanied by the raised LPO levels. In terms of immune response, activation of immune cells by MPs in aquatic organisms at environmentally relevant concentrations was documented. After mussels were exposed to 0.008 and 10 μg/L PE-PP MPs for 10 days, the activities of acid phosphatase, a biomarker to assess the immunity alteration, were significantly improved, accompanied the increased TDNA%, suggesting the simultaneous induction and the potential association of immune response and genotoxic effect induced by MPs (Revel et al., 2019). Consistently, Qiao et al. (2019) indicated that exposure to 20 μm of PP microfibers (10 μg/L) for 21 days activated the immune cells in zebrafish, which could be inferred through the significant increase in the expression level of interleukin-1α. Regarding DNA repair interference, Estrela et al. (2021) proposed that the ingested MPs could interfere with several DNA damage repair pathways/mechanisms. Brandts et al. (2018) also indicated that the exposure to MPs at environmentally relevant concentrations could reduce the expression of DNA damage repair genes to some extent in the gills of mussels, including P-53 tumor suppressor-like (*p53*) and damage inducible gene 45alpha (*gaadd45α*). Similarly, the expression of heat shock protein 70 (*hsp70*)

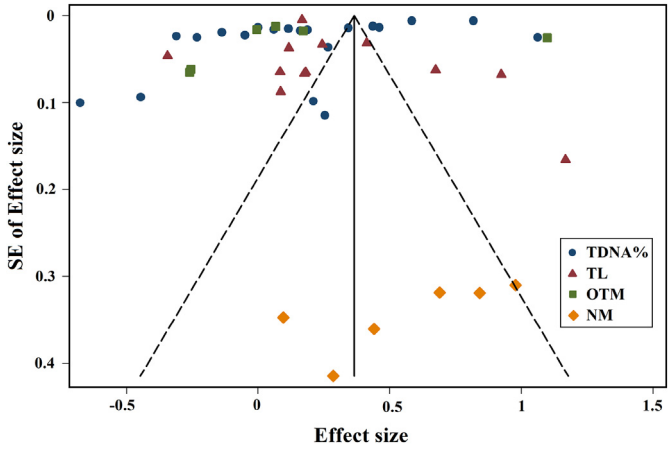

Fig. 2. Funnel plot of the publication bias assessment. The funnel plot was basically symmetrical, and Egger's test indicated that there was no obvious publication bias ($p > 0.05$).

Table 1
The post-hoc power analysis of each endpoint and the overall effect.

Endpoint	Effect size	Bootstrapped 95% CIs	Number of studies (*N*)	Sample size (*n*)	Distribution	Significant level (α)	Tail	Degree of freedom (*df*)	Noncentrality parameter (δ)	Critical *t* value	*P* of type II error	Statistical power
TDNA%	0.1807	(0.0158, 0.3497)	20	144	Normal/Gaussian	0.05	One	143	2.1684	1.6556	0.3039	0.6961
							Two	143	2.1684	1.9767	0.4231	0.5769
TL	0.2742	(0.0980, 0.4708)	12	97	Normal/Gaussian	0.05	One	96	2.7006	1.6609	0.1500	0.8500
							Two	96	2.7006	1.9850	0.2378	0.7622
OTM	0.1466	(−0.1499, 0.5398)	6	55	Normal/Gaussian	0.05	One	54	1.0872	1.6736	0.7161	0.2839
							Two	54	1.0872	2.0049	0.8126	0.1874
NM	0.5913	(0.3224, 0.8155)	6	24	Normal/Gaussian	0.05	One	23	2.8968	1.7139	0.1220	0.8780
							Two	23	2.8968	2.0687	0.2078	0.7922
Overall	0.2154	(0.1009, 0.3378)	44	320	Normal/Gaussian	0.05	One	319	3.8532	1.6496	0.0139	0.9861
							Two	319	3.8532	1.9674	0.0299	0.9701

that could facilitate the DNA damage repair (Sottile and Nadin, 2018) was decreased after the BL2.2 clone of *Daphnia magna* exposed to common plastic polymers for 48 h (Imhof et al., 2017).

However, it is worth noting that the above biochemical alterations might be the adaptive responses of aquatic organisms to cope with the temporal disruption of homeostasis caused by MP exposure (i.e. hormesis) (Calabrese and Mattson, 2017). Hormesis is a biphasic dose response characterized by low dose stimulation and high dose inhibition, which can not only reset the homeostatic setpoint, but also trigger an over-compensatory response to improve the biological resistance and protect the organism from more severe stress (Calabrese, 2001). Correspondingly, Chen et al. (2020b) identified the hormesis phenomenon in the growth of *Scenedesmus obliquus* induced by MPs. Therefore, the existing evidence is not enough to determine whether the above biochemical alterations contribute to the genotoxicity of MPs or enhance the biological resistance by hormesis, which needs to be clarified by future research. Moreover, the potential ecological risks caused by the strengthened biological performance also need to be further explored and clarified.

3.3. Potential link between genotoxicity and generational effects of MPs

Genotoxicity is a key toxicological endpoint due to the direct mechanistic association with the incidence of many adverse health consequences (Oliviero et al., 2019; Cortés et al., 2020). Recently, the within-, inter- and trans-generational effects of MPs at environmentally relevant concentrations in aquatic organisms have been reported and increasingly become a topic of interest (Martins and Guilhermino, 2018; Wang et al., 2019b; Zhang et al., 2019a; Qiang et al., 2020; Yu and Chan, 2020). Within-generational effect refers to the impact of a stimulus (e.g. MPs) on the parental generation (F_0), while inter-generational effect means that F_0 stimulation produces measurable outcomes in the next generation (F_1), and the trans-generational effect describes the impact of F_0 stimulation on the F_2 or F_3 generation (Heard and Martienssen, 2014; Perez and Lehner, 2019). For example, Martins and Guilhermino (2018) investigated the effects of a 21-day exposure to 0.1 mg/L of MPs (1–5 μm) on the growth and reproduction of 4 generations (F_0, F_1, F_2, F_3) of *Daphnia magna*. It was shown that after the exposure of F_0 to MPs, a significant decrease was not only observed in its mobile juveniles and growth by 41% and 8% (within-generational effect), respectively, but also in F_1 by 40% and 7% (inter-generational effect), F_2 by 33% and 4%, and F_3 by 10% and 4% (trans-generational effect), respectively (Fig. 3).

However, the exact mechanism underlying the generational effects of MPs in aquatic organisms has not been ascertained (Zhou et al., 2020). Yu and Chan (2020) proposed that parents might have a false sense of satiation when ingesting non-nutritive MPs, which would reduce the energy available for gonadal development and offspring production. In this work, the identification of genotoxicity of MPs at environmentally relevant concentrations suggested that the generational effects might be due to the breakage/damage of parental DNA induced by MPs, increasing the vulnerability and abnormality of the offspring. It should be noted that in this meta-analysis, the genotoxicity of MPs was determined by somatic cell assays, and whether it could induce genotoxicity to germ cells still needs further experimental verification. However, González-Soto et al. (2019) found that the ingested MPs could accumulate in the intestine and further transfer to the reproductive organs in *Mytilus galloprovincialis*, implying the potential to induce germ cell genotoxicity. Similarly, Sussarellu et al. (2016) also reported that MP exposure (23 μg/L) caused significantly differential expression of 46 transcripts in the gonad of *Crassostrea gigas*. Another evidence was that the exposure to MPs (32 μg/L) induced oxidative stress in the germ cells of mussels, which was manifested as the appearance of ceroids (lipofuscin pigments) in the gonad (Paul-Pont et al., 2016).

3.4. The genotoxic action mode of MPs at environmentally relevant concentrations

The group comparison revealed that the magnitudes of the genotoxicity of MPs to aquatic organisms at environmentally relevant concentrations were independent of selected endpoint (Fig. 4a), MP composition (Fig. 4b), morphology (Fig. 4c), exposure concentration (Fig. 4d) and duration (Fig. 4e) ($p > 0.05$), but closely correlated with particle size (Fig. 4f), living habitat (Fig. 4g) and tested species (Fig. 4h) ($p < 0.05$). These findings suggested that (i) the genotoxic effects of MPs were highly generalizable in response variables, MP composition and morphology, exposure concentration and duration; (ii) MPs could induce widespread genotoxicity even at low concentrations (≤20 μg/L) and short exposure time (≤4 days); (iii) the genotoxicity of MPs to aquatic organisms was species-specific, and freshwater organisms were more susceptible to MP stress, especially NP stress, than marine organisms; (iv) NPs could cause stronger genotoxicity than LMPs, which might be related to the high bioavailability of NPs (Zhang et al., 2019b). Consistently, Foley et al. (2018) also did not identify strong evidence that the effects of MPs on reproduction and survival of aquatic organisms were closely related to the MP morphology and exposure duration, and proposed that there might be a threshold concentration above which extra exposure did not increase the risk to organisms. The species-specific responses to MPs stress were widely documented in Long et al. (2017), Mouchi et al. (2019), Reichert et al. (2019), Mueller et al. (2020), Mendrik et al. (2021), Suckling (2021) and Zhang et al. (2021). As for the high toxicity of NPs, Chen et al. (2017b) also indicated that 1 mg/L NPs (50 nm) significantly decreased the

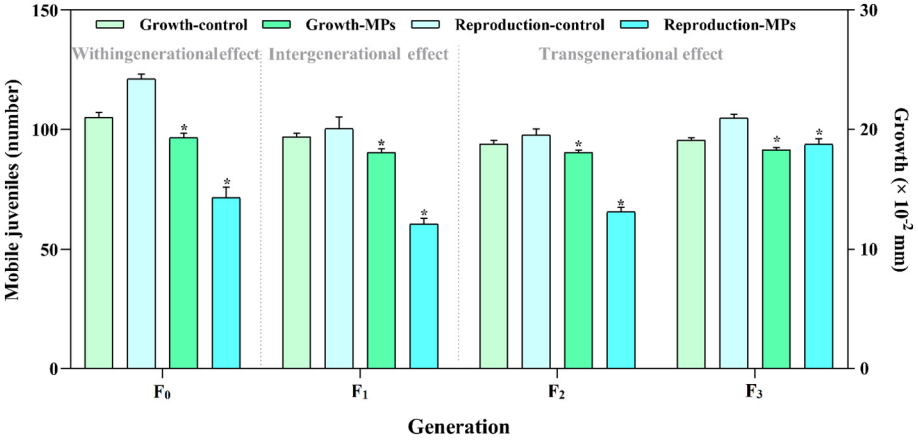

Fig. 3. The generational effects induced by MPs at environmentally relevant concentrations. Data were extracted from Martins and Guilhermino (2018). The asterisk (*) indicated statistical difference ($p < 0.05$) between control and MPs-treated groups in the original paper. Data were represented by mean with standard error.

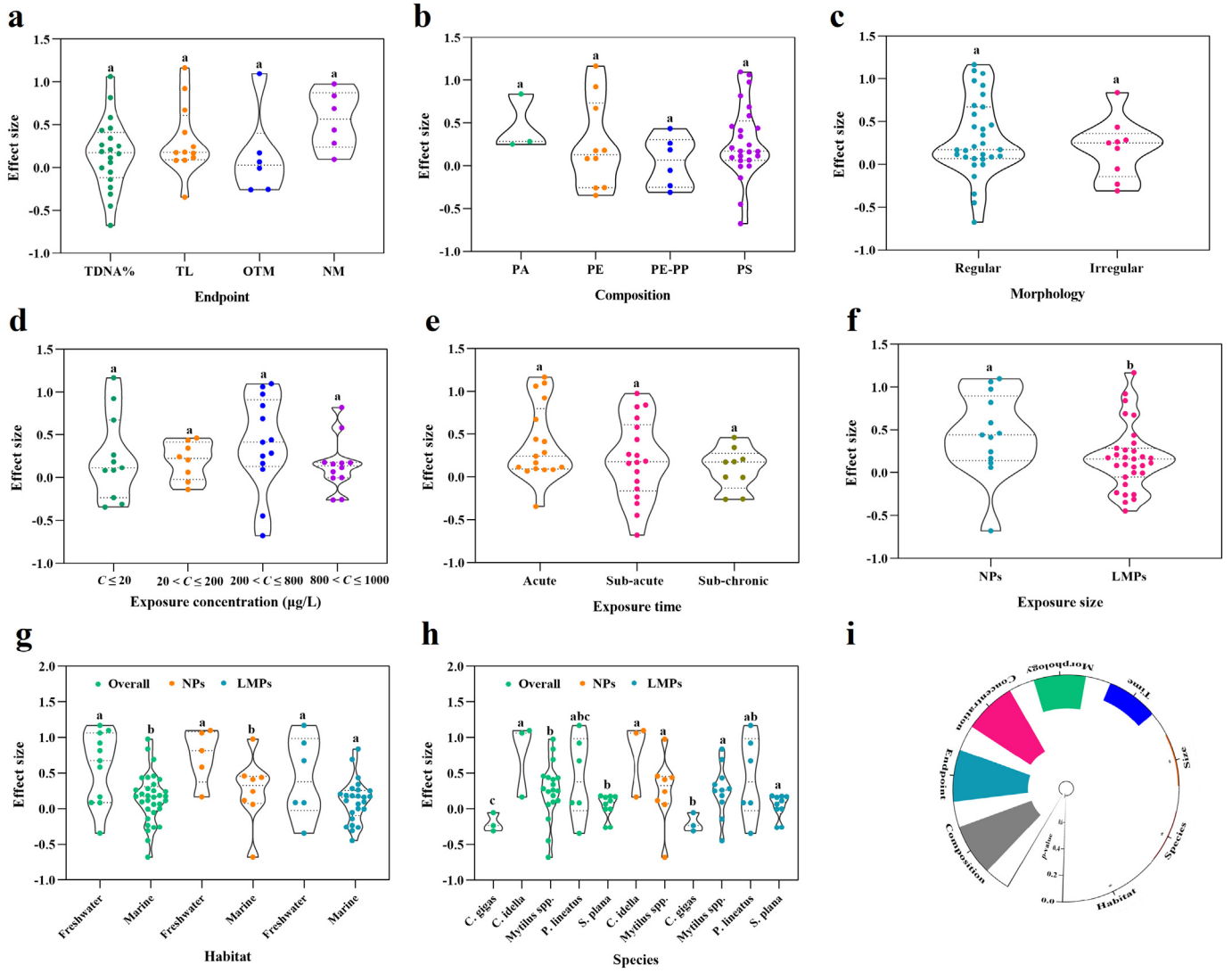

Fig. 4. The group comparison of factors potentially influencing the genotoxic effects of MPs. In order to improve the statistical power, each classification included at least three effect sizes. Different letter and the asterisk (*) denoted statistical difference ($p < 0.05$). Sub-figure (i) summarized the p values of these factors. Group comparisons of other factors (e.g. selected endpoint, composition, morphology, exposure concentration and exposure time) potentially influencing the genotoxicity of NPs and LMPs were displayed in Fig. S1 in the Supplementary data. Abbreviations: PA, polyamide; PE, polyethylene; PP, polypropylene; PS, polystyrene.

locomotor activity of zebrafish by 22%, reduced the body length by 6%, and inhibited the acetylcholinesterase activity by 40%, while 1 mg/L LMPs (45 μm) did not exhibit obvious behavioral toxicity, developmental toxicity and neurotoxicity.

The variable effects of MPs in different habitats indicated that freshwater species were more vulnerable to MP stress, especially NP stress, than marine species, reflecting the higher biological plasticity of marine organisms than freshwater organisms. Moyle et al. (2013) also stated that freshwater organisms were highly susceptible to human-caused changes. However, due to its proximity to urbanized and industrialized areas, freshwater environment is more likely to be a heavily-burdened sink of MPs, and LMPs can be degraded/fragmented into NPs by biotic (e.g. *Euphausia superba* and *Gammarus duebeni*) or abiotic processes (e.g. physical abrasion and UV photodegradation), posing a serious threat to the health of freshwater organisms (Eerkes-Medrano et al., 2015; Dawson et al., 2018; Mateos-cárdenas et al., 2020). Therefore, more efforts are needed to monitor and further remove the MPs in the freshwater environment, as well as in the marine environment for sure (Wagner et al., 2014; Auta et al., 2017; Picó and Barceló, 2019; Padervand et al., 2020). From another perspective, the results also indicated that the genotoxicity of MPs and NPs to fishes was significantly higher than that of aquatic invertebrates (Fig. 5). The difference in MPs-induced genotoxicity between fishes and aquatic invertebrates might be related to their different tolerance to MP/NP stress. Specifically, in this study, most involved aquatic invertebrates were benthos living in the sediment, and the abundance of MPs in the sediment was generally higher than that in the water phase (Scherer et al., 2020), which might lead to the higher tolerance of aquatic invertebrates to MP/NP stress than that of fishes.

In addition, the exposure conditions (e.g. temperature, pH, salinity, dissolved oxygen, and total dissolved solids) may influence the biological effects of MPs (Bhagat et al., 2021), and the exposure concentration may also vary with exposure time due to the deposition and aggregation of MPs (Alimi et al., 2018). However, sometimes these potential factors/parameters have not aroused enough attention and detailed records, which poses a great challenge for future evaluation and comparison between studies. Therefore, it is highly recommended that these factors/parameters can be monitored and reported detailedly in future studies to avoid speculation and increase comparability.

3.5. Limitations

Two limitations in this work should be noted: (i) all studies included in the meta-analysis were conducted under laboratory

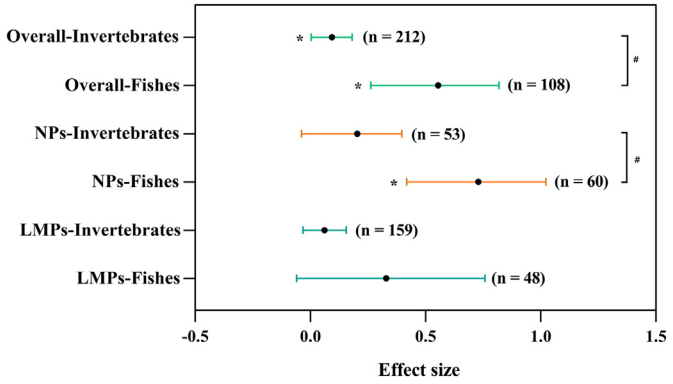

Fig. 5. The comparison of genotoxic responses between fishes and aquatic invertebrates. Data were represented by mean with 95% CIs. The asterisk (*) indicated the occurrence of significant genotoxicity induced by MP/NP exposure ($p < 0.05$). The pound (#) meant significant differences in genotoxic responses between fishes and aquatic invertebrates ($p < 0.05$). The lowercase letter *n* denoted the sample size.

conditions where organisms were isolated from the natural environment and inter- and intra-specific competition, which might weaken the biological resistance and adaptability of the tested animals, leading to the overestimation of the genotoxicity of MPs; (ii) although the meta-analytic method is relatively mature and widely used, potential bias may also occur due to the retrospective nature, for example, the results are influenced by the quality of the original research.

4. Conclusions

This study provided a robust evidence that the exposure to MPs at environmentally relevant concentrations (≤1 mg/L, median = 0.5 mg/L) induced obvious genotoxicity in aquatic organisms, which was reflected in the increase of all included genotoxic endpoints. Among them, TDNA%, TL and NM were remarkably increased by 20%, 32% and 81% compared with the control group, respectively. The overall genotoxic damage of aquatic organisms in MPs-treated groups was significantly increased by 24%, with high statistical power and no obvious publication bias. In addition, the magnitudes of the genotoxic effects of MPs were independent of selected endpoint, MP composition, morphology, exposure concentration and duration, but strongly related to particle size, living habitat and tested species. NPs could cause stronger genotoxicity than LMPs, and freshwater organisms were more susceptible to MP/NP stress than marine organisms.

CRediT authorship contribution statement

Tao Sun: Methodology, Formal analysis, Writing – original draft. **Junfei Zhan:** Formal analysis. **Fei Li:** Writing – review & editing. **Chenglong Ji:** Writing – review & editing. **Huifeng Wu:** Supervision, Writing – review & editing, Project administration.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.147076.

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