Research Paper

Environmentally relevant concentrations of microplastics influence the locomotor activity of aquatic biota

Tao Sun^{a,d}, Junfei Zhan^{a,d}, Fei Li^{a,c}, Chenglong Ji^{a,b,c}, Huifeng Wu^{a,b,c,*}

^a CAS Key Laboratory of Coastal Environmental Processes and Ecological Remediation, Yantai Institute of Coastal Zone Research (YIC), Chinese Academy of Sciences (CAS); Shandong Key Laboratory of Coastal Environmental Processes, YIC-CAS, Yantai 264003, PR China

^b Laboratory for Marine Fisheries Science and Food Production Processes, Qingdao National Laboratory for Marine Science and Technology, Qingdao 266237, PR China

^c Center for Ocean Mega-Science, Chinese Academy of Sciences (CAS), Qingdao 266071, PR China

^d University of Chinese Academy of Sciences, Beijing 100049, PR China

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ABSTRACT

The occurrence of microplastics (MPs) in various marine and freshwater matrices has attracted great attention. However, the effect of MPs in natural environment on the locomotor performance of aquatic biota is still controversial. Therefore, this meta-analysis was conducted, involving 116 effect sizes from 2347 samples, to quantitatively evaluate the alteration in locomotor behavior of aquatic organisms induced by MPs at environmentally relevant concentrations (≤ 1 mg/L, median = 0.125 mg/L). It was shown that MP exposure significantly inhibited the average speed and moved distance of aquatic organisms by 5% and 8% ($p < 0.05$), respectively, compared with the control, resulting in an obvious reduction of locomotor ability by 6% ($p < 0.05$). Egger's test indicated that the results were stable without publication bias ($p > 0.05$). The complex influence of MPs on the locomotor ability were characterized through random-effects meta-regression analyses, presenting size-, time-, concentration-dependent manners and multi-factors interactions. In addition, several physiological changes, including energy reserve reduction, metabolism disorder, gut microbiota dysbiosis, inflammation response, neurotoxic response, and oxidative stress, of aquatic organisms triggered by MP exposure at environmentally relevant concentrations were also provided, which might account for the MPs-induced locomotor activity decline.

1. Introduction

As an important sink, the aquatic environment has continually accumulated a large amount of pollutants, threatening the health and viability of aquatic organisms (Barboza et al., 2018a; Alomar et al., 2020; Patel et al., 2020). Among these pollutants, the ubiquitous microplastics (MPs, < 5 mm in size) have attracted global concerns (Li et al., 2020a; Miller et al., 2020). Numerous studies focus on the potential toxicity of MPs to aquatic organisms (Gambardella et al., 2017; Ziajahromi et al., 2018; De Felice et al., 2019; Naidoo and Glassom, 2019; Costa et al., 2020; Horn et al., 2020; Hu et al., 2021). However, due to the inconsistency in study design, and the mismatch between MP concentrations selected in laboratory studies and those monitored in realistic environments, the toxicity of MPs in the natural environment is still debatable (Burns and Boxall, 2018; Bucci et al., 2020).

For many species, behavioral alterations represent the first response to environmental stressors (Wong and Candolin, 2015). These intentional or unintentional behavioral changes directly influence the fitness indicators (e.g. growth, survival and reproduction), and play key roles in determining how individuals cope with the changing environment (Saaristo et al., 2018; Sievers et al., 2019). The locomotor activity quantified by the average speed and moved distance, as one of the most investigated behavioral responses of aquatic organisms to MP exposure, has been increasingly used as a sensitive indicator for assessing the impact of MPs (Chen et al., 2017a; Limonta et al., 2019; Zhang et al., 2020a). Recently, the contrary effects of MPs on the locomotor activity of aquatic biota at environmentally relevant concentrations have been widely reported, such as the hyperactive swimming behavior (Chen et al., 2020) versus the increased static duration (Bringer et al., 2020), and these arguments are confusing the public and the scientific

* Corresponding author at: CAS Key Laboratory of Coastal Environmental Processes and Ecological Remediation, Yantai Institute of Coastal Zone Research (YIC), Chinese Academy of Sciences (CAS); Shandong Key Laboratory of Coastal Environmental Processes, YIC-CAS, Yantai 264003, PR China.

E-mail address: hfwu@yic.ac.cn (H. Wu).

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community.

Moreover, many studies indicated that MPs ingested by aquatic organisms could be retained and accumulated in the gastrointestinal tract, which could trigger a series of adverse physiological reactions, such as energy reserve reduction (Yin et al., 2019), metabolism disorder (Zhao et al., 2020), gut microbiota dysbiosis (Wan et al., 2019), inflammation response (Jin et al., 2018), neurotoxic response (Tang et al., 2020) and oxidative stress (Barboza et al., 2018b). These adverse effects are closely related to energy supply, physical health and behavior modulation of aquatic organisms, suggesting the potential locomotor alteration induced by MPs (Mattsson et al., 2015; Limonta et al., 2019; Pirsaneh et al., 2020). Therefore, based on the current literature, we hypothesize that MPs can be ingested and accumulated by aquatic organisms, and subsequently cause toxic effects, thereby potentially alter the locomotor activity. However, there is still a lack of evidence-based assessment on the positive, negative or neutral effects of MPs on the locomotor activity of aquatic organisms at environmentally relevant concentrations.

Promisingly, meta-analysis is particularly powerful in integrating the conflicting findings with the same topic to determine a general trend and obtain a reliable estimator (Gurevitch et al., 2018). For example, Arciga and Soliman (2020) combined the existing positive/negative/neutral evidence by meta-analysis, identifying that MP exposure could reduce the growth rate of aquatic organisms. Based on the strong statistical power of meta-analysis, therefore, this work was conducted, aiming to (i) quantitatively evaluate the biological effect of MPs at environmentally relevant concentrations on the locomotor activity of aquatic organisms; (ii) explore the factors that may influence the effect of MPs; (iii) delineate potential mechanisms underlying MPs-induced locomotor activity decline. The biological effects of MPs on aquatic biota can be generally divided into two types: particle effect and chemical effect. The former is due to the direct interaction between MPs and organism tissues, while the latter is caused by the transfer of pollutants adsorbed on the surface of MPs or the leaching of plastic additives/monomers (Fossi et al., 2014; Pedà et al., 2016; Limonta et al., 2019). In this study, the effects of MPs refer to both particle effect and leachate-induced potential chemical effect.

2. Materials and methods

2.1. Literature retrieval

Following to the Cochrane Handbook v.6.1 (Higgins et al., 2020), three databases including *Web of Science Core Collection*, the *Cochrane Library* and *Medline* were systematically searched, using the terms and modifiers: *plastic*, *debris*, *behavio*, *alter*, *change*, *locomot*, *speed*, *velocity*, *distance*, *swim*, *mov*, *aquatic biota*, *aquatic organism*, *marine*, *sea*, *ocean*, *freshwater*. Gray literature, such as conference papers, dissertations and listed references, were also checked via *Index to Scientific and Technical Proceedings* and *Baidu Scholar*.

2.2. Inclusion criteria

The inclusion criteria for this meta-analysis were as follows: (i) original research paper; (ii) designed the control group; (iii) documented the effects of MPs on average speed and moved distance of aquatic organisms at environmentally relevant concentrations (≤ 1 mg/L) (Leslie et al., 2017; Besseling et al., 2019; Han et al., 2020; Garcés-Ordóñez et al., 2020); (iv) the effect was induced by MPs only (i.e. not combined exposure with other contaminants); (v) reported the mean, sample size, standard deviation (SD)/standard error (SE) for control and treatment groups.

2.3. Data extraction

After final screening, a total of 17 studies from 468 retrieved records were included in this meta-analysis (Table S1). The following

information were extracted from included studies: (i) the first author; (ii) publication year; (iii) the tested species; (iv) the selected morphology, composition, size and concentration of MPs, and exposure duration; (v) the endpoint with sample size, mean, and SD/SE. The Getdata Graph Digitizer v.2.24 software (<http://getdata-graph-digitizer.com/>) was used to measure the data presented in figures (Mazidi et al., 2017). The extracted data were cross-checked by two researchers, and any disagreement was resolved by discussion or consultation with the third researcher.

2.4. Data analysis

The effect size (ES) of MPs on the locomotor activity of aquatic biota was evaluated using the response ratio (RR) (Hedges et al., 1999; Sievers et al., 2019). The ES and its variance (v) were calculated by formulas (1) and (2), 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 were the arithmetic mean of the locomotor activity, standard deviation of the mean and the replicate number, of the control group, respectively. And x_t , SD_t and n_t represented the arithmetic mean, standard deviation of the mean and the replicate number, of the MPs-treated group, respectively.

Then, the overall ES ($\overline{ES^*}$) and its SE ($\overline{SE^*}$) were calculated by formulas (3) and (4), respectively.

$$\overline{ES^*} = \left(\sum_{i=1}^k w_i^* ES_i \right) / \left(\sum_{i=1}^k w_i^* \right) \quad (3)$$

$$\overline{SE^*} = 1 / \left(\sum_{i=1}^k w_i^* \right)^{1/2} \quad (4)$$

where i and k denoted i th endpoint and the number of included endpoints, respectively. And w_i^* was the reciprocal of the total variance of ES, which was calculated using the formula (5).

$$w_i^* = 1 / \left\{ v_i + \left[\sum_{i=1}^k (ES_i^2 / v_i) - \left(\sum_{i=1}^k (ES_i / v_i) \right)^2 / \sum_{i=1}^k (1 / v_i) - (k - 1) \right] / \left[\sum_{i=1}^k (1 / v_i) - \sum_{i=1}^k (1 / v_i)^2 / \sum_{i=1}^k (1 / v_i) \right] \right\} \quad (5)$$

A significant effect of MPs on the locomotor activity of aquatic biota was determined if the bootstrapped (64,999 iterations) 95% confidence interval of $\overline{ES^*}$ did not overlap with zero (Chen et al., 2017b; De Stefano and Jacobson, 2018; Cole, 2020).

The publication bias was visually detected by the funnel plot that was produced by Stata v.12.0 software (StataCorp, College Station, USA) (Higgins et al., 2020), and quantitatively assessed using the Egger's test (Egger et al., 1997). The random-effects meta-regression was employed via the **metareg** command in Stata to explore the potential factor that might influence the effect of MPs (see Chapter 10.11.4 of the Cochrane Handbook). The statistical significance was set at $\alpha = 0.05$.

3. Results and discussion

3.1. MPs-induced locomotor activity decline in aquatic biota

In this meta-analysis, aquatic organisms showed a significant decrease in locomotor activity by 6% after exposure to MPs at environmentally relevant concentrations (≤ 1 mg/L, median = 0.125 mg/L)

compared with the control ($p < 0.05$), which was manifested as the obvious reductions of average speed and moved distance by 5% and 8%, respectively ($p < 0.05$) (Fig. 1). The Egger's test indicated that this retrospective analysis was robust without evident publication bias ($p > 0.05$) (Fig. 2).

Locomotor behaviors play integral roles in feeding and social activities of aquatic organisms throughout their lifespan (Colwill and Creton, 2011; da Silva Souza et al., 2020). The locomotion speed was closely associated with the predator-avoidance behavior (Pang et al., 2014; Yin et al., 2019). The decrease of speed caused by MPs suggested that aquatic organisms ingesting MPs might not only reduce predation, but also be exposed to high risk of being preyed upon. Rist et al. (2017) reported that the microalgae ingestion rate of *Daphnia magna* significantly decreased by 21% after exposure to 1 mg/L polystyrene (PS) MPs for 24 h. Similarly, MPs-induced inhibition of feeding activity was also observed in *Neomysis japonica*, with a maximum of 20% at 250 µg/L PS MPs for 0.5 h (Wang et al., 2020). As proposed by Rotjan et al. (2019), MPs were barriers to food intake. In terms of the predation risk, Seuront (2018) evaluated the effect of environmentally relevant MP leachates on the predator (*Carcinus maenas*) – prey (*Littorina littorea*) interaction, indicating the increase of vulnerability and the decrease of escape response of *L. littorea*. In addition, the moved distance was a reflection of the explorative ability, and its reduction implied that after MP exposure, aquatic organisms tended to stay closer together in small-scale areas against the harsh environment, that was, the shoaling behavior (Gerlai, 2014; Mattsson et al., 2015; Wang et al., 2020). Correspondingly, the shoaling behaviors of zebrafish (*Danio rerio*) and black rockfish (*Sebastes schlegelii*) induced by MPs at environmentally relevant concentrations were revealed by Sarasamma et al. (2020) and Yin et al. (2019), respectively, which was reflected in the average inter-fish distance significantly decreased by 7–74% compared with control groups (Fig. 3). Therefore, the MPs in the natural environment could influence the locomotor activity of aquatic organisms and further produced a negative effect on their hunting behavior and exploration competence.

3.2. Factors influencing the biological effect of MPs

Previous studies on other endpoints suggested that the biological effects of MPs were influenced by multiple abiotic and biotic factors, such as the size-dependent reactive oxygen species (ROS) generation in the haemocytes of *Tegillarca granosa* (Shi et al., 2020), the dose-response relationship between MP concentration and dimethylsulfide production in *Tigriopus japonicus* (Yu et al., 2020), and the highly variable impacts of MPs on the growth of different aquatic biota (Foley et al., 2018). Consistently, a size-dependent pattern of locomotor activity was

reported by Chen et al. (2017a) through investigating the effects of two sizes of MPs (0.05 µm and 50 µm) on the locomotor activity of zebrafish. Results showed that 0.05 µm MPs significantly suppressed the larval locomotion by 22%, while 50 µm MPs did not exhibit obvious influence. However, whether the biological effects of MPs are affected by other factors (e.g. MP composition, concentration, and exposure duration), and to what extent these laboratory-based findings can be applied to evaluate the risk of MPs in the natural environment are still unclear. In this meta-analysis, a relatively complex exposure framework (e.g. various species, different concentrations, and dissimilar sizes) was established by combining diverse individual studies. Therefore, the time is ripe for understanding the mode of MP action by exploring the factors that may influence the biological effect of MPs on the locomotor activity of aquatic biota.

In this study, the univariate meta-regression analyses demonstrated that the effects of MPs on the locomotor activity presented size-, time- and concentration-dependent manners ($p < 0.05$) (Fig. 4a–c). Specifically, the locomotor performance of aquatic organisms enhanced significantly with the increase of MP size (Fig. 4a), suggesting that the smaller MPs could induce a stronger behavioral toxicity. The increased toxicity might be due to the higher availability of the smaller MPs (Jeong et al., 2016; Botterell et al., 2019). Interestingly, the locomotor activity of aquatic organisms increased significantly with the extension of exposure time (Fig. 4b), which challenged some previous observations on other endpoints, such as the negative relationship between lysosomal membrane stability and exposure duration (von Moos et al., 2012). This inconsistency could be explained by (i) the biological effect of MPs was closely related to the selected endpoint (Wright et al., 2013a; Anbumani and Kakkar, 2018); (ii) organisms had certain adaptability and self-healing potential. Prolonging exposure time enabled organisms to adapt and respond to MP stress, and this adaptive response could strengthen the biological performance by overcompensating for MPs-induced homeostasis disruption (i.e. the hormetic effect) (Calabrese, 2014). However, it could be speculated that if the exposure duration was extended above a certain threshold, the locomotor activity would be inhibited since MPs could impede the feeding and continuously reduce the energy gained (Cole et al., 2015). Expectedly, the locomotor ability declined obviously with the increase of exposure concentration (Fig. 4c), as the higher the MP concentration, the greater the adverse effect of induction (Zhang et al., 2019a; Tunali et al., 2020). The concentration-dependent effect of MPs presents a very interesting hypothesis, that is, when the concentration of MPs is very low, positive effects induced by MPs may be observed, which is particularly important for the risk assessment of MPs in relatively clean aquatic environment. However, this hypothesis needs further verification, due to the complex

Fig. 1. The alterations in locomotor activity of aquatic biota induced by MPs at environmentally relevant concentrations. The red arrows represented the decline of locomotor performance. The asterisk (*) indicated statistical difference ($p < 0.05$) between control and MP treatment groups.

Fig. 2. Funnel plots of publication bias assessments. Sub-figures (a), (b) and (c) represented the publication bias assessments for average speed, moved distance and all endpoints, respectively. There was no obvious publication bias when the p -value was greater than 0.05 (Egger et al., 1997).

action mode of MPs. Therefore, based on this meta-analysis, we call for more future studies to focus on the effects of MPs at low concentrations, especially the potential dose-response relationship. Furthermore, the impact of MPs on the locomotor activity of tested species did not exhibit species specificity, highlighting the widespread influence/threat/risk of MPs on the aquatic environment. In addition, the multivariate meta-regression analyses revealed the complex action mode of MPs on

Fig. 3. The shoaling behavior of aquatic organisms induced by MPs at environmentally relevant concentrations. Data were extracted from Sarasamma et al. (2020) and Yin et al. (2019). The asterisk (*) denoted the significant difference ($p < 0.05$) of inter-fish distance between the control and MP treatment groups in the original paper.

the locomotor performance of aquatic organisms, manifested as the interaction of multiple factors (Fig. 4d). Currently, the interactions between MPs and other contaminants, such as organic pollutants (Güven et al., 2018; Li et al., 2020b; Nobre et al., 2020), trace metals (Barboza et al., 2018a; Banaee et al., 2019; Sikdokur et al., 2020) and antibiotics (Fonte et al., 2016; Zhang et al., 2019b; Zhou et al., 2020), have been widely concerned. However, the interactions among MP characteristics, and between MPs and species have received limited attention, resulting in the mismatch and knowledge gap between the selected exposure condition and the complicated natural environment (Burns and Boxall, 2018; Akdogan and Güven, 2019; de Ruijter et al., 2020). Therefore, the interaction of multiple factors is necessary to be considered in the future MP relevant toxicity studies to address the mismatch and knowledge gap.

Additionally, methodological diversity is one of the potential contributors of variations between studies (Higgins et al., 2020). In this study, therefore, methodologies adopted by the locomotor activity assay were evaluated, indicating multiple inconsistencies, primarily embodied in six aspects: acclimation, test sequence, test paradigm, test time, test procedure, and data analysis (Table 1). These inconsistencies in study design could cause potential bias in assessing the biological effects of MPs (Savović et al., 2012; Fok et al., 2020). For example, in the test environment, unacclimated animals might be stimulated, which contributed to potential locomotor alternation. Similarly, different test time might produce different results, as circadian rhythm variation was observed in the locomotor activity (Vera et al., 2009; Ebrahimi et al., 2017; Krylov et al., 2020). Moreover, the absence of information on experimental methods (e.g. only four studies reported the test time) posed a challenge to the inter-study comparison of the effects of MPs. Therefore, it is highly recommended that a consistent and relatively standardized proposal, characterized (but not limited) by designing an adaptation period, giving priority to the behavior assay, using the same test paradigm, adopting the circadian rhythm matched test time, employing professional analysis software, and reporting detailed test information, should be considered for future studies to obtain less biased results and increase the comparability between studies.

3.3. Several physiological changes after exposure to MPs

Recently, some studies have reported several physiological changes, including energy reserve reduction, metabolism disorder, gut microbiota dysbiosis, inflammation response, neurotoxic response, and oxidative stress, of aquatic organisms triggered by MP exposure at

Fig. 4. The random-effects meta-regression analyses of factors that potentially influence the biological effects of MPs. The p -value less than 0.05 indicated that this factor contributed to the effect of MPs. The capital letters of A, T, M, S, D, and C in the sub-figure (d) represented the tested animals, selected MP composition, morphology, size, exposure duration, and exposure concentration, respectively.

Table 1

The locomotor activity assay design of included studies.

Included study	Acclimation (min)	Test sequence	Test paradigm	Test time	Test procedure ^a	Behavior analysis technology
Barboza et al., 2018a	–	First	Against water flow	–	ME → SR → DA → OR	–
Bringer et al., 2020	–	First	Free swimming	–	ME → SR → DA → OR	ImageJ v.1.52a software
Chen et al., 2017a	–	First	Free swimming	–	ME → SR → DA → OR	EthoVision XT 10 software
Chen et al., 2020	5	First	Free swimming	–	ME → AC → SR → DA → OR	EthoVision XT software
De Felice et al., 2019	30	First	Free swimming	–	ME → AC → SR → DA → OR	ImageJ plugin Animal Track
Gambardella et al., 2017	–	Second	Free swimming	–	ME → MA → DA → OR	Swimming Behavioral Recorder system
Gambardella et al., 2018	–	Second	Free swimming	–	ME → MA → DA → OR	Swimming Behavioral Recorder system
Gu et al., 2020	20	First	Free swimming	–	ME → AC → DA → OR	Zebralab Video-Track system
Hu et al., 2021	–	First	Free swimming	–	ME → DA → OR	Zebralab Video-Track system
Limonta et al., 2019	–	First	Free swimming	–	ME → SR → DA → OR	El Temps v.1.179 software
Qiang and Cheng, 2019	10	First	Free swimming	–	ME → AC → DA → OR	Zebralab Video-Track system
Sarasamma et al., 2020	3–5	First	Free swimming	10:00–16:00	ME → AC → SR → DA → OR	idTracker software
Wang et al., 2020	5	First	Free swimming	–	ME → AC → SR → DA → OR	ImageJ software
Yang et al., 2020	–	–	Free swimming	–	ME → SR → DA → OR	Image Pro Plus software
Yin et al., 2019	–	First	Free swimming	9:00–10:00	ME → SR → DA → OR	Image Pro software
Zhang et al., 2020b	10	First	Free swimming	11:30–12:30	ME → AC → SR → DA → OR	EthoVision 12 software
Zhang et al., 2021	10	First	Free swimming	13:30–16:00	ME → AC → SR → DA → OR	EthoVision XT 11.5 software

^a The abbreviations of ME, MA, AC, SR, DA and OR represented microplastic exposure, mortality analysis, acclimation, swimming recorded, data analysis and obtaining result, respectively.

environmentally relevant concentrations, which may provide potential mechanistic bases for understanding MPs-induced locomotor activity decline (Wright et al., 2013b; Paul-Pont et al., 2016; Barboza et al., 2018b; Ding et al., 2018; Jin et al., 2018; Limonta et al., 2019; Wan et al., 2019; Yin et al., 2019; Li et al., 2020c; Tang et al., 2020; Yang et al., 2020; Zhao et al., 2020).

3.3.1. Energy reserve reduction

Yin et al. (2019) investigated the effects of 14-day exposure to 15 μm PS MPs (190 $\mu\text{g/L}$) on the energy reserve and nutritional quality of *S. schlegelii*, indicating that the gross energy, crude protein and crude lipid dramatically reduced by 9%, 3% and 27%, respectively, compared with the control group. Similar effects on this species also appeared when exposed to 0.5 μm PS MPs for 14 days (Fig. 5a) (Yin et al., 2019). Consistently, the lugworm *Arenicola marina* significantly depleted the lipid reserves by up to 50% when exposed to polyvinyl chloride (PVC) MPs for 4 weeks at environmentally relevant concentrations, accompanied by obvious reduction of feeding activity (Wright et al., 2013b). Therefore, the ingestion and bioaccumulation of MPs could cause gut blockage and pseudo-physical satiety, inhibit food intake, thereby reducing the energy reserve, and consequently, limiting the necessary energy supply for the locomotor activity of aquatic organisms (Watts et al., 2015; Rotjan et al., 2019; da Costa Araújo and Malafaia, 2020).

3.3.2. Metabolism disorder

Zhao et al. (2020) exposed zebrafish to 100 $\mu\text{g/L}$ of 5 μm PS MPs for 21 days to evaluate the effect of MPs on the glycolipid metabolism in the liver. Results showed that the levels of major biomarkers tended to decrease in the MPs-treated group, and the levels of glucose, α -ketoglutaric acid, isocitrate dehydrogenase, aldehyde dehydrogenase 9 family member A1a (*ALDH9A1A*), enoyl-coenzyme A, hydratase/3-hydroxyacyl coenzyme A dehydrogenase (*EHHADH*) and aldehyde dehydrogenase-2*2 (*ALDH2*2*) obviously decreased by 47%, 40%, 33%, 71%, 73% and 70%, respectively, indicating the disruption of the hepatic glycolipid metabolism (Fig. 5b). Correspondingly, the transcriptome and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses identified that MP exposure mainly affected the carbon metabolism, amino acid metabolism and lipid metabolism pathways, with 1388 differentially expressed genes (756 down-regulated and 632 up-regulated). Similar findings were reported by Limonta et al. (2019), showing that one of the main effects of MPs on the gene transcription of zebrafish was down-regulation of the energy metabolism pathways. Therefore, MP exposure disrupted the energy homeostasis and inhibited the energy metabolism, resulting in the shortage of energy supply during the endurance locomotion of aquatic organisms (Cederwall et al., 2012; Mattsson et al., 2015; Zhao et al., 2020).

3.3.3. Gut microbiota dysbiosis

Gut microbiota is composed by various types of microorganisms inhabiting the intestines of animals (Kamada et al., 2013; Nieuwdorp et al., 2014). Wan et al. (2019) explored the effects of two sizes (5 and 50 μm) of PS MPs (1 mg/L) on the abundance and diversity of gut microbiota in zebrafish. It was shown that MP exposure obviously changed the structure of gut microbiome, which was reflected in the remarkably decreased abundances of *Bacteroidetes* and γ -*Proteobacteria* in both MPs-treated groups, and the significantly increased abundance of *Firmicutes* in 50 μm MPs-treated group (Fig. 5c). The following principal coordinate analysis (PCA) also revealed the alterations of the main components of gut microbiome in MP treatment groups. Consistently, Li et al., (2020c) also reported the dysbiosis of gut microbiota in *Mytilus edulis* induced by MPs at environmentally realistic concentration (200 $\mu\text{g/L}$), identifying 45 operational taxonomic units (OTUs) that had significant difference in the abundance of microbiota after MP exposure (20 OTUs increased and 25 OTUs decreased). Increasing evidence indicated that the gut microbiota had substantial influence on host energy harvest, energy metabolism, weight regulation and behavior

modulation, and its dysbiosis was harmful to host health and further influenced the locomotor activity (Mayer, 2011; Glennly et al., 2017).

3.3.4. Inflammation response

In order to determine the impacts of MPs on several inflammatory cytokines in the gut, zebrafish were exposed to 1 mg/L of two sizes (0.5 μm and 50 μm) of PS MPs for 14 days (Jin et al., 2018). It was shown that 0.5 μm MPs not only significantly increased the mRNA levels of interleukin-1 α (*IL-1 α*) (2.4-fold), interleukin-1 β (*IL-1 β*) (4.3-fold) and interferon (*IFN*) (2.4-fold), but also remarkably enhanced their protein levels (3.2-, 3.4-, 1.2-fold, respectively), and 50 μm MPs obviously improved the mRNA level of *IFN* (2.3-fold) and the protein level of IL-1 α (2.7-fold) (Fig. 5d), suggesting the occurrence of inflammation in the gut of zebrafish after MP exposure. It seemed that MPs could easily induce the inflammation response in zebrafish, because a remarkable expression of IL-1 α was observed at a very low exposure concentration (10 $\mu\text{g/L}$) (Qiao et al., 2019a). For other species, Wright et al. (2013b) also reported the appearance of inflammation response in *A. marina*, which was represented by the significantly increased phagocytic cells after MP exposure. The inflammation response might be due to the physical damage caused by the blockage of MPs in the gut (Wan et al., 2019). Qiao et al. (2019b) observed obvious histological changes (78%–86%) in the MPs-treated (50 $\mu\text{g/L}$ –500 $\mu\text{g/L}$) gut tissues, which were mainly manifested as bowel wall thinning, villi injury and epithelial damage. Besides that, the gut microbiota dysbiosis has been demonstrated to be closely associated with the inflammation response (Fackelmann and Sommer, 2019). Previous studies suggested that the inflammation response could inhibit the locomotor activity, as organisms with inflammation had decreased food intake, metabolic rate, body weight and fat content (Schneiders et al., 2015; Pirsaneh et al., 2020; Solomando et al., 2020).

3.3.5. Neurotoxic response

The locomotor activity is mainly regulated by the central nervous system (CNS) and generally serves as a sensitive indicator of neurotoxicity (Selderslaghs et al., 2010; Hajjima et al., 2017). The decline of locomotor performance suggested the occurrence of MP-induced neurotoxicity. Evidently, Ding et al. (2018) continuously monitored the changes of acetylcholinesterase (AChE) activity, a key neurotransmitter enzyme indicating neurotoxicity, in the brain of *Oreochromis niloticus* during a 14-day exposure to 0.1 μm PS MPs at the concentrations of 1, 10, and 100 $\mu\text{g/L}$. Obvious neurotoxicity was observed as indicated by the significant reductions of AChE activities at all exposure concentrations, with the maximum inhibition rates of 37.7% on the 3rd day after 10 and 100 $\mu\text{g/L}$ MP exposure (Fig. 5e). Similarly, when *Tegillarca granosa* was exposed to 1 mg/L PS MPs for 14 days, the expression levels of genes encoding neurotransmitter modulatory enzymes (AChE, monoamine oxidase (MAO) and gamma-aminobutyric acid transaminase (GABAT)) and receptors (muscarinic acetylcholine receptor M3 (*mAChR3*), dopamine receptor D3 (*DRD3*) and gamma-aminobutyric acid type A receptor subunit delta (*GABAD*)) tended to decrease, among which *DRD3* and *GABAD* decreased significantly, up to 49% and 69%, respectively, implying the evident neurotoxicity (Tang et al., 2020). The inhibitions of neurotransmitter modulatory enzymes and receptors would cause a marked accumulation of acetylcholine in the brain, and ultimately interfered with the function of neurons involved in the motor modulation (e.g. cholinergic and gamma-aminobutyric acidergic (GABAergic) neurons), resulting in the dysfunction of neural connections and the decline of muscular control, thereby impairing the locomotor performance of aquatic organisms (Pereira et al., 2012; Chen et al., 2017a; Zhang et al., 2020a).

3.3.6. Oxidative stress

The oxidative stress has been proposed as a key mechanism underlying MPs-induced locomotor activity decline in aquatic organisms (Paul-Pont et al., 2016; Chen et al., 2017a; Barboza et al., 2018b; Yang

Fig. 5. Several physiological changes in aquatic biota after exposure to MPs at environmentally relevant concentrations. Sub-figures (a) to (f) represented energy reserve reduction, metabolism disorder, gut microbiota dysbiosis, inflammation response, neurotoxic response, and oxidative stress, respectively, and the data were extracted from Yin et al. (2019), Zhao et al. (2020), Wan et al. (2019), Jin et al. (2018), Ding et al. (2018), and Yang et al. (2020), respectively. Data were presented by mean with standard error. The asterisk (*) indicated the statistical difference ($p < 0.05$) between control and MP treatment groups in the original paper. Abbreviations: α-KA, α-ketoglutaric acid; ACAT2, acyl-CoA: cholesterol acyltransferase 2; AChE, acetylcholinesterase; ALDH2*2, aldehyde dehydrogenase-2*2; ALDH9A1A, aldehyde dehydrogenase 9 family member A1a; ECHS1, enoyl-CoA hydratase; EHADH, enoyl-coenzyme A, hydratase/3-hydroxyacyl coenzyme A dehydrogenase; ICD, isocitrate dehydrogenase; IFN, interferon; IL-1α, interleukin-1α; IL-1β, interleukin-1β; PA, pyruvate acid; ROS, reactive oxygen species; T-CHO, total cholesterol.

et al., 2020). According to the PCA and multiple linear regression analyses by Chen et al. (2017a), after exposure to 1 mg/L MPs for 5 days, the major antioxidant biomarkers constituted a principal component that significantly influenced the locomotor performance of zebrafish. Consistently, Yang et al. (2020) reported the simultaneous appearances of velocity inhibition (6% on average) and ROS increase (21% on average) in *Carassius auratus* after exposure to two sizes of MPs (5 and 50 μm) at environmentally relevant concentrations (10, 100 and 1000 $\mu\text{g/L}$) for 3 and 7 days, implying a potential link between oxidative stress and locomotor defect (Fig. 5f). Paul-Pont et al. (2016) also indicated that exposure of mussels (*Mytilus* spp.) to 32 $\mu\text{g/L}$ PS MPs (2–6 μm) for 7 days could trigger the substantial modulation of oxidative balance, which was characterized by the increased production of ROS and enhanced expression of antioxidant and glutathione-related enzymes. In addition, the neurotoxicity of MPs is generally associated with oxidative stress, due to the high vulnerability of CNS to lipid peroxidation (LPO). LPO is a self-propagating autocatalytic reaction leading to the oxidative damage of lipoproteins, cell membranes and other lipid-containing structures (Khan et al., 2019). Barboza et al. (2018b) found that after *Dicentrarchus labrax* was exposed to 690 $\mu\text{g/L}$ MPs (1–5 μm) for 96 h, the LPO level in the brain significantly increased by 118%, while the AChE activity obviously decreased by 50%, indicating that MP exposure induced oxidative stress and lipid damage in the brain, and subsequently contributed to neurotoxicity affecting the locomotor activity (Salminen and Paul, 2014; Barboza et al., 2018b; Prokić et al., 2019).

4. Conclusions

This study provided a robust evidence that MPs at environmentally relevant concentrations (≤ 1 mg/L, median = 0.125 mg/L) significantly decreased the average speed and moved distance of aquatic biota by 5% and 8%, respectively, resulting in an obvious reduction of the locomotor performance by 6%. The MPs-induced alterations in locomotor activity were influenced by many factors, presenting size-, time-, concentration-dependent manners and multi-factors interactions. Moreover, the biological effect of MPs on locomotor activity was not species-specific among the tested organisms, highlighting the broader influence/threat/risk of MPs on the aquatic environment. In addition, the MPs-induced locomotor activity decline may be related to multiple physiological changes of aquatic organisms after exposure to MPs, including energy reserve reduction, metabolism disorder, gut microbiota dysbiosis, inflammation response, neurotoxic response, and oxidative stress. This work offered a robust analysis to assess the behavioral toxicity of MPs at environmentally relevant concentrations, contributing to understanding the health/environmental/ecological risk of MPs in the natural environment.

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. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2021.125581.

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