Science of the Total Environment 910 (2024) 168457

Contents lists available at ScienceDirect

Science of the Total Environment

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

Polystyrene nanoplastics affected the nutritional quality of *Chlamys farreri* through disturbing the function of gills and physiological metabolism: Comparison with microplastics

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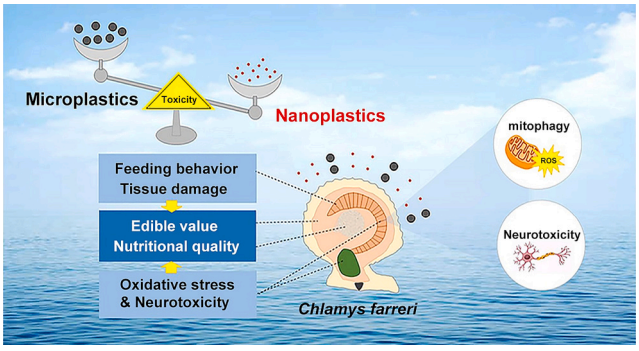
HIGHLIGHTS

- Plastic particles notably decreased the plumpness and protein content of scallops.
- NPs caused more notable impacts on the nutritional quality of scallops than MPs.
- NPs exhibited greater toxicity to the scallops than MPs based on IBR method.
- NPs significantly affected the physiological metabolism of scallops than MPs.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Damià Barceló

Keywords:
Nanoplastics
Microplastics
Scallops
Mariculture
Oxidative stress
Neurotoxicity

ABSTRACT

Although microplastics (MPs) and nanoplastics (NPs) have become a global concern because of their possible hazards to marine organisms, few studies have investigated the effects of MPs/NPs on the nutritional quality of marine economic species, and the toxicity mechanisms remain unclear. We therefore investigated the effects of polystyrene MPs (PS-MPs, 5 μm) and NPs (PS-NPs, 100 nm) at an environmentally relevant concentration on adult scallops *Chlamys farreri* through the determination of nutritional composition, physiological metabolism, enzymatic response, and histopathology. Results showed that plastic particles significantly decreased the plumpness (by 33.32 % for PS-MPs and 36.69 % for PS-NPs) and protein content of the adductor muscle (by 4.88 % for PS-MPs and 8.77 % for PS-NPs) in scallops, with PS-NPs causing more notable impacts than PS-MPs. Based on the integrated biomarker response analysis, PS-NPs exhibited greater toxicity than PS-MPs, suggesting a size-

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https://doi.org/10.1016/j.scitotenv.2023.168457
Received 30 August 2023; Received in revised form 14 October 2023; Accepted 7 November 2023
Available online 18 November 2023

dependent effect for plastic particle. Furthermore, PS-NPs significantly affected the physiological metabolism (e.g., filtration and ammonia excretion) than PS-MPs. Using gill transcriptomics analysis, the key toxicological mechanisms caused by NPs exposure included enrichment of the mitophagy pathway, responses to oxidative stress, and changes related to genes associated with nerves. This study provides new insights into the potential negative effects of MPs/NPs on the mariculture industry.

1. Introduction

Once plastic litter enters the marine environment, it can undergo degradation through mechanical abrasion, solar radiation, wave erosion, biological effects processes that result in the formation of microplastics (MPs, 1 μm–5 mm) and nanoplastics (NPs, 1 nm–1 μm) (Alimi et al., 2018; Moore, 2008). MPs derived from larger particles (secondary microplastics) or manufactured in small size (primary microplastics) are ubiquitous in marine environments and known to be ingested by a wide range of marine organisms (Gardon et al., 2018; Hidalgo-Ruz et al., 2012; Jeong et al., 2017; Xia et al., 2022). Importantly, MPs might represent a human health risk through exposure via the food chain (Cole et al., 2013; Peng et al., 2020). Despite the current analytical challenges associated with their detection and quantification (Ter Halle et al., 2017), NPs have also been reported to exist in marine environments (Li et al., 2023). Although direct evidence of toxicity of MPs/NPs to human is currently limited (Zhou et al., 2020), a series of studies investigating the toxicity of MPs/NPs using model species such as zebrafish suggest exposure could be deleterious, especially to NPs (Zhou et al., 2023a; Zhou et al., 2023b). When compared with MPs, the smaller size and larger specific surface area of NPs enables them to penetrate cell membranes and cross biological barriers (Besseling et al., 2014; Mattsson et al., 2017), consequently being transferred to the circulatory system (Kashiwada, 2006). Therefore, NPs have the potential to cause a greater impact on marine ecosystem than MPs.

Aquaculture plays an increasingly important role in the output of aquatic products (Duarte et al., 2009; Ray et al., 2019). In 2020, the contribution of aquaculture to global aquatic animal production reached 49.2 % (Wenning, 2020). Some studies have shown that MP/NP ingestion caused adverse effects on the physiological functions of fisheries organisms (Gardon et al., 2018; Kibria, 2023; Rist et al., 2016; Wegner et al., 2012). As mariculture species can be utilized as seafood for consumption by humans, it is therefore important to investigate whether exposure and ingestion of MPs/NPs can have any negative impacts on their nutritional quality. For example, polystyrene NPs altered muscle nutritional quality in the large yellow croaker (*Larimichthys crocea*) (Lai et al., 2021). Our previous study also reported that polystyrene MPs affected the nutritional composition of marine jacopever (*Sebastes schlegelii*) (Yin et al., 2018). To our knowledge, however, no studies have investigated the impacts of MPs/NPs on the nutritional composition of marine bivalves, hampering the risk assessment of MPs/NPs in the quality of seafood.

Gill is the primary route for plastic particle internalization via respiration or feeding (Duarte et al., 2009; Galimany et al., 2017). MPs/NPs can adhere to gill filaments upon ingestion, where they can be difficult to discharge. This can lead to a physical interference with the respiration process and a reduction in food intake, ultimately resulting in starvation (Watts et al., 2014; Wright et al., 2013). MPs have been shown to induce physiological and structural alterations in the gills of Indian green mussels through oxidative stress (Vasanthi et al., 2021). When dealing with an excess of reactive oxygen species (ROS), organisms depend not only on the catalytic activity of antioxidant enzymes, but also on the expression level of related genes (Zeng et al., 2023; Zhao et al., 2019). Futhermore, MPs (PS, 100 g/L, 5 ± 0.3 μm) have been shown to cause immune damage and changes in the DNA of gills of red swamp crayfish (Zeng et al., 2023). However, the underlying mechanisms on the effects of MPs/NPs on the nutritional quality and the edible value of marine bivalves remain unclear.

In this study, the marine scallop *Chlamys farreri* was selected as the experimental organism due to its ecological and economic value. We hypothesized that NPs caused greater impact on the nutritional quality of scallops than MPs by disturbing the gill function and physiological metabolism as the result of increased tissue damage, oxidative stress and neurotoxicity. To test this hypothesis, we investigated the impacts of polystyrene MPs (PS-MPs, 5 μm) and NPs (PS-NPs, 100 nm) at an environmentally relevant concentration (0.23 mg/L) on nutritional quality, physiological metabolism, and histopathological alteration of *Chlamys farreri*. The sublethal toxicity effects of PS-MPs/NPs were evaluated by measuring biomarkers for oxidative stress, lipid peroxidation levels, and neurotoxicity. Finally, gill transcriptome analysis allowed the underlying mechanisms to be elucidated for the first time.

2. Materials and methods

2.1. Test organisms

Adult scallops *Chlamys farreri* (mean fresh weight of 10.49 ± 1.02 g and mean shell length of 52.74 ± 1.42 mm) were collected from Laoshan Bay, Qingdao, China (120.544367°E, 36.104882°N). After carefully cleaning the epibionts, the scallops were acclimated in filtered and sterilized natural seawater (temperature of 22.88 ± 1.02 °C, pH of 8.13 ± 0.07, and salinity of 30.66 ± 0.64) with continuous aeration for 1 week. They were fed with the marine algae *Chlorella vulgaris* at a concentration of 6×10^5 cells m/L. The seawater was renewed daily to remove feces.

2.2. Test materials

PS beads (free of sodium azide; density: 1.064 g/cm^3, 10 mg/mL) with diameters of 100 nm and 5 μm were purchased from BaseLine ChromTech Research Centre (Tianjin, China). The polymer type was identified using Fourier transform infrared spectroscopy (Frontier FT-IR Spectrometer, PerkinElmer, U.S.). The morphology and primary size of PS-MPs and PS-NPs were characterized by Scanning electron microscopy (SEM, S-4800, Hitachi, Japan) and transmission electron microscope (TEM, JEM-2100, JEOL, Japan). The average hydrodynamic diameter and zeta potential of the samples (0.23 mg/L) in ultrapure water (pH = 7.02 ± 0.06) and filtered seawater (pH = 8.13 ± 0.07) were further characterized using a Nano laser particle size analyzer (NanoBrook 90Plus PALS, Brookhaven Instruments Corporation, U.S.).

2.3. Experimental design

After acclimation, the scallops were randomly divided into a control (only seawater without PS-NPs/MPs) and two exposure groups, each containing 18 individuals. During the exposure period, scallops were first fed with uncontaminated *Chlorella vulgaris* for 2 h daily, in which time the scallops typically consumed all the microalgae. Subsequently, the scallops were transferred to the aqueous PS-MPs/NPs solutions for 15 days at a concentration of 0.23 mg/L according to environmentally relevant concentrations (Zhang et al., 2019; Barboza et al., 2018; Goldstein et al., 2012), corresponding to 1.44×10^6 particles/L for PS-MPs and 4.19×10^{11} particles/L for PS-NPs. The control and both exposure groups were performed in triplicate.

On days 1, 3 and 7, the gill and digestive gland of nine scallops (three scallops per time node) were sampled, immediately frozen in liquid

nitrogen and stored at −80 °C until measurement of SOD, CAT, GPx, AChE activity, and MDA content. On day 15, three scallops were sampled for the above indicators, as well as histopathology and gene expression analysis. Three scallops were randomly sampled to measure filter-feeding behavior, physiological metabolism and morphological parameters. Finally, three scallops were randomly sampled to determine the nutritional composition. The physicochemical parameters of the seawater used in the exposure studies were identical to those used in acclimation period. No organism mortality was observed throughout the entire experimental period. A diagram outlining of the experimental design is shown in Fig. S1.

2.4. Feeding behavior

After 15 days, the feeding behavior was assessed using the filtration rate (FR) as an indicator, following the approach (Zhao et al., 2017). The dry weight of soft tissue was measured with an electronic balance (Sartorius, Germany). The FR (mg/(g·h)) was calculated with the following formula from a previous study (Coughlan, 1969).

$$FR = [V \times ln(C_0/C_t)]/(W \times t),$$

where C_0 is the initial algae concentration (cells/mL), C_t is the algae concentration at time t (cells/mL), V is the volume of seawater in the exposure container (L), W is the soft tissue dry weight (g) and t is the measurement time (h). Details of the method are provided in the SI.

2.5. Oxygen consumption rate, ammonia excretion rate, and oxygen-to-nitrogen ratio

Scallops were first starved after the 15 days of exposure for 12 h to empty their guts. The ammonium (NH_4^+) excretion was measured according to previous study (Solrzano, 1969), and the soft tissue dry weight was obtained as described above. The oxygen consumption rate (OR) and ammonia excretion rate (NR) were calculated using the following equation:

$$R = [(C_0 - C_t) \times V]/(W \times t),$$

where R is OR (or NR) (mg/(g·h)), C_0 and C_t are the dissolved oxygen (or ammonia) concentrations at the beginning and end of the experiment (mg/L), V is the volume of seawater in the exposure container (L), W is the soft tissue dry weight (g) and t is the measurement time (h). The O/N ratio was obtained through dividing OR by NR in atomic equivalents (Widdows, 1985). The details of the OR and NR methods are provided in the SI.

2.6. Nutritional composition and quality analysis

On day 15, the soft tissues and shells of scallops were dissected and dried in an oven at 65 °C for 72 h. The dry weight was determined using an electronic balance (Sartorius, Germany). The morphological parameters of scallops, including plumpness, visceral index and adductor index, were calculated as the ratio of dry weight of whole tissue, visceral mass, and adductor to dry shell weight of each sample (FW/SW × 100 %) (John and Chen, 1987). Given that adductor muscle is the most important edible part of scallop, the nutritional composition (e.g., moisture, ash, crude protein, and crude lipid) of adductor muscle was determined to further investigate the potential impacts of PS-MPs and PS-NPs on nutritional quality of scallops. Detailed information regarding the nutritional composition is presented in the SI.

2.7. Oxidative stress and neurotoxicity analysis

Samples of gill and digestive gland (approximately 0.5 g) was homogenized in 5 mL ice-cold phosphate buffered saline (0.01 M, pH = 7.4). After centrifuging at 2000 rpm for 10 min at 4 °C, the supernatant was used to measure enzyme activities, which were normalized to the total protein mass (mg). The total proteins were measured using a total protein quantitative assay kit (Coomassie blue staining), the antioxidant enzyme activity (e.g., Superoxide dismutase (SOD), Glutathione peroxidase (GPx), and Catalase (CAT)), Malondialdehyde (MDA)) and neurotoxic enzyme activity (e.g., Acetylcholinesterase (AChE)) of gills and digestive glands were determined using a commercial kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's protocols.

2.8. Integrated biomarker response analysis

The integrated biomarker response index (IBR) is a standardization process for series of biomarkers to compare environmental stress. The results are calculated according to the method described by Beliaeff and Burgeot and modified by Guerlet et al. (Beliaeff and Burgeot, 2002; Guerlet et al., 2010). In this study, we chose the SOD, CAT, GPx, AChE and MDA as biomarkers to calculate the IBR. Details for calculation of the IBR are given in the SI.

2.9. Histopathological and ultrastructural analysis

The gills and digestive glands of the scallops were fixed, dehydrated and sliced for histological analysis. Briefly, the samples were fixed with 10 % formalin and dehydrated through a graded series of ethanol (75 %, 85 %, 95 %, and 100 %) and toluene for intermediate impregnation. Next, the samples were embedded in paraffin, sectioned into 5 μm slices using a microtome, and stained with Hematoxylin and Eosin (H&E). The sections were observed and photographed using an Olympus BX-51 microscope with a digital camera. Histopathological alterations were ranked according to the severity of the lesions [grades 0 (−), 0.5 (+/−), 1 (+), 2 (++) and 3 (+++)] as described in previous studies (Riba et al., 2004). Comparisons of histopathological responses between treatments are expressed as the index of damage.

Ultrastructural analysis of the scallop gills was performed using TEM imaging according to the method (Xia et al., 2020). Briefly, the samples were dissected at about 5 mm in length on ice. The samples were then fixed in 2.5 % glutaraldehyde and post-fixed in 1 % osmium tetroxide at 4 °C for 1 h. They were then dehydrated in a graded series of ethanol and acetone solution and embedded in Epon 812 resin for 5 h. After sectioning using the Ultramicrotome, the ultrastructure of the gill and digestive gland was observed by TEM (JEM-2100, JEOL, Japan).

2.10. Transcriptome sequencing analysis and qRT-PCR validation

The total RNA of each gill sample was isolated using TRIzol Reagent (Invitrogen) and further treated with DNase I (Invitrogen) to remove DNA contamination following the manufacturer's protocol. Sequencing libraries were generated using the NEBNext UltraTM RNA Library Prep Kit for Illumina (NEB, USA). At least 7.06 Gb of Clean Data were generated for each sample where a minimum of 93.87 % of Clean Data achieved a quality score of Q30. The raw sequence reads have been deposited in the Sequence Read Archive (SRA) at the National Center for Biotechnology Information (NCBI) with accession number PRJNA904239. The transcriptome expression results were verified via quantitative real-time PCR (qRT-PCR) and the expression levels of 21 representative differentially expressed genes (DEGs) were determined. The primers used for qRT-PCR analysis and detailed information of the DEGs are listed in Table S1. Detailed information of the RNA extraction procedure and the qRT-PCR method are provided in the SI.

2.11. Statistical analysis

Data are presented as mean ± standard deviation. Before analysis, Levene's and Shapiro–Wilk's tests were used to verify the homogeneity and normality of variance, respectively. One-way ANOVAs with Tukey's

tests were conducted to compare differences among exposure groups. A $p < 0.05$ was considered to indicate statistical significance. All statistical analyses were performed using IBM SPSS Statistics 25.0.

3. Results

3.1. Characterization of polystyrene MPs and NPs

The polystyrene MPs (SEM imaging) and NPs (TEM imaging) are relatively regular in shape, mainly spherical, with a smooth surface (Fig. 1A and B). Particle size analysis showed the mean diameter of 5.08 ± 0.10 μm and 99.56 ± 3.80 nm for PS-MPs and PS-NPs, respectively. The most abundant particle size of PS-MPs ranged from 5.0 μm to 5.2 μm, which represented 76 % of the total particles (Fig. 1C). The two largest particle size abundances for the PS-NPs ranged from 98 nm to 100 nm and from 100 nm to 102 nm, which accounted for 27 % and 25 % of the total particles, respectively (Fig. 1D). The hydrodynamic diameters obtained from dynamic light scattering in ultrapure water and filtered seawater were 7.64 ± 0.57 μm and 13.60 ± 1.12 μm for PS-MPs (the polydispersity indices are 0.68 ± 0.019 and 0.46 ± 0.010, respectively) and 100.91 ± 1.94 nm and 153.81 ± 6.32 nm for PS-NPs (the polydispersity indices are 0.071 ± 0.0066 and 0.15 ± 0.012, respectively), respectively (Fig. S2). The zeta potential values of PS-MPs and PS-NPs were −44.24 ± 1.97 mV, −34.02 ± 0.36 mV in ultrapure water, and −4.13 ± 0.28 mV, −5.33 ± 0.36 mV in filtered seawater, respectively. Fourier transform infrared (FTIR) spectroscopy analysis confirmed that both the MPs and NPs were polystyrene (Fig. S3).

3.2. Nutritional quality and histological alterations

After exposure to PS-MPs/NPs for 15 days, the proportions (e.g., plumpness, visceral index, and adductor index) of the soft tissue in the scallops were significantly reduced relative to the controls (Fig. 2A). Nutritional composition (e.g., moisture, ash, crude protein, and crude lipid) determination of the adductor muscle (Fig. 2B–E) revealed that only the content of crude protein in MP and NP groups was significantly decreased by 4.88 % and 8.77 %, respectively, when compared to the controls (Fig. 2D). Compared to controls, the adductor muscles treated by PS-MPs and PS-NPs had no significant differences in overall morphology, except for endomysium in the NP groups, which had vacuoles in very few parts (Fig. 2F).

3.3. Feeding behavior and physiological metabolism

Compared to the controls, the filtration rates of scallops in the MP and NP groups were significantly reduced by 38.25 % and 67.01 %, respectively (Fig. 3A). There was no significant effect of PS-MPs/NPs on the oxygen consumption rate of scallops (Fig. 3B). However, the ammonia excretion rates of scallops in the MP and NP groups were significantly increased by 340.44 % and 166.19 % of the controls, respectively (Fig. 3C). Additionally, the oxygen-nitrogen ratios were significantly decreased in scallops exposed to PS-MPs and PS-NPs (Fig. 3D).

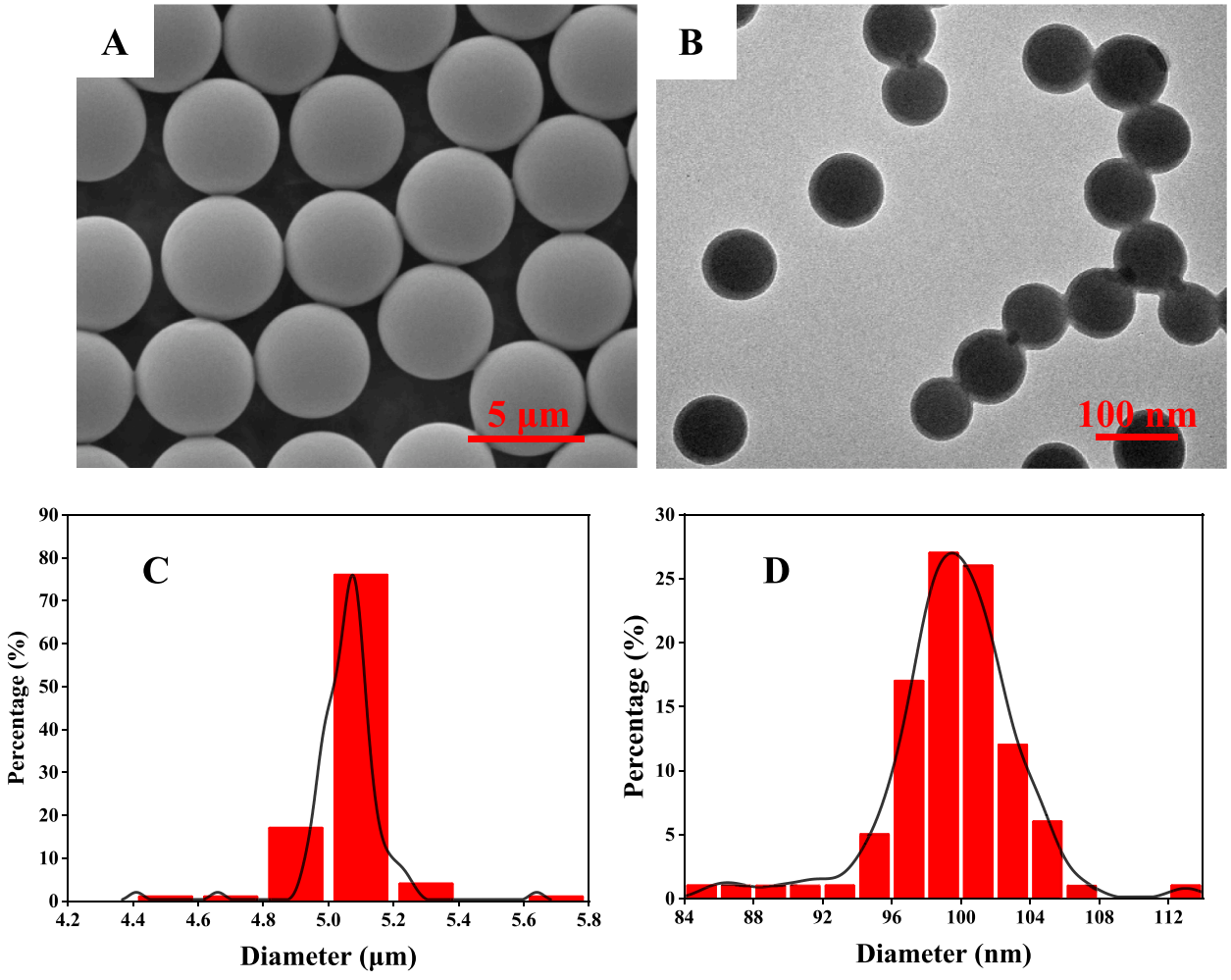


Fig. 1. Morphological characterization by SEM (A) and TEM (B), and size distribution histograms (C, D) of polystyrene MPs and NPs.

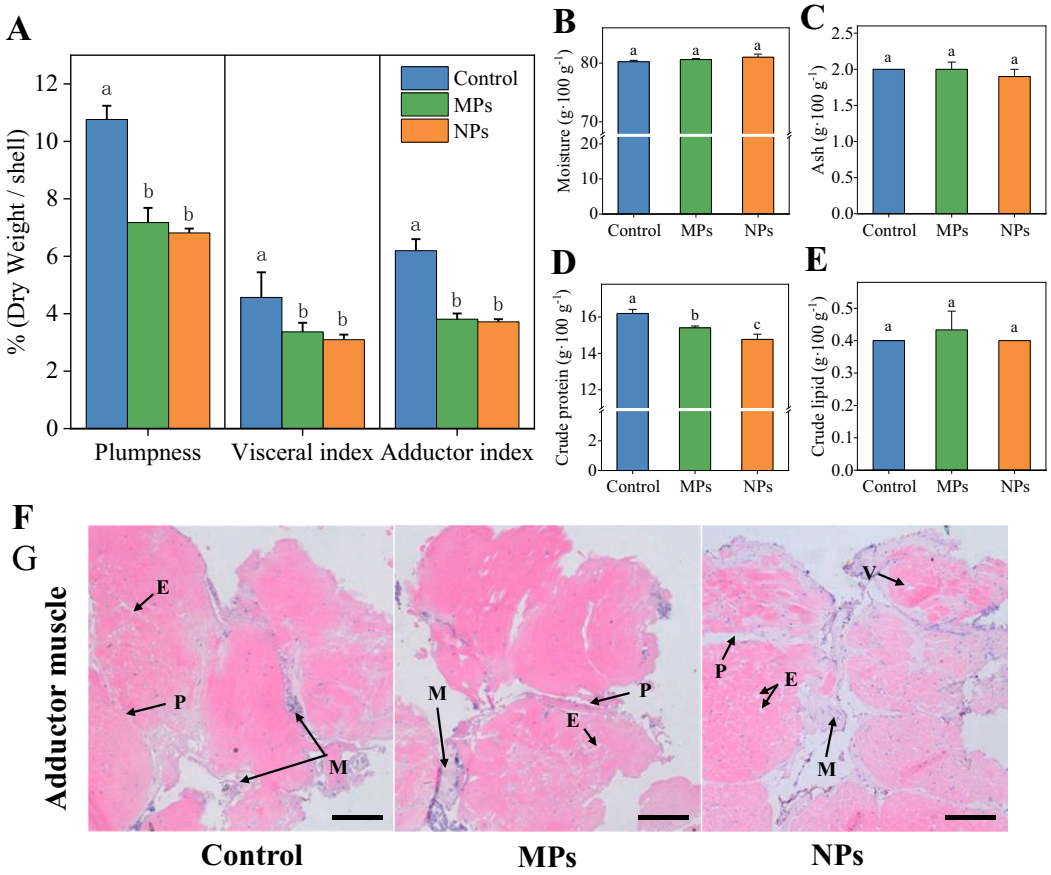


Fig. 2. (A) Morphological parameters of exposed and control scallops (plumpness, visceral index, adductor index) on day 15 ($n = 3$, Tukey's HSD, $^*p < 0.05$). Moisture (B), ash (C), crude protein (D), and crude lipid (E) of adductor muscle on day 15. Significant differences among different treatments are marked with different letters (a-c) (n = 3, Tukey's HSD, $p < 0.05$). The error bars represent SD. (F) Histological analysis of the adductor muscle of control and MPs and NPs exposed scallops on day 15. The collagen fibers and muscle fibers were pink, cell nuclei and ribosomes were blue, and collagen-based connective tissues were in descending order of muscle membrane, perimysium, and endomysium. M: muscle membrane P: perimysium E: endomysium. Bar: 50 μm.

3.4. Oxidative stress, neurotoxicity, and integrated biomarker response

In the gills on day 15, the activities of SOD and CAT in MP groups and NP groups were significantly decreased relative to the controls (Fig. 4A). In the digestive glands on day 15, only the activity of GPx in the MP groups and NP groups increased significantly compared with the controls (Fig. 4B). On day 15, the content of MDA in the MP groups (161.53 %) and NP groups (186.45 %) was significantly increased relative to the controls (Fig. 4A). In the digestive glands, only the content of MDA in NP groups increased significantly on day 7. On day 15, the content of MDA in MP groups (119.66 %) and NP groups (159.47 %) was significantly increased relative to the controls (Fig. 4B). The AChE activity in the gills of MP and NP groups increased significantly on day 7, and decreased significantly on day 15, compared to the controls (Fig. 4A). In the digestive glands, the AChE activity of MP and NP groups increased significantly on day 7. On day 15, only the AChE activity of NP groups (200.01 % of the control) still increased significantly (Fig. 4B). The transformed data from SOD, CAT, GPx, AChE activities and MDA contents of MP and NP groups on days 1, 3, 7 and 15 are presented as star plots (Fig. 5A). Although the digestive glands undergo metabolic regulation (e.g., excretion and detoxification) to relieve pressure on the gills, the oxidative stress and neurotoxicity of the gills are still more severe with increasing MP/NP exposure periods. Based on the calculated IBR values, the gills and digestive glands in the MP and NP groups responded to stress in order of MPs-digestive glands<MPs-gills<NPs-digestive glands<NPs-gills on day 15 (Fig. 5B).

3.5. Histological alterations

The normal morphology of the gills in control organisms exhibited a strong contact between the gill filaments surrounded by lateral and frontal cilia. In MP groups, cilium shedding, disruption of cilia structure and vacuoles were detected in the gills. In NP groups, more severe cilia shedding and damage was observed, as well as branchial adhesion and hemocyte infiltration (Fig. 4C). In the digestive glands of the controls, there were integral and tightly arranged digestive tubules, basophilic cells and eosinophilic cells were evenly distributed and the cytoplasm was intact. In MP groups, an increase in eosinophilic cells, cytoplasmic dispersion and a small amount of the atrophy of tubules were observed. In NP groups, a greater degree of cytoplasmic dispersion and atrophy of tubules was observed compared with the MP groups, while there was also hemocyte infiltration at the border of some digestive tubules (Fig. 4D). The degree of pathological damage in the digestive glands and gills was increased with decreasing PS particles size based on the damage index. In addition, the indexes of damage measured in the gills of scallops exposed to both PS particle sizes of polystyrene were higher than those measured in the digestive glands (Table S2).

3.6. Ultrastructural alterations

In controls, the cilia on gill filaments were arranged in an orderly manner, and the organelles were clear and complete in the columnar epithelial cells (Fig. 6A). Compared to controls, the structure of the cells and organelles in the MP groups was relatively complete, except for

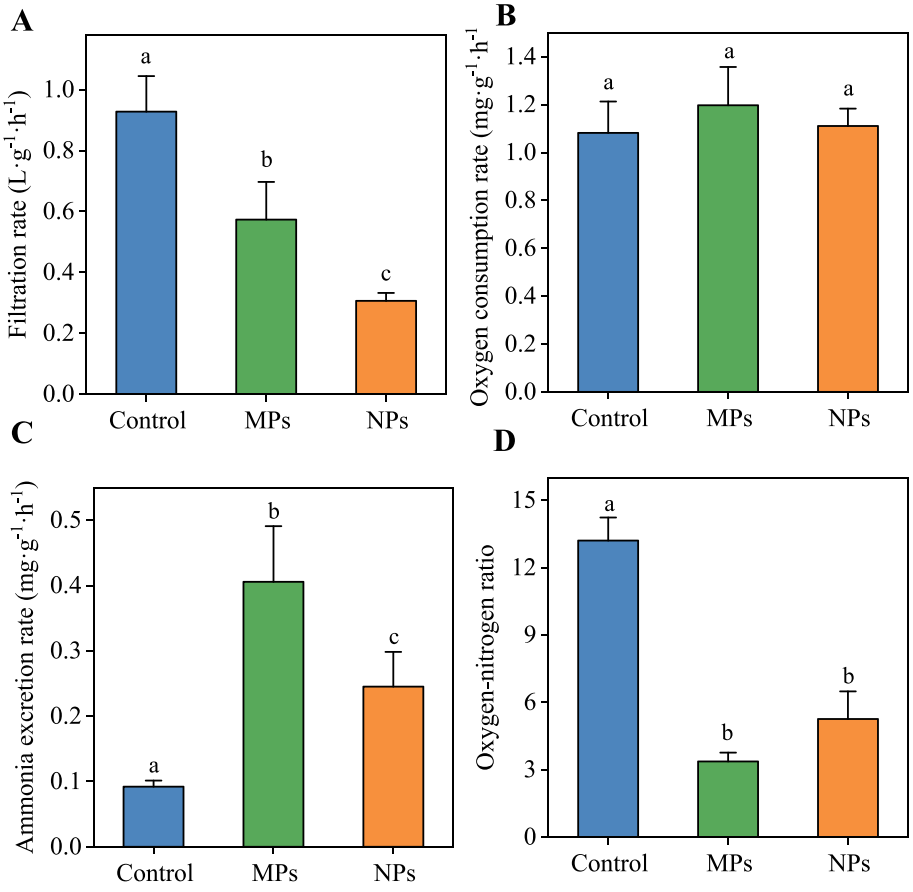


Fig. 3. Filtration rate (A), oxygen consumption rate (B), ammonia excretion rate (C), and oxygen-nitrogen ratio (D) of scallops on day 15. Significant differences among different treatments are marked with different letters (a–c) (n = 3, Tukey's HSD, $p < 0.05$). The error bars represent SD.

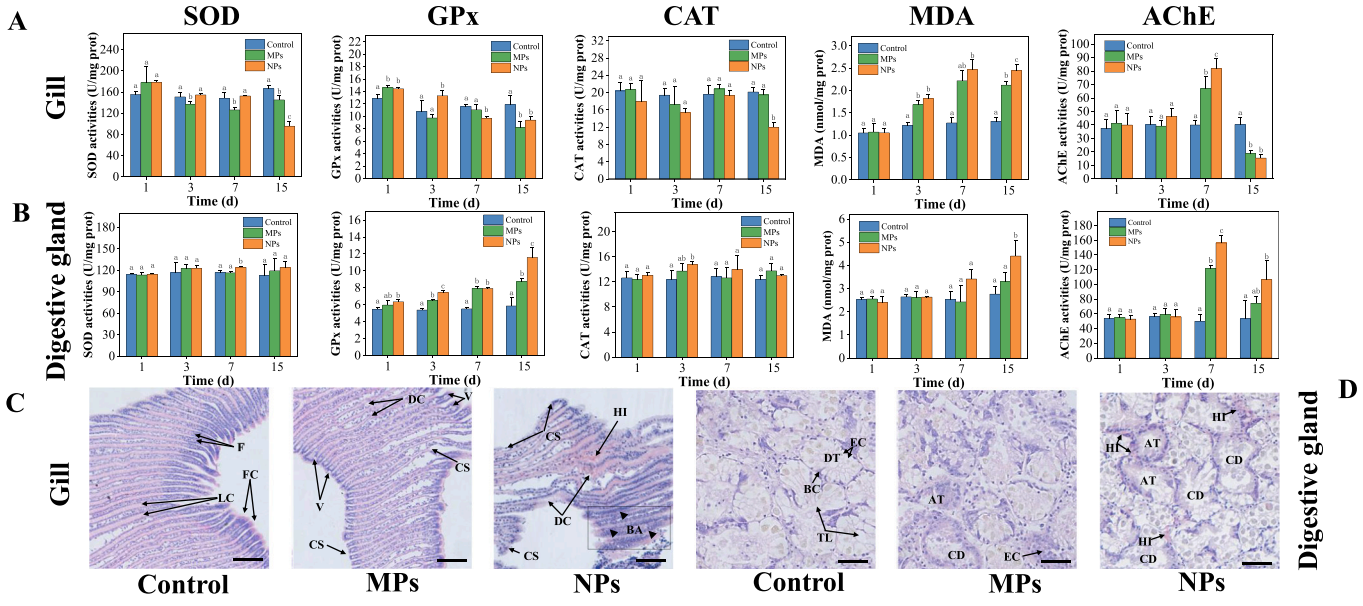
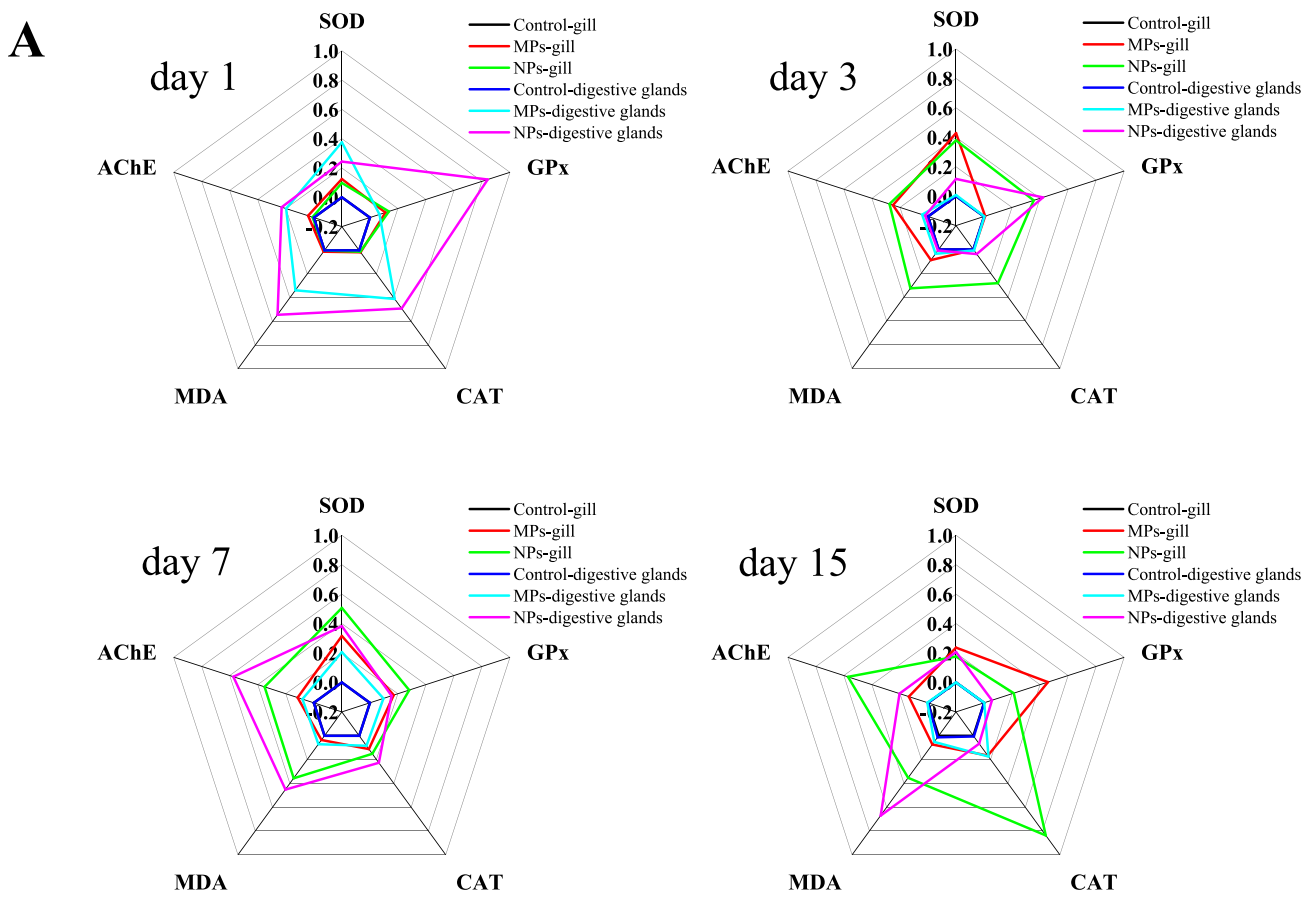


Fig. 4. SOD, GPx, CAT, and AChE activity and MDA content in gills (A) and digestive glands (B) of scallops. Significant differences between different treatments and same times are marked with different letters (a–c). ($n = 12$, Tukey's HSD, $p < 0.05$). Histological analysis of the gills (C) and digestive glands (D) of MPs-exposed and NPs-exposed scallops on day 15. F: gill filaments; LC: lateral cilia; FC: frontal cilia; CS: cilium shedding; DC: disruption of cilia structure; V: vacuole; HI: hemocyte infiltration; BA: branchial adhesion; DT: digestive tubules; TL: lumen of tubule; BC: basophilic cells; EC: eosinophilic cells; CD: cytoplasmic dispersion; AT: atrophy of tubules. Bar: 50 μm.



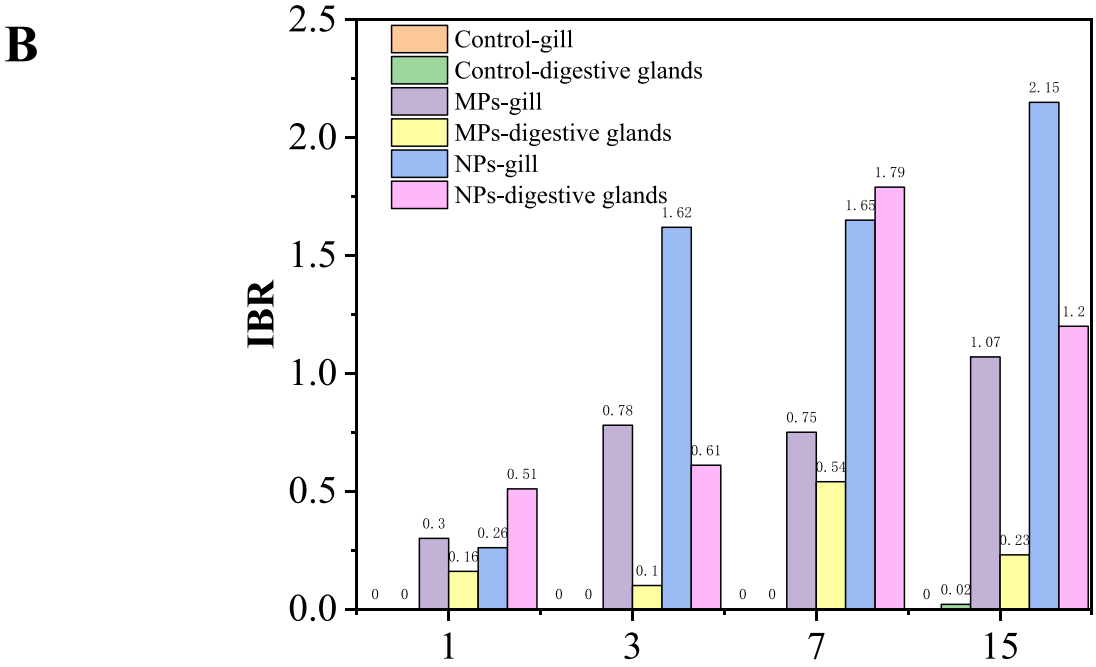


Fig. 5. (A) Star plots of standardized biomarker responses of SOD, GPx, CAT, and AChE activity and MDA content in the gills and digestive glands of scallops treated with MPs and NPs. (B) IBR values in the gills and digestive glands of scallops treated with MPs and NPs.

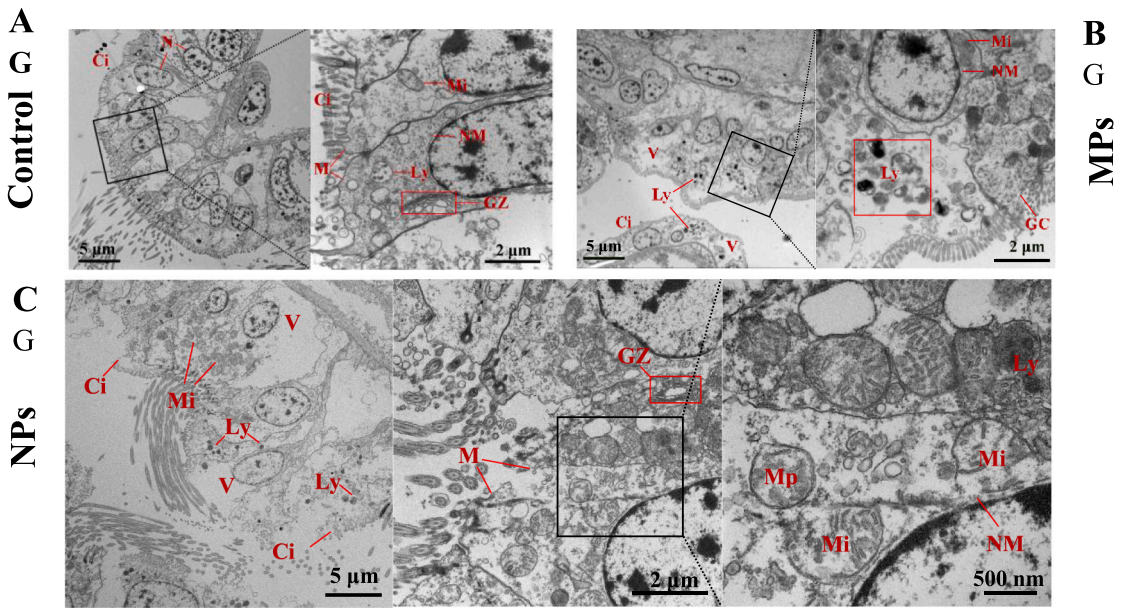


Fig. 6. Ultrastructural alteration analysis of the scallops after exposure to MPs and NPs for 15 days. (A) Gill in control; (B) Gill treated by MPs; (C) Gill treated by NPs. Ci: cilia; N: nucleus; NM: nuclear membrane; M: cell membrane; Mi: mitochondria; Mp: mitophagy; Ly: lysosome; GZ: golgi zone; GC: goblet cells.

some shortened cilia, a small amount of vacuolation and increased lysosomes (Fig. 6B). In the NP groups, cilia appeared broken and the gill filament structure was disordered with swelled, ruptured and vacuolated epithelial cells. Compared with controls, the internal structure of most cells was not clear and partly accompanied by the dissolution of the cell membrane and nuclear membrane. Moreover, the increased lysosomes, mitochondrial fragmentation and mitophagy were observed (Fig. 6C).

3.7. Gill transcriptomic responses

Clean reads of each sample were mapped to a specified reference genome. The mapping ratio ranged from 65.10 % to 74.35 % (Table S3). Linear fitting indicated that there was a strong positive linear correlation between the transcriptome sequencing analysis and qRT-PCR (R^2 = 0.9989, $p < 0.01$) (Fig. S4). These results confirmed that the transcriptome sequencing for the gill tissues of scallops was of high quality and reliable. RNA-seq analysis identified 821 significant DEGs (DESeqb<0.05) in the gills of control and NP groups after 15 days of exposure, where apparent differences in up-regulated and down-regulated genes were exhibited (up-regulated: 376, down-regulated: 445) (Fig. S5). The gill gene expression profiles of the scallops were significantly altered by NPs. Among the DEGs, 8 (6 up, 2 down) were potentially involved in lipid and energy metabolism (Table S4 and S5). In addition, a total of 13 DEGs (12 up, 1 down) putatively participating in transport and catabolism were significantly altered (Table S4 and S6). Additionally, 6 DEGs (5 up, 1 down) were potentially related to nerves (Table S4 and S7). Finally, a total of 9 DEGs (6 up, 3 down) potentially involved in response to oxidative stress were identified (Table S4 and S8).

The analysis results of GO enrichment and KEGG pathway enrichment in scallops exposed to NPs is described in Fig. 7. NPs had significant effects on neural-related biological processes, such as cell projection organization, neuron development, neuron projection morphogenesis, axonogenesis, axon guidance, axon choice point recognition, axon midline choice point recognition and cellular components on the neuronal cell body (Fig. 7A and B). In addition to the cellular components related to nerves (neuronal cell body), most of the enrichment pathways are related to membranes (Fig. 7B). Among molecular functions related to membranes, there was structural molecule activity and chitin synthase activity. Furthermore, the semaphorin receptor activity related to nerves, prostaglandin-endoperoxide synthase activity and glutathione peroxidase activity related to oxidative stress were also enriched (Fig. 7C). NP exposure was also found to alter the pathways involved in arachidonic acid metabolism and in the cholinergic synapse mediated by neuroactive ligand-receptor interaction. IP_3R is also involved in most of the pathways in the nervous system, such as dopaminergic synapse and retrograde endocannabinoid signaling. In terms of NPs impact on oxidative stress, exposure affects autophagy and mitophagy pathways mediated by the AMPK and PI3K-Akt signaling pathways, and might lead to degradation of the inner vesicle and inhibition of oxidative stress resistance and DNA repair. NP exposure also caused a notable up-regulation of endocytosis, phagosome and lysosome-related gene expression, along with oxidative phosphorylation and adhesion junctions (Figs. 7D, S6–S13 and Table S9).

4. Discussion

4.1. PS-MPs and PS-NPs decrease the edible value and nutritional quality of the scallops

The adductor muscle of scallops is widely consumed by humans in various forms for its rich nutrition and delicious taste (Zhao et al., 2021). The significant reduction in the morphological parameters of the scallops and the nutritional components of adductor muscle after a 15-day exposure to PS-MPs/NPs represents a measurable decrease in the edible value and nutritional quality of scallops. To explore the underlying causes of this reduction in nutrition quality of scallops following exposure to PS-MPs and PS-NPs, the feeding behavior and physiological metabolism were investigated. The observed decrease in filtration rate demonstrates that the feeding behavior of the scallops was significantly inhibited by PS-MPs and PS-NPs, which subsequently reduces the energy acquisition capacity from food. The similar results have been reported for blue mussels (*Mytilus edulis*) exposed to polystyrene NPs (30 nm) and clams (*Atactodea striata*) exposed to MPs (63 μm–250 μm) (Wegner et al., 2012; Xu et al., 2017). Bivalve molluscs can adjust their feeding rate according to the duration and intensity of an external stressor (Wu et al., 2019). Under chronic exposure conditions, the nutrient intake of organisms can be reduced for long periods, which may lead to potential starvation (Yin et al., 2018), inhibiting the tissue growth and protein

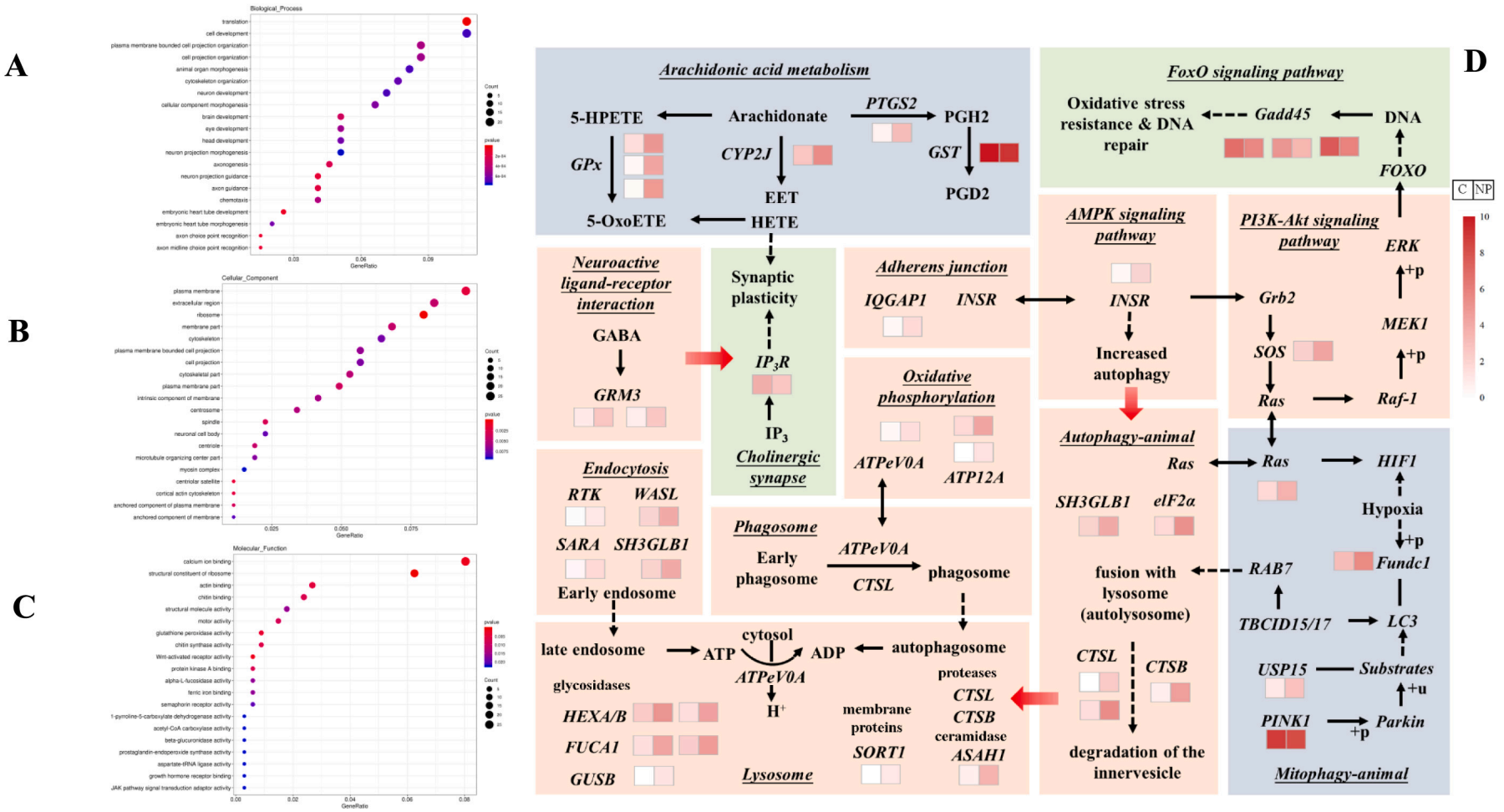


Fig. 7. Transcriptome analysis of scallops exposed to NPs. The top 20 GO terms with significant enrichment of DEGs for biological process (A), cellular component (B), and molecular function (C). A summary of differentially expressed genes in the key pathways of scallops in response to NPs exposure relative to control (D). The up-/down-regulated metabolic processes and related genes are shown in red/white, respectively. The color intensity of the scale indicates the relative expression of each gene based on the $\log_2$ fold change value.

synthesis in the animals. It is suggested that the weakened feeding behavior of scallops exposed to MPs and NPs might be responsible for the observed reduction in their nutritional quality.

Consistent with our findings, polyvinyl chloride MPs had no significant effect on the respiration/oxygen consumption rate of Mediterranean mussels (*Mytilus galloprovincialis*) (Yap et al., 2020), while there was no change in the oxygen respiration rate of European flat oysters (*Ostrea edulis*) exposed to MPs (Green, 2016). Although there was no significant alteration in respiration observed in the MP and NP groups, ammonia excretion was significantly increased and the O/N ratio was significantly decreased. The O/N is an indicator for the respective fraction of metabolic substrates (i.e., carbohydrates, lipids and proteins) used in energy metabolism (Dall and Smith, 1986). Generally, a higher O/N indicates a higher fraction of catabolism of carbohydrates and lipids, whereas the lower one suggests an elevated turnover of proteins. Thus, the increased ammonium excretion and reduced O/N observed for the scallops indicate a shift toward protein catabolism upon exposure to PS-MPs and PS-NPs. This may also contribute to the overall reduction in the nutritional quality of scallops, especially the reduction in protein content of the adductor muscle.

4.2. PS-NPs cause greater toxicity to scallops than PS-MPs

In the context of MP/NP exposure, there is increasing evidence for the existence of oxidative stress and damage caused by the excessive ROS in organisms (Li et al., 2020; Paul-Pont et al., 2016; Qiao et al., 2019). Eventually, antioxidant enzymes can be completely deactivated, disrupted, or even destroyed (Li et al., 2020). Excessive ROS levels can induce lipid peroxidation, which is reflected by the MDA content (Qiao et al., 2019). The results of this study indicate that the PS-NPs cause greater oxidative damage than PS-MPs.

AChE is a key enzyme present in the nervous system (Fulton and Key, 2001). An accumulation of the neurotransmitter acetylcholine (ACh) in the synaptic cleft results in an over-stimulation of receptors, impedes neurotransmission and can even lead to paralysis and death (Chen et al., 2017). AChE is responsible for the removal of ACh from the synaptic cleft through hydrolysis (Worek et al., 2002). The significant increase in AChE activity in the gills and digestive glands of the two exposure groups may reflect that PS-MPs/NPs exposure at environmentally relevant concentrations reduces high levels of neurotransmitters, thereby affecting the binding efficiency of acetylcholine receptors, In turn, this might lead to disorders in the nervous system. The IBR results also showed that the toxic effect of the PS-NPs is significantly greater than that of PS-MPs in the gills and digestive glands of scallops.

4.3. PS-MPs and PS-NPs inhibit feeding behavior through dysfunction of the gills

The gill damage noted in the current study indicates that PS-MPs and PS-NPs inhibit scallop feeding behavior through dysfunction of gills, supported by changes in AChE activity. A previous study showed that polyethylene MPs caused adverse neurological effects on the common goby (*Pomatoschistus microps*), evidenced by the significantly reduced AChE activity (Oliveira et al., 2013). Similar changes in AChE activity have been reported in the gills of mussels (*M. galloprovincialis*) and peppery furrow shells (*Scrobicularia plana*) after exposure to MPs (Avio et al., 2015; Ribeiro et al., 2017). As discussed above, exposure of scallops to MPs/NPs may cause neurotoxicity that can lead to motor dysfunction. The inhibition of AChE activity in scallop gills may therefore partially explain the inhibition of filtration capacity observed in scallops exposed to PS-MPs/NPs.

The IBR results of oxidative stress response and neurotoxicity calculated for the gills were more severe than those of the digestive glands. The gills are the first organ in contact with PS-MPs/NPs. Once in contact with the gills, PS-MPs/NPs are transported by endocytosis, which is considered to be the main pathway for small particle transport (Sendra et al., 2021). Our previous study found that the gill may be more susceptible and more sensitive than the digestive gland following exposure of *Chlamys farreri* to TiO_2 nanoparticles (Xia et al., 2017). Recent research has highlighted that the molecular mechanism(s) of toxicity of plastic debris, including MPs and NPs, on bivalves remains unclear and should be clarified in depth (Li et al., 2022). Therefore, we performed gill transcriptome analysis in an attempt to elucidate the toxic mechanism of PS-NPs to marine scallops.

4.4. The underlying mechanisms through gill transcriptome analysis

RNA-seq can provide gene expression information for organisms placed under environmental stress, which helps to predict the toxicity of pollutants and elucidate the mechanisms of chemical action (Mu et al., 2018; Qiu et al., 2020). It has been reported that phosphatidylinositol 3′-kinase(PI3K)-Akt and AMP-activated protein kinase (AMPK) pathways are related to cell growth, survival and apoptosis, which can protect against oxidative damage caused by H_2O_2 and regulate mitochondrial homeostasis, respectively (Herzig and Shaw, 2018; Lee et al., 2006). The forkhead box transcription factor subfamily and main transcription factor of AMPK were involved in mediating various ROS regulatory processes. Among them, FOXO3a is mainly regulated by combining with the promoter to modify the expression of genes such as SOD, CAT and GPx (Ronnebaum and Patterson, 2010; Storz, 2011). As such, AMPK/FOXO3a may be an effective pathway against oxidative damage. However, when ROS are excessive, the current study indicates that down-regulation of Gadd45 may affect oxidative stress resistance and DNA repair. This may be one of the mechanisms through which PS-NPs cause oxidative and DNA damage to scallop gills. Furthermore, autophagy can maintain essential cellular functions by recycling building blocks such as amino acids, where double-membrane vesicles are responsible for transporting macromolecules or organelles into lysosomes to degrade and reuse the resulting macromolecules, enabling cells to survive starvation (Herzig and Shaw, 2018; Yang and Klionsky, 2010). It is reported that FUNDC1-mediated mitophagy promoted the mitigation of ROS (Huang et al., 2020). In this study, ultrastructural analysis of scallop gills from the NP groups also confirmed the occurrence of mitochondrial aggregation and mitophagy, which ultimately led to the increased lysosomes. A similar increase of lysosomes also occurred in the gills of scallops exposed to PS-MPs, indicating a similar compensation mechanism. However, no mitophagy was observed, indicating that the toxic effect of PS-MPs on scallop gills was less than that of PS-NPs.

The process of autophagy ultimately leads to degeneration of the inner vesicle, and the formation of autophagosomes involves complex membrane reorganization (Mizushima et al., 2011). Most lipids are primarily distributed on different cell membranes, and the transport of the inner vesicle is also related to the transport of intracellular lipids. Ultimately, the cell membrane and plasma membrane may be adversely affected. Cell vacuolization involves endoplasmic reticulum or lysosome compartments and other numerous cell organelles (Carella et al., 2015). In the ultrastructure, the dissolution of nuclear membranes and cell membranes, as well as cell rupture, vacuolization and even mitochondrial rupture (Lin et al., 2020), may cause irreversible damage to cells. The activation of the Adherens junction, which is important for maintaining tissue structure and cell polarity, may be one reason for the appearance of tissue damage, such as gill filament adhesions in the microstructure. It is well known that Gamma-aminobutyric acid (GABA) functions as a major inhibitory neurotransmitter in animals (Mody et al., 1994). GABA-mediated inhibitory neurotransmission is regulated by ROS (González et al., 2020). Similarly, the down-regulation of IP_3R in cholinergic synaptic pathways mediated by neuroactive ligand-receptor interactions affects synaptic plasticity. This may be the damage mechanism of neurotoxicity to the gills of scallops exposed to PS-NPs. Therefore, the enrichment of genes related to oxidative stress and nerves in the cytosol, as well as the activation of lysosome and autophagy, were the main causes of toxicity.

5. Conclusion

The present study showed that exposure of *Chlamys farreri* to environmentally relevant concentrations of PS-MPs/NPs decreased the edible value and nutrition quality through inhibiting their feeding behavior and causing changes in physiological metabolism. Although the scallops were able to adopt some compensatory responses and activate antioxidant defense systems in response to MP and NP exposure, the inhibition of feeding behavior was mainly attributed to tissue damage and neurotoxicity induced by the PS-MP/NP exposure. Importantly, PS-NPs caused greater toxic effects on the scallops than PS-MPs for most of the toxicological endpoints studied. After uptake of NPs by gill cells, excessive oxidative stress was observed and the regulation of nerve cells could not be compensated. Associated with this gill damage, the feeding behavior and physiological metabolism of scallops were affected, ultimately leading to a decrease in nutritional quality. From a commercial fisheries perspective, MPs/NPs could adversely affect the sustainable development of bivalve aquaculture. In future studies, the ecological impact of MPs/NPs in a realistic marine environment merits further investigation.

Author statement

The authors approve the final version of the manuscript.

CRediT authorship contribution statement

Yejiao Sun: Methodology, Investigation, Writing - original draft. **Xinguo Zhao**: Methodology, Investigation, Methodology, Formal analysis. **Bin Xia** and **Andy M. Booth**: Conceived the framework, Writing - review & editing, Funding acquisition. **Qi Sui**: Methodology, Investigation; **Xuemei Sun and Lin Zhu**: Formal analysis, Writing - review & editing; **Bijuan Chen and Keming Qu**: Supervision.

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.

Data availability

Data will be made available on request.

Acknowledgement

This work was supported by the NSFC-Shandong Joint Fund for Marine Ecology and Environmental Sciences (U2106213), the Laoshan Laboratory (LSKJ202203903), the Key Technology Research and Development Program of Shandong province (2022CXPT013), Shandong Provincial Natural Science Foundation (ZR2020QC209), Taishan Scholars Program of Shandong Province (tsqn202211267), Central Public-interest Scientific Institution Basal Research Fund, YSFRI, CAFS (20603022021005), and Central Public-interest Scientific Institution Basal Research Fund, CAFS (2023TD13).

Appendix A Supplementary data

Additional details on feeding behavior, oxygen consumption rate and ammonia excretion rate, nutritional composition, oxidative stress and neurotoxicity analysis, calculation of the IBR and RNA Extraction and qRT-PCR; 13 figures; 9 tables, and additional references 7. Supplementary data to this article can be found online at doi: https://doi.org/10.1016/j.scitotenv.2023.168457.

References

Alimi, O.S., Farner Budarz, J., Hernandez, L.M., Tufenkji, N., 2018. Microplastics and nanoplastics in aquatic environments: aggregation, deposition, and enhanced contaminant transport. Environ. Sci. Technol. 52 (4), 1704–1724.
Avio, C.G., Gorbi, S., Milan, M., Benedetti, M., Fattorini, D., d'Errico, G., Pauletto, M., Bargelloni, L., Regoli, F., 2015. Pollutants bioavailability and toxicological risk from microplastics to marine mussels. Environ. Pollut. 198, 211–222.
Barboza, L.G.A., Vieira, L.R., Branco, V., Figueiredo, N., Carvalho, F., Carvalho, C., Guilhermino, L., 2018. Microplastics cause neurotoxicity, oxidative damage and energy-related changes and interact with the bioaccumulation of mercury in the European seabass, *Dicentrarchus labrax* (Linnaeus, 1758). Aquat. Toxicol. 195, 49–57.
Beliaeff, B., Burgeot, T., 2002. Integrated biomarker response: a useful tool for ecological risk assessment. Environ. Toxicol. Chem. 21 (6), 1316–1322.
Besseling, E., Wang, B., Lurling, M., Koelmans, A.A., 2014. Nanoplastic affects growth of *S. obliquus* and reproduction of *D. magna*. Environ. Sci. Technol. 48 (20), 12336–12343.
Carella, F., Feist, S.W., Bignell, J.P., De Vico, G., 2015. Comparative pathology in bivalves: aetiological agents and disease processes. J. Invertebr. Pathol. 131, 107–120.
Chen, Q., Gundlach, M., Yang, S., Jiang, J., Velki, M., Yin, D., Hollert, H., 2017. Quantitative investigation of the mechanisms of microplastics and nanoplastics toward zebrafish larvae locomotor activity. Sci. Total Environ. 584-585, 1022–1031.
Cole, M., Lindeque, P., Fileman, E., Halsband, C., Goodhead, R., Moger, J., Galloway, T. S., 2013. Microplastic ingestion by zooplankton. Environ. Sci. Technol. 47 (12), 6646–6655.
Coughlan, J., 1969. The estimation of filtering rate from the clearance of suspensions. Mar. Biol. 2 (4), 356–358.
Dall, W., Smith, D.M., 1986. Oxygen consumption and ammonia-N excretion in fed and starved tiger prawns, *Penaeus esculentus Haswell*. Aquaculture 55 (1), 23–33.
Duarte, C.M., Holmer, M., Olsen, Y., Soto, D., Marbà, N., Guiu, J., Black, K., Karakassis, I., 2009. Will the oceans help feed humanity? Bioscience 59 (11), 967–976.
Fulton, M.H., Key, P.B., 2001. Acetylcholinesterase inhibition in estuarine fish and invertebrates as an indicator of organophosphorus insecticide exposure and effects. Environ. Toxicol. Chem. 20 (1), 37–45.
Galimany, E., Wikfors, G.H., Dixon, M.S., Newell, C.R., Meseck, S.L., Henning, D., Li, Y., Rose, J.M., 2017. Cultivation of the ribbed mussel (*Geukensia demissa*) for nutrient bioextraction in an urban estuary. Environ. Sci. Technol. 51 (22), 13311–13318.
Gardon, T., Reisser, C., Soyez, C., Quillien, V., Le Moullac, G., 2018. Microplastics affect energy balance and gametogenesis in the pearl oyster *Pinctada margaritifera*. Environ. Sci. Technol. 52 (9), 5277–5286.
Goldstein, M.C., Rosenberg, M., Cheng, L., 2012. Increased oceanic microplastic debris enhances oviposition in an endemic pelagic insect. Biol. Lett. 8 (5), 817–820.
González, A., Pazos, M., Calvo, D.J., 2020. Reactive oxygen species in the regulation of the GABA mediated inhibitory neurotransmission. Neuroscience 439, 137–145.
Green, D.S., 2016. Effects of microplastics on European flat oysters, *Ostrea edulis* and their associated benthic communities. Environ. Pollut. 216, 95–103.
Guerlet, E., Vasseur, P., Giamberini, L., 2010. Spatial and temporal variations of biological responses to environmental pollution in the freshwater zebra mussel. Ecotox. Environ. Safe. 73 (6), 1170–1181.
Herzig, S., Shaw, R.J., 2018. AMPK: guardian of metabolism and mitochondrial homeostasis. Nat. Rev. Mol. Cell Bio. 19 (2), 121–135.
Hidalgo-Ruz, V., Gutow, L., Thompson, R.C., Thiel, M., 2012. Microplastics in the marine environment: a review of the methods used for identification and quantification. Environ. Sci. Technol. 46 (6), 3060–3075.
Huang, J., Zhu, T., Rong, R., You, M., Ji, D., Li, H., 2020. FUN14 domain-containing 1-mediated mitophagy suppresses interleukin-1 beta production in macrophages. Int. Immunopharmacol. 88, 106964.
Jeong, C.B., Kang, H.M., Lee, M.C., Kim, D.H., Han, J., Hwang, D.S., Souissi, S., Lee, S.J., Shin, K.H., Park, H.G., Lee, J.S., 2017. Adverse effects of microplastics and oxidative stress-induced MAPK/Nrf2 pathway-mediated defense mechanisms in the marine copepod *Paracyclopina nana*. Sci. Rep. 7, 41323.
John, D., Chen, X., 1987. A comparison of methods for the assessment of condition in the mussel (*Mytilus edulis L.*). J. Mollus. Stud. 3 (3).
Kashiwada, S., 2006. Distribution of nanoparticles in the see-through medaka (*Oryzias latipes*). Environ. Health. Persp. 114 (11), 1697–1702.
Kibria, G., 2023. Impacts of microplastic on fisheries and seafood security — global analysis and synthesis. Sci. Total Environ. 904, 166652.
Lai, W., Xu, D., Li, J., Wang, Z., Ding, Y., Wang, X., Li, X., Xu, N., Mai, K., Ai, Q., 2021. Dietary polystyrene nanoplastics exposure alters liver lipid metabolism and muscle nutritional quality in carnivorous marine fish large yellow croaker (*Larimichthys crocea*). J. Hazard. Mater. 419, 126454.
Lee, Y.J., Lee, J.H., Han, H.J., 2006. Extracellular adenosine triphosphate protects oxidative stress-induced increase of p21(WAF1/Cip1) and p27(Kip1) expression in primary cultured renal proximal tubule cells: role of PI3K and Akt signaling. J. Cell. Physiol. 209 (3), 802–810.
Li, Z., Feng, C., Wu, Y., Guo, X., 2020. Impacts of nanoplastics on bivalve: fluorescence tracing of organ accumulation, oxidative stress and damage. J. Hazard. Mater. 392, 122418.
Li, Z., Chang, X., Hu, M., Fang, J.K., Sokolova, I.M., Huang, W., Xu, E.G., Wang, Y., 2022. Is microplastic an oxidative stressor? Evidence from a meta-analysis on bivalves. J. Hazard. Mater. 423 (Pt B), 127211.

Li, Q., Sun, H., Bai, Q., Li, P., Lai, Y., Yu, S., Liu, X., Yao, Z., Cai, Y., Liu, J., 2023. Spatial distribution of polystyrene nanoplastics and small microplastics in the Bohai Sea. China. Sci. Total Environ. 881, 163222.
Lin, Y., Weng, K., Liang, J., Zhou, Y., Hao, Q., Hao, Y., Yao, K., Zou, J., 2020. The extracellular and intracellular regions of Crb2a play distinct roles in guiding the formation of the apical zonula adherens. Biomed. Pharmacother. 125, 109942.
Mattsson, K., Johnson, E.V., Malmendal, A., Linse, S., Hansson, L.A., Cedervall, T., 2017. Brain damage and behavioural disorders in fish induced by plastic nanoparticles delivered through the food chain. Sci. Rep. 7 (1), 11452.
Mizushima, N., Yoshimori, T., Ohsumi, Y., 2011. The role of Atg proteins in autophagosome formation. Annu. Rev. Cell Dev. Bi. 27, 107–132.
Mody, I., De Koninck, Y., Otis, T.S., Soltesz, I., 1994. Bridging the cleft at GABA synapses in the brain. Trends Neurosci. 17 (12), 517–525.
Moore, C.J., 2008. Synthetic polymers in the marine environment: a rapidly increasing, long-term threat. Environ. Res. 108 (2), 131–139.
Mu, X., Huang, Y., Li, X., Lei, Y., Teng, M., Li, X., Wang, C., Li, Y., 2018. Developmental effects and estrogenicity of bisphenol A alternatives in a zebrafish embryo model. Environ. Sci. Technol. 52 (5), 3222–3231.
Oliveira, M., Ribeiro, A., Hylland, K., Guilhermino, L., 2013. Single and combined effects of microplastics and pyrene on juveniles (0+group) of the common goby *Pomatoschistus microps* (Teleostei, Gobiidae). Ecol. Indic. 34, 641–647.
Paul-Pont, I., Lacroix, C., Gonzalez Fernandez, C., Hegaret, H., Lambert, C., Le Goic, N., Frere, L., Cassone, A.L., Sussarellu, R., Fabioux, C., Guyomarch, J., Albentosa, M., Huvet, A., Soudant, P., 2016. Exposure of marine mussels *Mytilus* spp. to polystyrene microplastics: toxicity and influence on fluoranthene bioaccumulation. Environ. Pollut. 216, 724–737.
Peng, L., Fu, D., Qi, H., Lan, C.Q., Yu, H., Ge, C., 2020. Micro- and nano-plastics in marine environment: source, distribution and threats — a review. Sci. Total Environ. 698, 134254.
Qiao, R., Sheng, C., Lu, Y., Zhang, Y., Ren, H., Lemos, B., 2019. Microplastics induce intestinal inflammation, oxidative stress, and disorders of metabolome and microbiome in zebrafish. Sci. Total Environ. 662, 246–253.
Qiu, W., Chen, B., Greer, J.B., Magnuson, J.T., Xiong, Y., Zhong, H., Andrzejczyk, N.E., Zheng, C., Schlenk, D., 2020. Transcriptomic responses of bisphenol S predict involvement of immune function in the cardiotoxicity of early life-stage zebrafish (*Danio rerio*). Environ. Sci. Technol. 54 (5), 2869–2877.
Ray, N.E., Maguire, T.J., Al-Haj, A.N., Henning, M.C., Fulweiler, R.W., 2019. Low greenhouse gas emissions from oyster aquaculture. Environ. Sci. Technol. 53 (15), 9118–9127.
Riba, I., Gonzalez de Canales, M., Forja, J.M., DelValls, T.A., 2004. Sediment quality in the Guadalquivir estuary: sublethal effects associated with the aznalcollar mining spill. Mar. Pollut. Bull. 48 (1–2), 153–163.
Ribeiro, F., Garcia, A.R., Pereira, B.P., Fonseca, M., Mestre, N.C., Fonseca, T.G., Ilharco, L.M., Bebianno, M.J., 2017. Microplastics effects in *Scrobicularia plana*. Mar. Pollut. Bull. 122 (1–2), 379–391.
Rist, S.E., Assidqi, K., Zamani, N.P., Appel, D., Perschke, M., Huhn, M., Lenz, M., 2016. Suspended micro-sized PVC particles impair the performance and decrease survival in the Asian green mussel *Perna viridis*. Mar. Pollut. Bull. 111 (1–2), 213–220.
Ronnebaum, S.M., Patterson, C., 2010. The FoxO family in cardiac function and dysfunction. Annu. Rev. Physiol. 72, 81–94.
Sendra, M., Sparaventi, E., Novoa, B., Figueras, A., 2021. An overview of the internalization and effects of microplastics and nanoplastics as pollutants of emerging concern in bivalves. Sci. Total Environ. 753, 142024.
Solrzano, L., 1969. Determination of ammonia in natural waters by the phenol-hypochlorite method. Limnol. Oceanogr. 14 (5), 799–801.
Storz, P., 2011. Forkhead homeobox type O transcription factors in the responses to oxidative stress. Antioxid. Redox. Sign. 14 (4), 593–605.
Ter Halle, A., Jeanneau, L., Martignac, M., Jarde, E., Pedrono, B., Brach, L., Gigault, J., 2017. Nanoplastic in the North Atlantic Subtropical Gyre. Environ. Sci. Technol. 51 (23), 13689–13697.
Vasanthi, R.L., Arulvasu, C., Kumar, P., Srinivasan, P., 2021. Ingestion of microplastics and its potential for causing structural alterations and oxidative stress in Indian green mussel *Perna viridis*- a multiple biomarker approach. Chemosphere 283, 130979.
Watts, A.J., Lewis, C., Goodhead, R.M., Beckett, S.J., Moger, J., Tyler, C.R., Galloway, T. S., 2014. Uptake and retention of microplastics by the shore crab *Carcinus maenas*. Environ. Sci. Technol. 48 (15), 8823–8830.
Wegner, A., Besseling, E., Foekema, E.M., Kamermans, P., Koelmans, A.A., 2012. Effects of nanopolystyrene on the feeding behavior of the blue mussel (*Mytilus edulis* L.). Environ. Toxicol. Chem. 31 (11), 2490–2497.
Wenning, R., 2020. The state of world fisheries and aquaculture (Sofia) 2020 report. Integr. Environ. Asses. 16 (5), 800–801.
Widdows, J., 1985. Physiological procedures. In: Bayne, B.L. (Ed.), The Effect of Stress and Pollution on Marine Animals. Praeger, New York, pp. 161–178.
Worek, F., Reiter, G., Eyer, P., Szinicz, L., 2002. Reactivation kinetics of acetylcholinesterase from different species inhibited by highly toxic organophosphates. Arch. Toxicol. 76 (9), 523–529.
Wright, S.L., Rowe, D., Thompson, R.C., Galloway, T.S., 2013. Microplastic ingestion decreases energy reserves in marine worms. Curr. Biol. 23 (23), R1031–R1033.
Wu, Y., Gu, E., Li, H., Tian, C., Feng, C., 2019. Oxidative stress and histological changes in *Corbicula fluminea* exposed to nano-Al_{13} and monomeric Al coagulants. Environ. Sci. Nano 6 (9), 2736–2748.
Xia, B., Zhu, L., Han, Q., Sun, X., Chen, B., Qu, K., 2017. Effects of TiO_2 nanoparticles at predicted environmental relevant concentration on the marine scallop *Chlamys farreri*: an integrated biomarker approach. Environ. Toxicol. Phar. 50, 128–135.
Xia, B., Zhang, J., Zhao, X., Feng, J., Teng, Y., Chen, B., Sun, X., Zhu, L., Sun, X., Qu, K., 2020. Polystyrene microplastics increase uptake, elimination and cytotoxicity of decabromodiphenyl ether (BDE-209) in the marine scallop *Chlamys farreri*. Environ. Pollut. 258, 113657.
Xia, B., Sui, Q., Du, Y., Wang, L., Jing, J., Zhu, L., Zhao, X., Sun, X., Booth, A.M., Chen, B., Qu, K., Xing, B., 2022. Secondary PVC microplastics are more toxic than primary PVC microplastics to *Oryzias melastigma* embryos. J. Hazard. Mater. 424, 127421.
Xu, X.Y., Lee, W.T., Chan, A.K.Y., Lo, H.S., Shin, P.K.S., Cheung, S.G., 2017. Microplastic ingestion reduces energy intake in the clam *Atactodea striata*. Mar. Pollut. Bull. 124 (2), 798–802.
Yang, Z., Klionsky, D.J., 2010. Eaten alive: a history of macroautophagy. Nat. Cell Biol. 12 (9), 814–822.
Yap, V.H.S., Chase, Z., Wright, J.T., Hurd, C.L., Lavers, J.L., Lenz, M., 2020. A comparison with natural particles reveals a small specific effect of PVC microplastics on mussel performance. Mar. Pollut. Bull. 160, 111703.
Yin, L., Chen, B., Xia, B., Shi, X., Qu, K., 2018. Polystyrene microplastics alter the behavior, energy reserve and nutritional composition of marine jacopever (*Sebastes schlegelii*). J. Hazard. Mater. 360, 97–105.
Zeng, Q., Yang, Q., Chai, Y., Wei, W., Luo, M., Li, W., 2023. Polystyrene microplastics enhanced copper-induced acute immunotoxicity in red swamp crayfish (*Procambarus clarkii*). Ecotox. Environ. Safe. 249, 114432.
Zhang, C., Jeong, C.B., Lee, J.S., Wang, D., Wang, M., 2019. Transgenerational proteome plasticity in resilience of a marine copepod in response to environmentally relevant concentrations of microplastics. Environ. Sci. Technol. 53 (14), 8426–8436.
Zhao, X., Shi, W., Han, Y., Liu, S., Guo, C., Fu, W., Chai, X., Liu, G., 2017. Ocean acidification adversely influences metabolism, extracellular pH and calcification of an economically important marine bivalve, *Tegillarca granosa*. Mar. Environ. Res. 125, 82–89.
Zhao, D., Zhang, X., Liu, D., Ru, S., 2019. Cu accumulation, detoxification and tolerance in the red swamp crayfish *Procambarus clarkii*. Ecotox. Environ. Safe. 175, 201–207.
Zhao, L., Lian, S., Ren, Q., Yang, Z., Guo, Z., Lou, J., Kong, X., Li, M., Bao, Z., Hu, X., 2021. In vivo and rapid assessment of scallop muscle trait. Aquaculture 530, 735817.
Zhou, W., Han, Y., Tang, Y., Shi, W., Du, X., Sun, S., Liu, G., 2020. Microplastics aggravate the bioaccumulation of two waterborne veterinary antibiotics in an edible bivalve species: potential mechanisms and implications for human health. Environ. Sci. Technol. 54 (13), 8115–8122.
Zhou, W., Shi, W., Du, X., Han, Y., Tang, Y., Ri, S., Ju, K., Kim, T., Huang, L., Zhang, W., Yu, Y., Tian, D., Yu, Y., Chen, L., Wu, Z., Liu, G., 2023a. Assessment of nonalcoholic fatty liver disease symptoms and gut-liver axis status in zebrafish after exposure to polystyrene microplastics and oxytetracycline, alone and in combination. Environ. Health Perspect. 131 (4), 47006.
Zhou, W., Tong, D., Tian, D., Yu, Y., Huang, L., Zhang, W., Yu, Y., Lu, L., Zhang, X., Pan, W., Shen, J., Shi, W., Liu, G., 2023b. Exposure to Polystyrene Nanoplastics Led to Learning and Memory Deficits in Zebrafish by Inducing Oxidative Damage and Aggravating Brain Aging. Healthc. Mater, Adv. https://doi.org/10.1002/adhm.202301799e2301799.