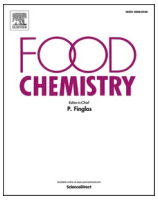
Food Chemistry 462 (2025) 140961

Contents lists available at ScienceDirect

Food Chemistry

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



Metabolic profiles and protein expression responses of Pacific oyster (*Crassostrea gigas*) to polystyrene microplastic stress

Yu Liu [a,b], Shuai Wu [b], Lipin Chen [a,b,*], Xiaoyu Teng [b], Haohao Shi [a,b], Changhu Xue [b], Zhaojie Li [b,*]

[a] School of Food Science and Technology, Hainan University, Hainan 570228, PR China
[b] College of Food Science and Engineering, Ocean University of China, No.1299, San Sha Road, Qingdao, Shandong Province 266003, PR China

ARTICLE INFO

Keywords:
Oysters
Microplastics
Metabolomics profiling
Enzymatic biomarkers
Oxidative stress

ABSTRACT

The underlying toxicity mechanisms of microplastics on oysters have rarely been explored. To fill this gap, the present study investigated the metabolic profile and protein expression responses of oysters to microplastic stress through metabolomics and biochemical analyses. Oysters were exposed to microplastics for 21 days, and the results indicated that the microplastics induced oxidative stress, with a significant decrease in SOD activity in the 0.1 mg/L exposure group. Metabolomics revealed that exposure to microplastics disturbed many metabolic pathways, such as amino acid metabolism, lipid metabolism, biosynthesis of amino acids, aminoacyl-tRNA biosynthesis, and that different concentrations of microplastics induced diverse metabolomic profiles in oysters. Overall, the current study provides new reference data and insights for assessing food safety and consumer health risks caused by microplastic contamination.

1. Introduction

Bivalve mollusks are abundant in marine ecosystems, which play significant roles in ecosystem functioning and place important economic value on commercial markets. Among them, oysters are widely distributed worldwide as commercially important species and constitute a wealth of premium ingredients, including proteins, polysaccharides, polyunsaturated fatty acids, taurine, zinc, and other bioactive compounds (Du et al., 2023; Liu et al., 2024). The Pacific oyster (*Crassostrea gigas*) is one of the most important cultured shellfish species worldwide and has gained increasing popularity in both the domestic and international shellfish markets. As popular seafood products, oysters are widely favored by consumers worldwide owing to their unique fragrance and tender texture, and the estimated annual global production value is US$1.24 billion (Negara et al., 2022).

Since it was first proposed in 2004 by Thompson, microplastic (MPs) pollution has been one of the main environmental issues globally (Thompson et al., 2009). MPs are generally termed tiny plastic particles smaller than 5 mm in size and can be classified into primary and secondary MPs on the basis of their production methods and origin (Liu et al., 2023). High concentrations of MPs have been detected in various marine media, including offshore, mid-ocean, deep-sea, and even polar regions. For instance, in offshore Pacific water, the concentrations of MPs range from 8 to 9200 particles m^{-3}, and an average abundance of 2500 particles km^{-2} has been reported in the northwestern Atlantic (Auta et al., 2017; Law et al., 2010). Lonnstedt and Eklov (2016) reported that the density of MPs in the surface water of the Swedish Coast ranged from 150 to 2400 particles m^{-3} to 68,000–102,000 particles m^{-3}. MPs can travel globally through wind and oceanic currents (Su et al., 2022), and the small dimensions of MPs make them readily available for ingestion by a variety of species, such as fish (Neves et al., 2015), oysters (Teng et al., 2019), mussels (Li et al., 2016), shrimp (Gurjar et al., 2021), and zooplankton (Botterell et al., 2022), in the aquatic environment. In marine ecosystems, bivalves are among the most susceptible species to MPs ingestion because of their filtering feeding style. Among them, oysters tend to have a greater likelihood of MPs bioaccumulation because of their intensive filtering-feeding activities and inferior motility. MPs are present in almost all oysters (94.4%) sampled worldwide, yielding an average of 1.41 ± 0.33 MPs/g soft tissue, and wild oysters accumulate more than twice the amounts of MPs than farmed samples do (Wootton et al., 2022). Some previous studies have reported the behavioral and physiological impacts of MPs on

* Corresponding authors at: School of Food Science and Engineering, Hainan University, Haikou 570228, China; College of Food Science and Engineering, Ocean University of China, No.1299, San Sha Road, Qingdao, Shandong Province, 266003, PR China.
E-mail addresses: chenlipin@hainanu.edu.cn (L. Chen), lizhaojie@ouc.edu.cn (Z. Li).

https://doi.org/10.1016/j.foodchem.2024.140961
Received 1 April 2024; Received in revised form 1 August 2024; Accepted 21 August 2024
Available online 22 August 2024

oysters. For example, Gardon et al. (2018) demonstrated that oysters can ingest 6- and 10-μm polystyrene (PS) microbeads under laboratory conditions and that the ingestion of PS affects the energy balance and gametogenesis of oysters. Similarly, the ingestion of polyethylene (PE) MPs of different sizes (4–6, 11–13 and 20–25 μm) impaired the swimming activity and development of Pacific oyster D-larvae (Bringer et al., 2020). Oxidative stress caused by MPs ingestion in oysters has been reported in many studies (Bringer et al., 2022; Liu et al., 2023). Metabolic dysfunction, including energy metabolism, lipid metabolism, protein metabolism, and amino acid metabolism induced by MPs accumulation, has also been reported in several studies (Du et al., 2023; Gardon et al., 2018; Liu et al., 2024; Teng et al., 2021b). MPs ingested by oysters can be transferred to higher trophic levels via trophic interactions, posing potential threats to human safety, which has elicited global concern (Liu et al., 2023). Although MPs are known to have deleterious effects on the physiological functions and biochemical status of oysters, there is limited information concerning their underlying toxicity mechanisms.

In recent years, extensive metabolomics research has been carried out on the physical and biological influences of MPs on various species. Metabolomic analyses can depict changes in the functional status of organisms in response to exogenous pollutant exposure by detecting and quantifying alterations in various metabolites to reveal the underlying toxicity mechanisms induced by contaminants (Bhagat et al., 2022). Some studies concerning metabolomic profiles have focused on the exploration of the mechanisms of MPs toxicity in model organisms such as zebrafish, tilapia, mussels, and rice (Huang et al., 2021; Jia et al., 2022; Wu et al., 2020; Zhao et al., 2021). However, the mechanisms underlying the metabolite profiles of oysters in response to MPs exposure remain unclear.

Therefore, in this study, metabolomics analysis was performed to investigate the changes in the metabolic profiles of oysters under MPs stress, and the levels of a series of biomarkers were measured to evaluate alterations in their antioxidant abilities. This study aimed to explore the underlying toxicological mechanisms of MPs under different exposure concentrations in oysters, and the overall results are expected to help advance the knowledge of MPs-related toxicity mechanisms in marine species.

2. Materials and methods

2.1. Sample collection and acclimation

Two hundred adult oysters, *Crassostrea gigas* (*C. gigas*), weighing 80–110 g, were sampled from Jiaozhou Bay in Qingdao (Shandong Province, 36° 16′ 49″N, 120° 00′ 36″E), China. The oysters were washed with seawater to remove the external silt attached to them. The oysters were subsequently transferred to the laboratory and acclimated for 3 days in seawater before the experiment. Seawater was sampled from the same harvesting area as *C. gigas*. After collection, the seawater was filtered through membrane filters (20 μm, 5 μm, and 1 μm), and then ultraviolet pumps were used to eliminate the microorganisms. The acclimation conditions were as follows: the salinity was maintained at 31 g/L, and the temperature was maintained at 15 ± 1 °C through temperature-controlled devices.

2.2. Characterization and preparation of MPs

Commercially available PS powder was purchased from RuiXiang polymer material, Dongguan City, Guangdong Province, China. PS-MPs are a common type of MPs widely identified in marine environments and organisms and are widely utilized in toxicity assessments of MPs (Liu et al., 2023). To characterize the MPs better, the infrared spectra of the PS-MPs were analyzed via a Thermo Nicolet iS50 Fourier transform infrared (FTIR) spectroscopy system (Thermo Fisher Scientific Co., Madison, WI, USA) in the following settings: the range of 400–4000 cm^{-1}, with a spectral resolution of 4 cm^{-1}, and 64 scans were acquired for each spectrum. Emission scanning electron microscopy (SEM) was performed via an F.E.I. Nova NanoSEM 450, operating with a tension of 20 kV and amplification of 50–10,000 times to obtain the morphology and sizes of the PS-MPs. The results are shown in Fig. S1 in the supplemental materials.

As shown in Fig. S1, pellets with sizes of 3–8 μm were chosen to be as close as possible to the sizes of the food particles of *C. gigas* (i.e., microalgae, with sizes of 4–8 μm) to make them easier to ingest (Du et al., 2024; Gardon et al., 2020). Suspensions of PS powder was prepared in ultrapure water. To prevent polymer aggregation and adhesion to the tank walls, the surfactant Tween-80 (0.00001% in seawater, *v/v*) was added to prepare stock suspensions of MPs. Tween 80 at this concentration was demonstrated to be nontoxic to organisms (Kilic et al., 2023). The MPs suspensions were stored at 4 °C and sonicated before use.

2.3. MPs exposure experiment

After the acclimation period, intact and active oysters were randomly chosen and placed in 4 experimental 60-L glass aquarium tanks (30 individuals per tank). Since the poor polydispersity of the MPs used in this study makes accurate quantification of their number difficult, the concentration of MPs is defined via mass-based units. Three concentrations (0.1, 1, and 10 mg/L) of MPs were set as the exposure groups, of which 1 mg/L was the concentration set on the basis of previous toxicological studies and is a common exposure dose under laboratory conditions (Liu et al., 2023). The use of dose–response tests can be helpful in assessing toxicity thresholds for a given pollutant and organism; therefore, the concentration of 1 mg/L was decreased and increased, respectively, producing 0.1 mg/L and 10 mg/L to target a response window with a dose–effect relationship (Gardon et al., 2020). During the exposure period, half of the seawater was renewed daily, and subsequently, MPs stock suspensions were added. *C. gigas* were fed oyster food purchased from a local market at a ratio equal to 7–8% dry weight algae/dry weight oyster once daily. To avoid interactions between algae and MPs, microalgae were not fed within 2 h after daily addition of MPs. *C. gigas* was subjected to a 12 h:12 h light–dark cycle under light-intensity conditions, and air stones were used for aeration and to maintain the MPs particles in suspension. The exposure assay lasted for 21 days, and no mortality was observed during exposure. As filter-feeding species, oysters filter seawater through gills to obtain nutrients and oxygen, and gill tissues are more sensitive to the environment and external stress than are digestive glands and other tissues. Therefore, the gills were separated and collected after the exposure experiments, and the samples were immediately frozen in liquid nitrogen and then stored at −40 °C for further analyses.

2.4. Biochemical analysis

The gill weights of the three oysters were measured separately, and precooled normal saline (0.9% NaCl) was added at a ratio of weight (g): volume (mL) = 1:9. The homogenization was then carried out, and the samples were centrifuged at 2500 rpm for 10 min at 4 °C. The supernatants were collected and collected for analysis of biochemical parameters. The levels of superoxide dismutase (SOD, catalog number A001–3), catalase (CAT, A007–2-1), peroxidase (POD, A084–1-1), glutathione peroxidase (GSH-Px, A005–1), glutathione S-transferases (GSH-ST, A004–1-1), glutathione (GSH, A006–2-1), malondialdehyde (MDA, A003–1), and acetylcholinesterase (AChE, A024–1-1) were examined via commercial kits from Nanjing Jiancheng Bioengineering Institute (Nanjing Jiancheng) with a Spark 10 M microplate reader (Tecan, Männedorf, Switzerland).

2.5. Metabolite extraction and analysis

The gill samples used for metabolomic analysis were weighed (50±5 mg per sample) and then transferred to 400 μL of precooled methanol: water (4:1, *v/v*) solution containing 0.02 mg/mL L-2-chlorophenylalanine as an internal standard. A 6 mm tungsten bead was added to three samples in each group separately, and the mixture was ground with a crusher (Shanghai Wanbo Biotechnology Co., Ltd.) at 50 Hz for 6 min at −10 °C, followed by ultrasonication at 40 kHz for 30 min at 5 °C. The samples were subsequently stored at −20 °C for 30 min for protein precipitation. After centrifugation at 13000 ×*g* for 15 min at 4 °C, the supernatants were collected for further analysis. The separation and identification of metabolites in the samples were performed via a Thermo Ultra High-Performance Liquid Chromatography system (UHPLC) system equipped with an ACQUITY UPLC HSS T3 (100 mm × 2.1 mm i.d., 1.8 μm; Waters, Milford, USA) and a Thermo UHPLC-Q Exactive Mass Spectrometer (Thermo Fisher Scientific, USA) equipped with an electrospray ionization (ESI) source. Detailed information on the parameters of the UHPLC and MS analyses can be found in the supplemental materials.

2.6. Statistical analysis

All analyses were performed in triplicate, and the data were analyzed by one-way analysis of variance with Duncan's multiple comparison method using the SPSS 25.0 software (SPSS Inc., Chicago, USA) and visualized using Origin 2023b software (OriginLab Inc., USA). The data are shown as the mean ± standard deviation (SD), with $p < 0.05$ indicating a statistically significant difference. SIMCA-P + software (version 16.0, Umetrics) was used for plotting the principal component (PCA) and the orthogonal partial least squares–discriminant (OPLS–DA) methods. The significantly different metabolites were determined by the VIP value of the OPLS-DA model and the *p* value of the student's *t*-test, and metabolites with variable importance in projection (VIP) > 1 and $p < 0.05$ were chosen as significantly differentially abundant metabolites (DMs) through the ropls (Version 1.6.2) R package. The identified DMs were searched for categorization and pathway information in the human metabolome (HMDB) database and the Kyoto Encyclopedia of Genes and Genomes (KEGG) database.

3. Results and discussion

3.1. Biomarkers in gills of C. gigas exposed to micro-PS

The activities of a series of enzymes in the gills of *C. gigas* were measured (Fig. 1) to investigate the oxidative stress, lipid peroxidation, and neurotoxicity induced by three different concentrations of micro-PS. Antioxidant enzyme systems such as SOD, CAT, and POD have diverse but synergistic effects on the elimination of reactive oxygen species (ROS). Among them, the SOD system is generally considered the first line of defense against the formation of ROS in response to environmental stress. The superoxide radical can be transformed to H_2O_2 under SOD catalysis, while CAT and POD further convert H_2O_2 into water and oxygen (Baihetiyaer et al., 2023). It is generally believed that oxidative stress induced by pollutants increases antioxidant enzyme activity in organisms to offset damage caused by ROS. However, in this study, the activity of SOD was significantly inhibited in the 0.1 mg/L group ($p < 0.05$), and the activity of CAT was also downregulated after MPs exposure, while the degree of inhibition was independent of the concentration of MPs, with a significant difference observed between the 0.1 mg/L and 10 mg/L groups ($p < 0.05$). Similarly, Zhang et al. (2021) reported that exposure to PS-MPs with an average size of 17 μm for 8 days significantly inhibited SOD activity in clams ($p < 0.05$), and Paul-Pont et al. (2016) reported a significant decrease in CAT activity in mussels exposed to 2–6 μm micro-PS for 7 days compared with that in the controls ($p < 0.05$). However, some research results regarding changes in SOD and CAT activity may contradict the findings mentioned above. Teng et al. (2021a) reported that SOD activity in oysters significantly increased in gills exposed to 0.01 mg/L PE particles but significantly decreased following exposure to 1 mg/L PE MPs for 21 days. Wang et al. (2024) reported that the activities of both SOD and CAT in oysters increased following exposure to 0.1, 0.5, and 2.5 mg/L PS at a size of 80 nm for 7 days. Several possible reasons may explain this response. First, long-term exposure to MPs results in severe oxidative stress, which inhibits the activities of antioxidant enzymes and reduces the scavenging capacity of ROS. Second, direct interactions between MPs and antioxidants might occur, inhibiting or damaging enzymes (Parra et al., 2021). In addition, the downregulation of SOD activity increases the stress level of organisms, leading to a lower elimination of ROS than their formation; thus, the redox balance is disturbed by substantial ROS and

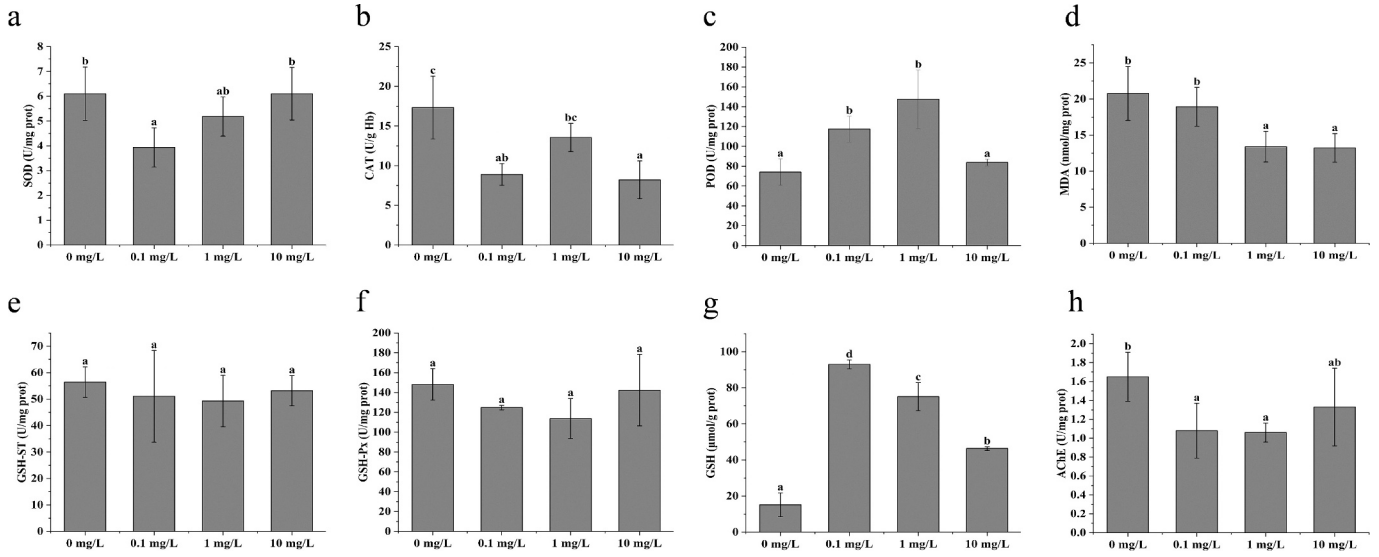


Fig. 1. Parameters of the enzymatic biomarkers in the gills of *C. gigas* after 21 days of exposure to different concentrations of micro-PS, in which a represents superoxide dismutase (SOD); b represents catalase (CAT); c represents peroxidase (POD); d represents malondialdehyde (MDA); e represents glutathione S-transferase (GSH-ST); f represents glutathione peroxidase (GSH-Px); g represents glutathione (GSH); and h represents acetylcholinesterase (AChE). The values are means ± SE. Means with different letters (a, b, c, d) are significantly different ($p < 0.05$).

inhibited activities of other antioxidant enzymes (Baihetiyaer et al., 2023).

Considering that the function of an antioxidant enzyme can be replaced by other antioxidants, the decrease in CAT activity could be partially compensated for by the increase in POD activity. The activity of POD in gills increased in all three experimental groups after exposure to MPs, with a significant increase observed in gills exposed to MPs at concentrations of 0.1 and 1 mg/L ($p < 0.05$). GSH-ST is one of the most efficient phase II biotransformation pathways for potentially harmful substances in invertebrates and can offset the damage caused by ROS to organisms by combining with contaminants and generating compounds that are more readily excreted, and GSH-Px can catalyze the reduction of various hydroperoxides, such as H_2O_2, to alcohol and water through the transformation of GSH into its disulfide form to protect organisms from oxidative stress (Mkuye et al., 2022; Zhang et al., 2021). However, the results for GSH-ST and GSH-Px were not significantly different among the three experimental groups compared with those of the control, although slight decreases in the activities of GSH-ST and GSH-Px were noted in all the experimental groups. Similarly, a study by Nobre et al. (2020) revealed no significant alterations in GSH-ST or GSH-Px activity when oysters were exposed to PET-MPs (150–250 μm, 25 mg/L) for 7 days. These results may suggest a weak overall response of GSH-dependent enzymes to MPs when organisms are exposed to relatively high concentrations of contaminants for long periods. However, in this study, the activities of GSH-ST and GSH-Px were measured only 21 days after exposure, and the lack of data on changes in GSH-ST and GSH-Px activity before and after 21 days made it impossible to observe the dynamics of enzyme activity changes and difficult to analyze the reasons for their changes; therefore, these changes need further research and explanation.

Surprisingly, in all the oysters treated with MPs, regardless of the dose, the concentration of MDA was lower than that in the control group, with significant differences observed between the 1 mg/L and 10 mg/L groups ($p < 0.05$). Similarly, Wang et al. (2024) reported that the MDA content in oysters significantly decreased following exposure to 0.1, 0.5, and 2.5 mg/L PS with a size of 80 nm for 7 days. Hamm and Lenz (2021) reported that the levels of MDA in mussels exposed to micro-PS reached 30% of the levels reported in the control group. As a final product of lipid peroxidation, the degradation of polyunsaturated fatty acids by ROS (MDA) is currently recognized as an indirect biomarker of oxidative stress that can cause damage to the cell membrane. Previous studies have demonstrated that the levels of MDA generally increase when organisms experience continuous stress events that last for days or longer. For example, the MDA content in pearl oysters significantly increased after exposure to 1.5 mg/L and 15 mg/L polyvinyl chloride (PVC) MPs with a size of 50 μm for 15 days (Lu et al., 2024). These contradictory results might be due to several factors. First, the variability in experimental conditions among studies, including the type of MPs, exposure dose, and exposure time, might result in a relatively high or low degree of oxidative stress and a high level of MDA in organisms exposed to MPs. Second, MDA production can be influenced by factors such as the age and nutritional status of organisms, resulting in instability and abnormal changes in indicators (Zhang et al., 2021). The downregulation of SOD and CAT activity and the observed MDA levels could therefore also indicate that other enzymatic or nonenzymatic systems were upregulated at the expense of these systems, which alleviated the oxidative stress caused by MPs (Wei et al., 2021). This could be explained by the significant increase in GSH levels observed in the gills of *C. gigas* after MPs exposure. GSH is not an enzyme but can form powerful reducing radicals (GSSG) through a series of reactions to scavenge xenobiotic-induced ROS and alleviate oxidative stress (Mkuye et al., 2022). GSH can also eliminate excess ROS in organisms by combining with GSH-Px and GSH-ST or directly reacting with some reactive oxygen molecules. Therefore, the minor changes in GSH-ST and GSH-PX activity in this study may have occurred because GSH reacts directly with certain reactive oxygen molecules rather than binding to GSH-ST and GSH-Px after exposure to MPs for a long period of time.

Surprisingly, the inhibitory effect of 0.1 mg/L MPs on SOD activity was more pronounced than that of higher concentrations of MPs. Similarly, the levels of GSH and MDA in the 0.1 mg/L exposure group were much greater than those in the 1 and 10 mg/L groups. These results may reflect the adaptation and restoration of oysters to a toxic environment over time or the reduction in toxicity through nonenzymatic or enzymatic antioxidant and immune response modulation at relatively high concentrations of MPs. This notion was further supported by the changes in metabolites, with greater quantities of differentially abundant metabolites being observed in the 0.1 mg/L exposure group than in the 1 and 10 mg/L groups. However, there are many types of detoxifying enzymes in organisms, and the specific reasons for these changes need further research and clarification.

AChE can hydrolyze acetylcholine into choline and acetic acid, and the consequence of perturbing acetylcholine metabolism is its accumulation in synapses and neuromuscular junctions, resulting in disturbance of nerve impulses (Prokic et al., 2019). Therefore, the level of AChE has been extensively used as a potential marker for evaluating neurotoxicity in organisms induced by various pollutants. In this study, in comparison with those of SOD and CAT, the activity of AChE displayed a different pattern: a U-shaped pattern was observed. The activity of AChE did not significantly change in the gills of *C. gigas* exposed to 10 mg/L MPs but significantly decreased after exposure to MPs at concentrations of 0.1 and 1 mg/L ($p < 0.05$) compared with the control, indicating that MPs might induce neurotoxicity in *C. gigas*.

3.2. Metabolomic alterations in the gills of C. gigas

Metabolomic analysis was performed to investigate the alterations in the gill metabolic profile after exposure to MPs at different concentrations, and a total of 867 metabolites were identified and characterized in the gills. A PCA model was initially applied to visualize the differences between the micro-PS-exposed groups and the control group. As shown in the PCA score plots (Fig. 2A), the metabolic profiles of gills exposed to three concentrations of MPs could be separated from those of the control. To further inspect the differences between and within the control and experimental groups, orthogonal partial least squares discriminant analysis (OPLS-DA) was performed (Fig. 2B). As a supervised discriminant analysis statistical method, OPLS-DA can effectively exclude irrelevant independent variables for categorization and identify characteristic variables in samples compared with PCA (Kang et al., 2022). The results of the OPLS-DA model significantly distinguished the experimental groups from the control, which suggested that MPs exposure caused marked metabolite alterations in the gills of *C. gigas*. Moreover, samples treated with different concentrations of MPs were also successfully separated. These findings indicated that three concentrations of micro-PS induced different metabolomic profiles, which supported the results of hierarchical clustering.

3.3. Selection of DMs induced by micro-PS

Multidimensional OPLS-DA was used to screen for DMs, and metabolites with VIP > 1 and $p < 0.05$ were deemed statistically significant. A volcano plot (Fig. 3) revealed that the levels of numerous metabolites significantly differed between the experimental groups and the control group. Compared with those in the control group, 106 DMs were observed, of which 63 metabolites were upregulated and 42 metabolites were downregulated in the 0.1 mg/L exposure group; 72 DMs were acquired, of which 48 metabolites were upregulated and 24 metabolites were downregulated in the 1 mg/L group; and 75 significant DMs were identified, of which 51 metabolites were upregulated and 24 metabolites were downregulated in the 10 mg/L group. In addition, the results presented on the hierarchical clustering heatmap (Fig. S2) revealed different profiles of metabolite changes in the different treatment groups, revealing the upregulated and downregulated DMs in the gills of

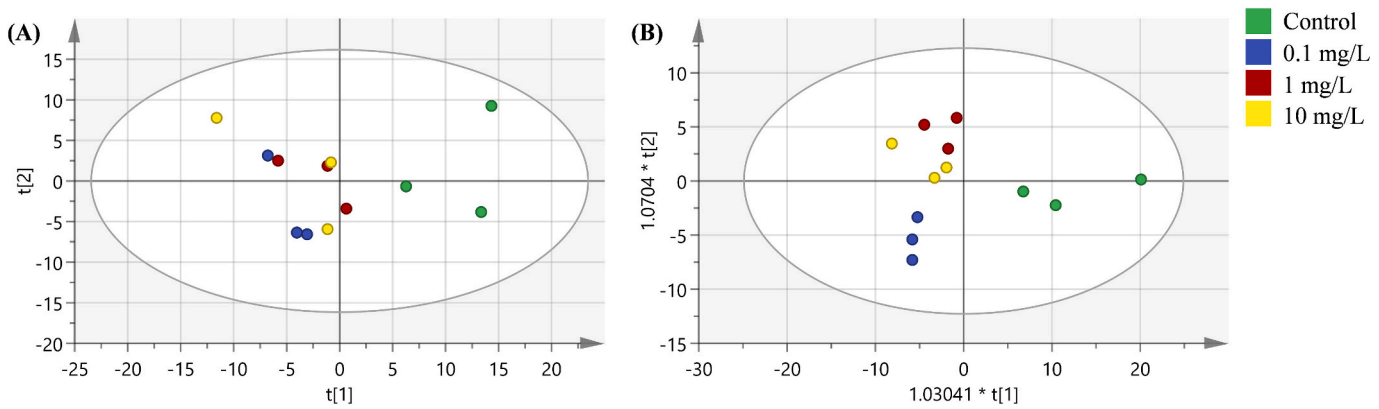


Fig. 2. The PCA model score plots (A) and OPLS-DA model results (B) from data of metabolites extracted from gills of *C. gigas* exposed to different concentrations of micro-PS.

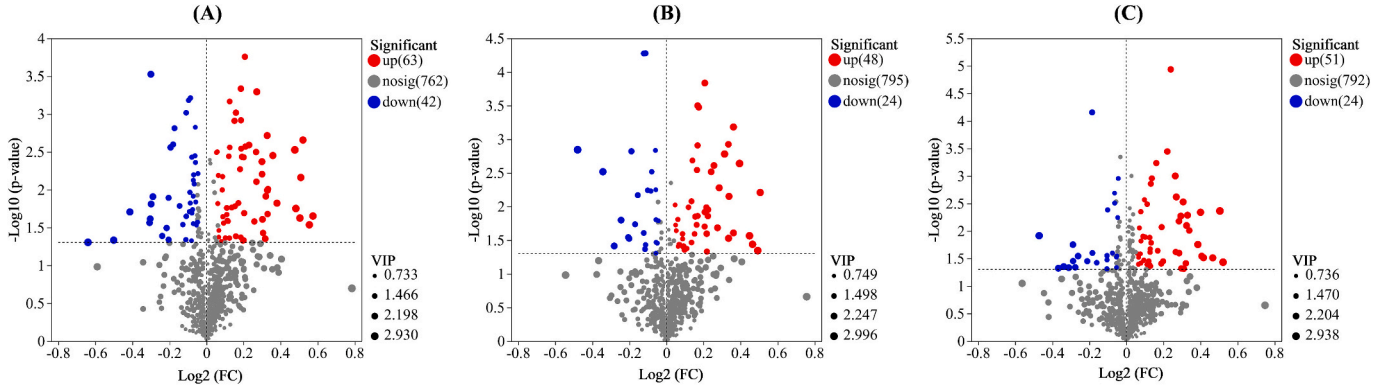


Fig. 3. The different metabolite volcano plots between the control and three exposure groups (A: 0.1 mg/L; B: 1 mg/L; C: 10 mg/L). The red points represent significantly upregulated metabolites, and the blue points represent significantly downregulated metabolites. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

C. gigas after exposure to micro-PS compared with those in the control. The identified DMs were then searched for classes in the HMDB database. DMs that could be annotated in metabolic pathways were selected via the KEGG database and are presented in Table 1. A total of 20 DMs were selected from the 0.1 mg/L group, 11 DMs were selected from the 1 mg/L group, and 16 DMs were selected from the 10 mg/L group.

3.4. Pathways perturbed by micro-PS

Pathway enrichment analysis was carried out to identify perturbed metabolic pathways associated with micro-PS exposure. KEGG pathway enrichment analysis revealed significantly impacted pathways, including lipid metabolism, amino acid metabolisms, metabolism of vitamins and cofactors, translation, and membrane transport, in the three MPs treatment groups (Table 2). The bubble plots in Fig. 4 illustrated the top 20 disturbed metabolic pathways derived from the KEGG metabolic pathway analysis. In the 0.1 mg/L group, the perturbed metabolic pathways included mainly cysteine and methionine metabolism, alpha-linolenic acid metabolism, aminoacyl-tRNA biosynthesis, arginine and proline metabolism, neuroactive ligand-receptor interaction, and biosynthesis of cofactors. In the 1 mg/L group, the main metabolic pathways of DMs were cysteine and methionine metabolism, aminoacyl-tRNA biosynthesis, biosynthesis of cofactors, arginine and proline metabolism, and oxidative phosphorylation. In the 10 mg/L group, the main perturbed metabolic pathways were cysteine and methionine metabolism, beta-alanine metabolism, histidine metabolism, aminoacyl-tRNA biosynthesis, ABC transporters, neuroactive ligand-receptor interaction, D-amino acid metabolism, arginine and proline metabolism, and the biosynthesis of cofactors.

Free amino acids, which make up a large proportion of the metabolome in marine bivalves, are necessary substrates for body proteins and play a key role in controlling the protein turnover process (Cappello et al., 2021). The current investigation revealed that MPs caused a significant decrease in the concentrations of tyrosine and aspartic acid in oysters, suggesting a disturbance in amino acid metabolism. L-aspartic acid, a crucial amino acid, serves as an intermediate in the tricarboxylic acid (TCA) cycle and plays a variety of roles in the metabolism and function of leukocytes (Zeng et al., 2023). L-aspartic acid also serves as a neurotransmitter; thus, the downregulation of L-aspartic acid might induce neurotoxicity in *C. gigas* (Zeng et al., 2023). Tyrosine is also involved in the TCA cycle, and alterations in this metabolite can interfere with the TCA cycle and affect the energy production of cells, as well as the immune, metabolic, and growth activities of organisms (Huang et al., 2021). Compared with those of the control, the levels of histidine were significantly lower under high-concentration MPs exposure (10 mg/L). The observed differences in several amino acids could be explained in several ways. On the one hand, amino acids are not only build blocks but also breakdown products of proteins, and fluctuations in their levels could be ascribed to an increase in anabolism or a decrease in catabolism, leading to a decrease in metabolite abundance (Lu et al., 2023). Another reason is that the intake of MPs might increase satiety and reduce the feeding activity of oysters, which, in turn, could lead to diminished energy reserves and reduced pools of some primary metabolites to maintain the turnover of amino acid metabolism (Liu et al., 2023). In addition, these alterations indicate a growing need for the utilization of existing metabolite reservoirs to channel them into diverse

Table 1
Differential metabolites associated with metabolic pathways after exposure to three concentrations of micro-PS.

Group	Metabolites	VIP	FC	*p*-value	Regulate
0.1 mg/L	LysoPC(18:1(11Z)/0:0)	1.1009	0.9686	0.006132	down
	Pregnenolone	1.1815	1.0456	0.006494	up
	Biotin	2.4343	1.255	0.001926	up
	S-Adenosylmethionine	2.398	1.2308	0.004262	up
	Liquiritigenin	2.2123	0.8104	0.02424	down
	Farnesylcysteine	1.7983	0.8679	0.04576	down
	5-Acetylamino-6-formyl amino-3-methyl-uracil	1.4549	1.091	0.002763	up
	L-Tyrosine	2.5437	0.7509	0.0197	down
	L-4-Hydroxyglutamate semialdehyde	1.3214	1.0634	0.02277	up
	L-Aspartic acid	1.1934	0.9533	0.006359	down
	5′-Methylthioadenosine	1.3092	1.0734	0.04343	up
	Morph	2.6904	1.4895	0.02228	up
	2-n-Propyl-4-oxopentanoic acid	1.7685	0.8678	0.01282	down
	7-Epijasmonic acid	1.3133	0.9259	0.04577	down
	LysoPC(16:0/0:0)	1.1518	0.958	0.003566	down
	Cholic Acid	2.6536	1.4234	0.006899	up
	(3Z,6Z)-3,6-Nonadienal	1.1029	1.0578	0.04818	up
	2-Hydroxyethanesulfonate	1.257	0.9381	0.01955	down
	3-Methyl-2-Oxovaleric Acid	1.0642	0.9518	0.02815	down
	Octadec-9-enoic Acid	1.0296	0.9456	0.04731	down
1 mg/L	S-Adenosylmethionine	2.3507	1.2105	0.02071	up
	Liquiritigenin	2.3107	0.8227	0.03863	down
	5-Acetylamino-6-formyl amino-3-methyl-uracil	1.487	1.086	0.01028	up
	L-Tyrosine	2.9958	0.7173	0.001434	down
	L-4-Hydroxyglutamate semialdehyde	1.433	1.062	0.02549	up
	PC(14:1(9Z)/20:5 (5Z,8Z,11Z,14Z,17Z))	2.8528	1.4073	0.04524	up
	L-Aspartic acid	1.3874	0.9466	0.003047	down
	5′-Methylthioadenosine	1.7252	1.0984	0.00836	up
	2-n-Propyl-4-oxopentanoic acid	1.7222	0.8894	0.01839	down
	Oxidized Glutathione	2.591	1.2617	0.0297	up
	Flavin Mononucleotide	1.1172	1.0515	0.03664	up
10 mg/L	Biotin	2.3712	1.2525	0.007963	up
	S-Adenosylmethionine	2.3522	1.2261	0.005361	up
	Liquiritigenin	2.0213	0.8259	0.04577	down
	N-Methyl-L-Glutamic Acid	1.1442	1.0464	0.04028	up
	L-4-Hydroxyglutamate semialdehyde	1.428	1.0725	0.01291	up
	PC(14:1(9Z)/20:5 (5Z,8Z,11Z,14Z,17Z))	2.8606	1.4359	0.03698	up
	2,3,4,5-Tetrahydro-2-pyridine carboxylic acid	1.0585	0.9702	0.001114	down
	L-Aspartic acid	1.1444	0.9574	0.003117	down
	Adenosine 3′-monophosphate	1.087	0.9651	0.02854	down
	5′-Methylthioadenosine	1.5537	1.0971	0.01641	up
	Morph	2.0133	1.2653	0.009805	up
	2-n-Propyl-4-oxopentanoic acid	1.6452	0.8806	0.02511	down
	Oxidized Glutathione	2.6181	1.3065	0.01764	up
	2-Hydroxyethanesulfonate	1.4629	0.8949	0.03776	down
	L-Histidine	1.1593	0.949	0.02521	down
	3-Methyl-2-Oxovaleric Acid	1.2805	0.9323	0.004145	down

Table 2
Top ten significantly perturbed metabolic pathways derived from the KEGG database on the basis of selected differential metabolites.

Group	Metabolic pathway	*p*-value	Differential metabolites	Regulate
0.1 mg/L	Cysteine and methionine metabolism	0.003371	L-Aspartic acid	down
			S-Adenosylmethionine	up
			5′-Methylthioadenosine	up
	alpha-Linolenic acid metabolism	0.01771	7-Epijasmonic acid	down
			(3Z,6Z)-3,6-Nonadienal	up
	Neuroactive ligand–receptor interaction	0.02427	L-Aspartic acid	down
			Morph	up
	Aminoacyl-tRNA biosynthesis	0.02427	L-Tyrosine	down
			L-Aspartic acid	down
	Arginine and proline metabolism	0.04096	S-Adenosylmethionine	up
			L-4-Hydroxyglutamate semialdehyde	up
	Sulfur relay system	0.05072	S-Adenosylmethionine	up
	Drug metabolism - cytochrome P450	0.06213	Morph	up
			2-n-Propyl-4-oxo pentanoic acid	down
	Biosynthesis of cofactors	0.06292	Biotin	up
			S-Adenosylmethionine	up
			L-Tyrosine	down
			L-Aspartic acid	down
	Caffeine metabolism	0.09899	5-Acetylamino-6-formyl amino-3-methyl-uracil	up
	Arginine biosynthesis	0.1033	L-Aspartic acid	down
1 mg/L	Cysteine and methionine metabolism	0.0006304	PC(14:1(9Z)/20:5 (5Z,8Z,11Z,14Z,17Z))	up
	Biosynthesis of cofactors	0.001061	S-Adenosylmethionine	up
			L-Tyrosine	down
			L-Aspartic acid	down
			Oxidized Glutathione	up
			Flavin Mononucleotide	up
	Aminoacyl-tRNA biosynthesis	0.008338	L-Tyrosine	down
			L-Aspartic acid	down
	Arginine and proline metabolism	0.01438	S-Adenosylmethionine	up
			L-4-Hydroxyglutamate semialdehyde	up
	Sulfur relay system	0.02965	S-Adenosylmethionine	up
	Oxidative phosphorylation	0.04287	Flavin Mononucleotide	up
	Caffeine metabolism	0.05851	5-Acetylamino-6-formyl amino-3-methyl-uracil	up
	Arginine biosynthesis	0.06109	L-Aspartic acid	down
	Riboflavin metabolism	0.06367	Flavin Mononucleotide	up
	Alanine, aspartate and glutamate metabolism	0.07391	L-Aspartic acid	down
10 mg/L	Cysteine and methionine metabolism	0.002018	S-Adenosylmethionine	up
			L-Aspartic acid	down
			5′-Methylthioadenosine	up
	beta-Alanine metabolism	0.006839	L-Aspartic acid	down
			L-Histidine	down
	Histidine metabolism	0.0144	L-Aspartic acid	down
			L-Histidine	down
	ABC transporters	0.01586	Biotin	up
			L-Aspartic acid	down
			L-Histidine	down
	Neuroactive ligand–receptor interaction	0.01746	L-Aspartic acid	down
			Morph	up
	Aminoacyl-tRNA biosynthesis	0.01746	L-Aspartic acid	down
			L-Histidine	down
	D-Amino acid metabolism	0.02735	L-Aspartic acid	down
			L-Histidine	down
	Arginine and proline metabolism	0.0297	S-Adenosylmethionine	up
			L-4-Hydroxyglutamate semialdehyde	up

(*continued on next page*)

Table 2 (*continued*)

Group	Metabolic pathway	*p*-value	Differential metabolites	Regulate
	Biosynthesis of cofactors	0.03579	Biotin	up
			S-Adenosylmethionine	up
			L-Aspartic acid	down
			Oxidized Glutathione	up
	Sulfur relay system	0.04287	S-Adenosylmethionine	up

metabolic pathways (Lu et al., 2023). As shown in this study, the metabolomics results revealed that MPs resulted in metabolite disorders related to changes in amino acid metabolism. To be specific, the synthesis and metabolism of several amino acids were mainly influenced by exposure to micro-PS. For example, cysteine and methionine metabolism, arginine and proline metabolism, arginine biosynthesis, valine, leucine, and isoleucine biosynthesis, taurine and hypotaurine metabolism, alanine, aspartate and glutamate metabolism, and histidine metabolism were induced. Similar results were also reported by Teng et al. (2021b), who demonstrated that MPs can disturb the metabolism of amino acids in the digestive gland of *C. gigas,* such as the metabolism of arginine and proline, the metabolism of beta-alanine, and the metabolism of histidine. Huang et al. (2021) also reported that micro-PS induced amino acid metabolism disorders in mussels such as phenylalanine metabolism. Amino acids play crucial roles in energy metabolism and serve as oxidative substrates to promote the formation of ATP during osmoregulation in organisms, and the dysfunction of amino acid metabolism may be the key factor that leads to oxidative stress in oysters and other bivalves. These changes in amino acid metabolism indicated that the ingestion of MPs could impair the antioxidant system in *C. gigas*.

Significant effects of all three levels of micro-PS on aminoacyl-tRNA biosynthesis were revealed via KEGG pathway enrichment analysis. Aminoacyl-tRNA biosynthesis involves the process of direct aminoacylation of tRNA and amino acids, which is closely linked to the production of polypeptides and proteins within cells (Wu et al., 2023). Research on aminoacyl-tRNA biosynthesis has shown that oysters might deal with oxidative stress induced by MPs by regulating the translation process (Wang et al., 2021). However, metabolites enriched in such pathways, including tyrosine and aspartic acid, were significantly downregulated, indicating that the formation of polypeptide chains and the synthesis of proteins in oysters might be disrupted after exposure to MPs.

Lipid metabolism was also perturbed after exposure to MPs. Lysophosphatidylcholines (LysoPCs) are derived from the cleavage of phosphatidylcholine in the circulation by phospholipase A2 (Liu et al., 2020) and it has a significant function in lipid metabolism and the transportation of glycerophospholipid components, including fatty acids, phosphatidylglycerol, and choline (Wei et al., 2021). Moreover, LysoPC is the main component of oxidized low-density lipoproteins and is considered a group of proinflammatory lipids that are correlated with several chronic inflammatory diseases (Law et al., 2019). Teng et al. (2021b) reported that the levels of LysoPCs were elevated after exposure to MPs compared with those in the control, indicating that an inflammatory response might occur in oysters. However, in this study, the levels of LysoPCs, such as LysoPC (18:1(11Z)/0:0) and LysoPC (16:0/0:0) were significantly lower in the low-concentration exposure group (0.1 mg/L), whereas no significant changes in these two metabolites were observed in the 1 mg/L and 10 mg/L exposure groups. The changes in such metabolites are unclear and need further investigation. Moreover, α-linolenic acid biosynthesis was enriched under MPs at concentrations of 0.1 and 1 mg/L compared with the control. α-linolenic acid usually functions as a structural component and metabolism regulator in regulating the lipid metabolism of organisms. It typically manifests under inflammations and serves as a crucial substance of cells and the mitochondrial membrane to regulate associated enzymes (Han et al., 2023). In addition to α-linolenic acid metabolism, the linoleic acid metabolism pathway was identified in the 10 mg/L exposure group. The disturbed pathway of α-linolenic acid and linoleic acid metabolism indicated the inflammatory response and oxidative stress induced by MPs in oysters.

The cytochrome P450 is a heme-thiolate protein that encodes a family of genes that play important roles in the biosynthesis of endogenous compounds and the biotransformation of several xenobiotics and therapeutic drugs. Drug metabolism - cytochrome P450 is commonly related to xenobiotic biotransformation and the response to environmental stress in diverse organisms (Luchmann et al., 2015; Siebert et al., 2017). Drug metabolism-cytochrome P450 was enriched in the 0.1 and 10 mg/L MPs groups, with significant enrichment noted in the 10 mg/L exposure group. The leading enrichment of Drug metabolism-cytochrome P450 indicated that exposure to MPs induced toxic effects on oysters. Nevertheless, MPs cannot be metabolized or absorbed by oysters. It was speculated that the enrichment of Drug metabolism - cytochrome P450 was caused by the leaching of organic chemical additives from PS since certain flame retardants and plasticizers, such as bisphenol A, 2,4,6-tribromophenol, and tetrabromobisphenol A, are commonly added to plastics to improve their properties for use during manufacturing processes (Barhoumi et al., 2023).

Subsequently, six DMs, including aspartic acid, shikimic acid, LysoPC (16:0/0:0), LysoPC (18:1(11z)/0:0), biotin, and 5-acetylamino-6-formylamino-3-methyluracil, were selected to investigate whether alterations in the abundance of metabolites exhibit the same mode of changes in response to increasing micro-PS concentrations. As shown in Fig. S3, the concentrations of shikimic acid increased with increasing doses of micro-PS, whereas the abundance of LysoPC (18:1(11z)/0:0) increased with increasing levels of micro-PS, indicating a relationship between the concentrations of such metabolites and the MPs dose. However, except for shikimic acid and LysoPC (18:1(11z)/0:0), the other four selected metabolites demonstrated no special mode of change as a function of increasing doses of MPs. These results showed that

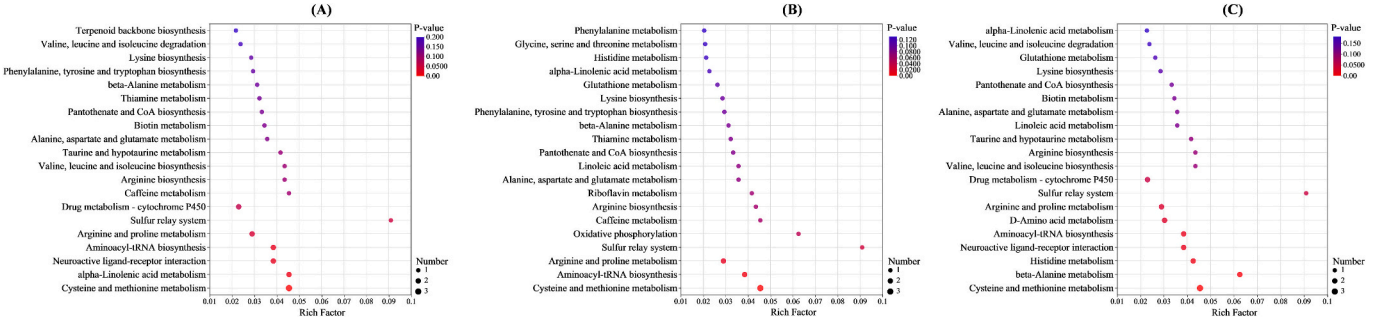


Fig. 4. The bubble plots of metabolic enrichment of differential metabolites. A, B, and C show the top 20 metabolic pathways enriched in the 0.1, 1, and 10 mg/L micro-PS exposure groups. The abscissa represents the enrichment factor (rich factor = metabolite count/pathway pop hits). The larger the bubbles, the greater the number of different metabolites, and the color indicates the significance of enrichment; a darker color indicates that the pathway is more significantly enriched.

different doses of micro-PS induced diverse metabolomic profiles in oysters.

4. Conclusion

MPs are hazardous contaminants that threaten marine organisms and ecosystems worldwide. Currently, knowledge regarding the effects of MPs on various aquatic organisms is insufficient. As filtering-feeding species, oysters tend to have a relatively high likelihood of MPs ingestion, and there is an urgent need to clarify the impacts of MPs on oysters. This study evaluated the disruption of metabolic systems in oysters induced by PS-MPs employing LC-MS non-target metabolomics. In this study, MPs exposure resulted in severe oxidative stress and impaired the enzymatic antioxidant systems of oysters. The metabolomics results revealed that all three doses of MPs significantly changed the metabolomics profiles of oysters and that the metabolomics profiles varied according to the concentrations of the MPs. These altered pathways were related primarily to amino acid metabolism, lipid metabolism, amino acid biosynthesis, energy metabolism, and oxidative stress. Overall, the results provide some new evidences and explanations for the environmental risks of MPs to marine shellfish.

CRediT authorship contribution statement

Yu Liu: Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Shuai Wu:** Writing – review & editing, Methodology. **Lipin Chen:** Writing – review & editing, Visualization, Investigation, Conceptualization. **Xiaoyu Teng:** Writing – review & editing, Methodology. **Haohao Shi:** Writing – review & editing, Methodology. **Changhu Xue:** Supervision, Project administration. **Zhaojie Li:** Validation, Project administration, Conceptualization.

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.

Acknowledgment

This work was supported by the earmarked fund for Modern Agro-industry Technology Research System (CARS-49).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2024.140961.

References

Auta, H. S., Emenike, C. U., & Fauziah, S. H. (2017). Distribution and importance of microplastics in the marine environment: A review of the sources, fate, effects, and potential solutions. *Environment International, 102*, 165–176. https://doi.org/10.1016/j.envint.2017.02.013

Baihetiyaer, B., Jiang, N., Li, X., He, B., Wang, J., Fan, X., Sun, H., & Yin, X. (2023). Oxidative stress and gene expression induced by biodegradable microplastics and imidacloprid in earthworms (*Eisenia fetida*) at environmentally relevant concentrations. *Environmental Pollution, 323*, Article 121285. https://doi.org/10.1016/j.envpol.2023.121285

Barhoumi, B., Metian, M., Oberhaensli, F., Mourgkogiannis, N., Karapanagioti, H. K., Bersuder, P., & Tolosa, I. (2023). Extruded polystyrene microplastics as a source of brominated flame retardant additives in the marine environment: Long-term field and laboratory experiments. *Environment International, 172*, Article 107797. https://doi.org/10.1016/j.envint.2023.107797

Bhagat, J., Zang, L., Nishimura, N., & Shimada, Y. (2022). Application of omics approaches for assessing microplastic and nanoplastic toxicity in fish and seafood species. *Trac-Trends in Analytical Chemistry, 154*, Article 116674. https://doi.org/10.1016/j.trac.2022.116674

Botterell, Z. L. R., Bergmann, M., Hildebrandt, N., Krumpen, T., Steinke, M., Thompson, R. C., & Lindeque, P. K. (2022). Microplastic ingestion in zooplankton from the Fram Strait in the Arctic. *Science of the Total Environment, 831*, Article 154886. https://doi.org/10.1016/j.scitotenv.2022.154886

Bringer, A., Cachot, J., Dubillot, E., Prunier, G., Huet, V., Clérandeau, C., Evin, L., & Thomas, H. (2022). Intergenerational effects of environmentally aged microplastics on the *Crassostrea gigas*. *Environmental Pollution, 294*, Article 118600. https://doi.org/10.1016/j.envpol.2021.118600

Bringer, A., Thomas, H., Prunier, G., Dubillot, E., Bossut, N., Churlaud, C., Clérandeau, C., Le Bihanic, F., & Cachot, J. (2020). High density polyethylene (HDPE) microplastics impair development and swimming activity of Pacific oyster D-larvae, *Crassostrea gigas*, depending on particle size. *Environmental Pollution, 260*, Article 113978. https://doi.org/10.1016/j.envpol.2020.113978

Cappello, T., De Marco, G., Oliveri, C. G., Giannetto, A., Ferrante, M., Mauceri, A., & Maisano, M. (2021). Time-dependent metabolic disorders induced by short-term exposure to polystyrene microplastics in the Mediterranean mussel *Mytilus galloprovincialis*. *Ecotoxicology and Environmental Safety, 209*, Article 111780. https://doi.org/10.1016/j.ecoenv.2020.111780

Du, Y., Teng, J., Zhao, J., Ren, J., Ma, H., Zhang, T., Xia, B., Sun, S., & Wang, Q. (2024). Effects of ocean acidification and polystyrene microplastics on the oysters *Crassostrea gigas*: An integrated biomarker and metabolomic approach. *Marine Environmental Research, 196*, Article 106434. https://doi.org/10.1016/j.marenvres.2024.106434

Du, Y., Zhao, J., Teng, J., Ren, J., Shan, E., Zhu, X., Zhang, W., Wang, L., Hou, C., & Wang, Q. (2023). Combined effects of salinity and polystyrene microplastics exposure on the Pacific oysters *Crassostrea gigas*: Oxidative stress and energy metabolism. *Marine Pollution Bulletin, 193*, Article 115153. https://doi.org/10.1016/j.marpolbul.2023.115153

Gardon, T., Morvan, L., Huvet, A., Quillien, V., Soyez, C., Le Moullac, G., & Le Luyer, J. (2020). Microplastics induce dose-specific transcriptomic disruptions in energy metabolism and immunity of the pearl oyster *Pinctada margaritifera*. *Environmental Pollution, 266*, Article 115180. https://doi.org/10.1016/j.envpol.2020.115180

Gardon, T., Reisser, C., Soyez, C., Quillien, V., & Le Moullac, G. (2018). Microplastics affect energy balance and gametogenesis in the pearl oyster *Pinctada margaritifera*. *Environmental Science & Technology, 52*(9), 5277–5286. https://doi.org/10.1021/acs.est.8b00168

Gurjar, U. R., Xavier, M., Nayak, B. B., Ramteke, K., Deshmukhe, G., Jaiswar, A. K., & Shukla, S. P. (2021). Microplastics in shrimps: A study from the trawling grounds of north eastern part of Arabian Sea. *Environmental Science and Pollution Research, 28* (35), 48494–48504. https://doi.org/10.1007/s11356-021-14121-z

Hamm, T., & Lenz, M. (2021). Negative impacts of realistic doses of spherical and irregular microplastics emerged late during a 42 weeks-long exposure experiment with blue mussels. *Science of the Total Environment, 778*, Article 146088. https://doi.org/10.1016/j.scitotenv.2021.146088

Han, Y., Fu, M., Wu, J., Zhou, S., Qiao, Z., Peng, C., Zhang, W., Liu, F., Ye, C., & Yang, J. (2023). Polylactic acid microplastics induce higher biotoxicity of decabromodiphenyl ethane on earthworms (*Eisenia fetida*) compared to polyethylene and polypropylene microplastics. *Science of the Total Environment, 862*, Article 160909. https://doi.org/10.1016/j.scitotenv.2022.160909

Huang, W., Wang, X., Chen, D., Xu, E. G., Luo, X., Zeng, J., … Wang, Y. (2021). Toxicity mechanisms of polystyrene microplastics in marine mussels revealed by high-coverage quantitative metabolomics using chemical isotope labeling liquid chromatography mass spectrometry. *Journal of Hazardous Materials, 417*, Article 126003. https://doi.org/10.1016/j.jhazmat.2021.126003

Jia, Y., Wang, P., Yang, S., Huang, Z., Liu, Y., & Zhao, J. (2022). Influence of microplastics on triclosan bioaccumulation and metabolomics variation in Tilapia fish tissues. *Environmental Science and Pollution Research, 29*(42), 62984–62993. https://doi.org/10.1007/s11356-022-20278-y

Kang, C., Zhang, Y., Zhang, M., Qi, J., Zhao, W., Gu, J., Guo, W., & Li, Y. (2022). Screening of specific quantitative peptides of beef by LC–MS/MS coupled with OPLS-DA. *Food Chemistry, 387*, Article 132932. https://doi.org/10.1016/j.foodchem.2022.132932

Kilic, O., Belivermis, M., Sikdokur, E., Sezer, N., Aksut, Y., Pekmez, M., … Gercek, Y. C. (2023). The combined effects of polyethylene microplastics and benzoanthracene on Manila clam *Ruditapes philippinarum*. *Chemosphere, 329*, Article 138664. https://doi.org/10.1016/j.chemosphere.2023.138664

Law, K. L., Moret-Ferguson, S., Maximenko, N. A., Proskurowski, G., Peacock, E. E., Hafner, J., & Reddy, C. M. (2010). Plastic accumulation in the North Atlantic subtropical gyre. *Science, 329*(5996), 1185–1188. https://doi.org/10.1126/science.1192321

Law, S. H., Chan, M. L., Marathe, G. K., Parveen, F., Chen, C. H., & Ke, L. Y. (2019). An updated review of Lysophosphatidylcholine metabolism in human diseases. *International Journal of Molecular Sciences, 20*(5), 1149. https://doi.org/10.3390/ijms20051149

Li, J., Qu, X., Su, L., Zhang, W., Yang, D., Kolandhasamy, P., Li, D., & Shi, H. (2016). Microplastics in mussels along the coastal waters of China. *Environmental Pollution, 214*, 177–184. https://doi.org/10.1016/j.envpol.2016.04.012

Liu, P., Zhu, W., Chen, C., Yan, B., Zhu, L., Chen, X., & Peng, C. (2020). The mechanisms of lysophosphatidylcholine in the development of diseases. *Life Sciences, 247*, Article 117443. https://doi.org/10.1016/j.lfs.2020.117443

Liu, Y., Shi, H., Chen, L., Teng, X., Xue, C., & Li, Z. (2023). An overview of microplastics in oysters: Analysis, hazards, and depuration. *Food Chemistry, 422*, Article 136153. https://doi.org/10.1016/j.foodchem.2023.136153

Liu, Y., Teng, X., Chen, L., Wu, S., Xue, C., & Li, Z. (2024). Changes in flavor-related biomarkers in Pacific oysters (*Crassostrea gigas*). *Following Microplastic Exposure. Foods, 13*(5). https://doi.org/10.3390/foods13050765

Lonnstedt, O. M., & Eklov, P. (2016). RETRACTED: Environmentally relevant concentrations of microplastic particles influence larval fish ecology. *Science, 352* (6290), 1213–1216. https://doi.org/10.1126/science.aad8828

Lu, F., Guo, C., Mkuye, R., Chen, W., Yang, X., Zhou, Z., He, Y., Yang, C., & Deng, Y. (2024). Effects of polyvinyl chloride microplastic on pearl oyster (*Pinctada fucata martensii*). *Regional Studies in Marine Science, 69*, Article 103313. https://doi.org/10.1016/j.rsma.2023.103313

Lu, H. C., Kumar, A., Melvin, S. D., Ziajahromi, S., Neale, P. A., & Leusch, F. (2023). Metabolomic responses in freshwater benthic invertebrate, *Chironomus tepperi*, exposed to polyethylene microplastics: A two-generational investigation. *Journal of Hazardous Materials, 459*, Article 132097. https://doi.org/10.1016/j.jhazmat.2023.132097

Luchmann, K. H., Clark, M. S., Bainy, A. C., Gilbert, J. A., Craft, J. A., Chipman, J. K., … Schroeder, D. C. (2015). Key metabolic pathways involved in xenobiotic biotransformation and stress responses revealed by transcriptomics of the mangrove oyster *Crassostrea brasiliana*. *Aquatic Toxicology, 166*, 10–20. https://doi.org/10.1016/j.aquatox.2015.06.012

Mkuye, R., Gong, S., Zhao, L., Masanja, F., Ndandala, C., Bubelwa, E., Yang, C., & Deng, Y. (2022). Effects of microplastics on physiological performance of marine bivalves, potential impacts, and enlightening the future based on a comparative study. *Science of the Total Environment, 838*, Article 155933. https://doi.org/10.1016/j.scitotenv.2022.155933

Negara, B. F. S. P., Mohibbullah, M., Sohn, J., Kim, J., & Choi, J. (2022). Nutritional value and potential bioactivities of Pacific oyster (*Crassostrea gigas*). *International Journal of Food Science and Technology, 57*(9), 5732–5749. https://doi.org/10.1111/ijfs.15939

Neves, D., Sobral, P., Ferreira, J. L., & Pereira, T. (2015). Ingestion of microplastics by commercial fish off the Portuguese coast. *Marine Pollution Bulletin, 101*(1), 119–126. https://doi.org/10.1016/j.marpolbul.2015.11.008

Nobre, C. R., Moreno, B. B., Alves, A. V., de Lima, R. J., Da, R. F. H., Abessa, D., … Pereira, C. (2020). Effects of microplastics associated with Triclosan on the oyster *Crassostrea brasiliana*: An integrated biomarker approach. *Archives of Environmental Contamination and Toxicology, 79*(1), 101–110. https://doi.org/10.1007/s00244-020-00729-8

Parra, S., Varandas, S., Santos, D., Felix, L., Fernandes, L., Cabecinha, E., … Monteiro, S. M. (2021). Multi-biomarker responses of Asian clam *Corbicula fluminea* (Bivalvia, Corbiculidea) to cadmium and microplastics pollutants. *Water, 13*(4), 15. https://doi.org/10.3390/w13040394

Paul-Pont, I., Lacroix, C., González Fernández, C., Hégaret, H., Lambert, C., Le Goïc, N., Frère, L., Cassone, A., 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. *Environmental Pollution, 216*, 724–737. https://doi.org/10.1016/j.envpol.2016.06.039

Prokic, M. D., Radovanovic, T. B., Gavric, J. P., & Faggio, C. (2019). Ecotoxicological effects of microplastics: Examination of biomarkers, current state and future perspectives. *Trac-Trends in Analytical Chemistry, 111*, 37–46. https://doi.org/10.1016/j.trac.2018.12.001

Siebert, M. N., Mattos, J. J., Toledo-Silva, G., Razzera, G., & Bainy, A. (2017). Candidate cytochrome P450 genes for ethoxyresorufin O-deethylase activity in oyster *Crassostrea gigas*. *Aquatic Toxicology, 189*, 142–149. https://doi.org/10.1016/j.aquatox.2017.06.004

Su, L., Xiong, X., Zhang, Y., Wu, C., Xu, X., Sun, C., & Shi, H. (2022). Global transportation of plastics and microplastics: A critical review of pathways and influences. *Science of the Total Environment, 831*, Article 154884. https://doi.org/10.1016/j.scitotenv.2022.154884

Teng, J., Wang, Q., Ran, W., Wu, D., Liu, Y., Sun, S., Liu, H., Cao, R., & Zhao, J. (2019). Microplastic in cultured oysters from different coastal areas of China. *Science of the Total Environment, 653*, 1282–1292. https://doi.org/10.1016/j.scitotenv.2018.11.057

Teng, J., Zhao, J., Zhu, X., Shan, E., & Wang, Q. (2021a). Oxidative stress biomarkers, physiological responses and proteomic profiling in oyster (*Crassostrea gigas*) exposed to microplastics with irregular-shaped PE and PET microplastic. *Science of the Total Environment, 786*, Article 147425. https://doi.org/10.1016/j.scitotenv.2021.147425

Teng, J., Zhao, J. M., Zhu, X. P., Shan, E. C., Zhang, C., Zhang, W. J., & Wang, Q. (2021b). Toxic effects of exposure to microplastics with environmentally relevant shapes and concentrations: Accumulation, energy metabolism and tissue damage in oyster *Crassostrea gigas*. *Environmental Pollution, 269*, Article 116169. https://doi.org/10.1016/j.envpol.2020.116169

Thompson, R. C., Moore, C. J., Vom, S. F., & Swan, S. H. (2009). Plastics, the environment and human health: Current consensus and future trends. *Philosophical Transactions of the Royal Society B-Biological Sciences, 364*(1526), 2153–2166. https://doi.org/10.1098/rstb.2009.0053

Wang, B., Junaid, M., Imran, M., Wei, L., Chen, G., & Wang, J. (2024). Environmental and sublethal concentrations of polystyrene Nanoplastics induced antioxidant system, transcriptomic responses, and disturbed gut microbiota in oyster *Magallana Hongkongensis*. *Journal of Agricultural and Food Chemistry, 72*(24), 13581–13592. https://doi.org/10.1021/acs.jafc.4c02895

Wang, Q., Wen, J., Zheng, J., Zhao, J., Qiu, C., Xiao, D., Mu, L., & Liu, X. (2021). Arsenate phytotoxicity regulation by humic acid and related metabolic mechanisms. *Ecotoxicology and Environmental Safety, 207*, Article 111379. https://doi.org/10.1016/j.ecoenv.2020.111379

Wei, Q., Hu, C., Zhang, R., Gu, Y., Sun, A., Zhang, Z., Shi, X., Chen, J., & Wang, T. (2021). Comparative evaluation of high-density polyethylene and polystyrene microplastics pollutants: Uptake, elimination and effects in mussel. *Marine Environmental Research, 169*, Article 105329. https://doi.org/10.1016/j.marenvres.2021.105329

Wootton, N., Sarakinis, K., Varea, R., Reis-Santos, P., & Gillanders, B. M. (2022). Microplastic in oysters: A review of global trends and comparison to southern Australia. *Chemosphere, 307*, Article 136065. https://doi.org/10.1016/j.chemosphere.2022.136065

Wu, X., Li, M., Yang, S., Dong, J., Pan, W., Ning, Y., Hua, X., Dong, D., & Liang, D. (2023). Liver metabolic dysregulation induced by polypropylene Nano- and Microplastics in Nile Tilapia using internal extractive electrospray ionization mass spectrometry. *Analytical Chemistry, 95*(20), 7863–7871. https://doi.org/10.1021/acs.analchem.2c05672

Wu, X., Liu, Y., Yin, S., Xiao, K., Xiong, Q., Bian, S., Liang, S., Hou, H., Hu, J., & Yang, J. (2020). Metabolomics revealing the response of rice (*Oryza sativa* L.) exposed to polystyrene microplastics. *Environmental Pollution, 266*, Article 115159. https://doi.org/10.1016/j.envpol.2020.115159

Zeng, Y., Deng, B., Kang, Z., Araujo, P., Mjos, S. A., Liu, R., … Qu, Y. (2023). Tissue accumulation of polystyrene microplastics causes oxidative stress, hepatopancreatic injury and metabolome alterations in *Litopenaeus vannamei*. *Ecotoxicology and Environmental Safety, 256*, Article 114871. https://doi.org/10.1016/j.ecoenv.2023.114871

Zhang, X., Wang, X., & Yan, B. (2021). Single and combined effects of phenanthrene and polystyrene microplastics on oxidative stress of the clam (*Mactra veneriformis*). *Science of the Total Environment, 771*, Article 144728. https://doi.org/10.1016/j.scitotenv.2020.144728

Zhao, Y., Qiao, R., Zhang, S., & Wang, G. (2021). Metabolomic profiling reveals the intestinal toxicity of different length of microplastic fibers on zebrafish (*Danio rerio*). *Journal of Hazardous Materials, 403*, Article 123663. https://doi.org/10.1016/j.jhazmat.2020.123663