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Polystyrene microplastics induce metabolic disturbances in marine medaka (*Oryzias melastigmas*) liver

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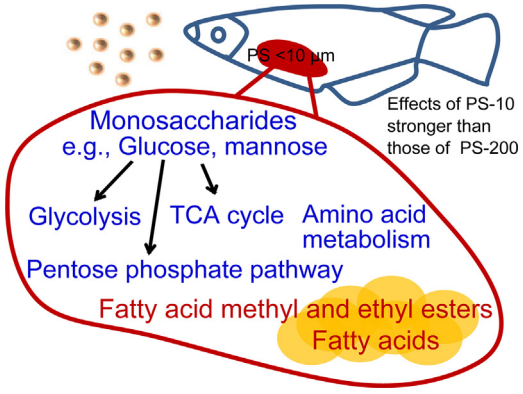
HIGHLIGHTS

- PS disturbed pentose phosphate pathway and nucleotide metabolism in the liver.
- PS suppressed hepatic organic acids in the tricarboxylic acid cycle and glycolysis.
- PS suppressed most monosaccharides and amino acids in the liver.
- PS induced accumulation of hepatic fatty acids, fatty acid methyl, and ethyl esters.
- PS size affected hepatic metabolism and PS accumulation.

GRAPHICAL ABSTRACT



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ABSTRACT

Microplastics are toxic to aquatic organisms. Metabolic disturbances in the liver are highly correlated with the pathophysiology of diseases and toxicology of contaminants. However, the effects of microplastics on the comprehensive metabolic responses of aquatic animal livers are unclear. Therefore, an untargeted metabolomics approach using gas chromatography–mass spectrometry was employed to discover metabolic disorders in marine medaka (*Oryzias melastigmas*) liver after microplastic exposure. We found that 83 metabolites were significantly altered in marine medaka liver after 10-μm and/or 200-μm polystyrene microplastic (PS) exposure. Subsequent metabolic pathway analysis revealed that 33 and 28 metabolic pathways, such as glycerolipid metabolism, biosynthesis of unsaturated fatty acids, glycolysis/gluconeogenesis, fructose and mannose metabolism, and glycine, serine and threonine metabolism, were significantly altered after 10-μm (PS-10) and 200-μm (PS-200) PS exposure, respectively. Most monosaccharides (e.g., glucose, mannose, and ribose), organic acids (e.g., lactate, fumarate, and malate) and amino acids (e.g., alanine, serine, and leucine) significantly decreased, while most fatty acids and fatty acid methyl and ethyl esters significantly increased in marine medaka liver due to PS-10 and/or PS-200 exposure. The results indicated that PS exposure triggered inhibition of the monosaccharide metabolism, tricarboxylic acid cycle, glycolysis, pentose phosphate pathway, and amino acid metabolism, and accumulation of

Abbreviations: PS, polystyrene microplastics; PS-10, 10-μm PS; PS-200, 200-μm PS.

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fatty acids, fatty acid methyl and ethyl esters in marine medaka liver. The varying influences of PS-10 and PS-200 on hepatic metabolism were observed. Changes in the carbohydrate, amino acid, and lipid metabolism induced by PS-10 were greater than those induced by PS-200. However, PS-200 induced the hepatic accumulation of most disaccharides and trisaccharides, including lactose, maltose, mannobiose and maltotriose, while PS-10 did not. The increased toxicity caused by PS-10 than that by PS-200 may be associated with the hepatic accumulation observed only in the group exposed to PS-10. This study provides novel insights into microplastic ecotoxicology.

1. Introduction

Microplastics, defined as plastic particles with diameters ranging from 1 μm to 5 mm, are found in waterbodies (such as inland freshwaters, coastal waters, and seawater), commercial salts, freshwater and marine organisms, and even in humans who ingest it via the food chain and/or drinking water (Cole and Galloway, 2015; Luo et al., 2019a; Toussaint et al., 2019). Due to their small size, microplastics can be easily ingested by aquatic organisms, and consequently cause physical, chemical, and biological damages, such as digestive tract blockage, ulcerative lesions, increased mortality, lower growth and development rates, abnormal hepatic metabolism, endocrine disorders, oxidative stress, genotoxicity and disturbed immunological responses (Gardon et al., 2018; Pannetier et al., 2020). Therefore, the investigation of microplastic impact of aquatic organisms and ecosystems is a vital issue.

Metabolism is a crucial physiological process, and its abnormalities are closely related to the pathophysiology of diseases and toxicology of contaminants (Hirano and Suzuki, 1996; Luo et al., 2019a; Castegna et al., 2020; Tardelli et al., 2020). The accumulated evidence has demonstrated that polystyrene microplastics (PS) exposure disrupts the metabolic pathways associated with energy metabolism, thus affecting biological functions in the exposed organisms (Antao Barboza et al., 2018; Qiao et al., 2019a; Xie et al., 2020; Zhao et al., 2020). For example, PS exposure triggered significant decreases in succinate dehydrogenase and lactate dehydrogenase (key enzymes in the tricarboxylic acid cycle and glycolysis, respectively) levels in the testicular tissue of mice, suggesting sperm cell energy deficits in mice with low amount and quality of mature sperms and high sperm deformity rates due to PS exposure (Xie et al., 2020). Additionally, PS exposure triggered significant increases in lactate dehydrogenase and isocitrate dehydrogenase levels in the muscle tissue of the European seabass (*Dicentrarchus labrax*, Linnaeus, 1758) and a significant decrease in the lactate level in the intestine of zebrafish, and vacuolization and cilia defects in the gut mucosa (Antao Barboza et al., 2018; Qiao et al., 2019a). Hepatic transcriptome analysis has demonstrated that expression levels of genes involved in glycolysis/gluconeogenesis (*Glut2*, *GK*, *HK*, *PK*, and *PEPCKC*) and fatty acid oxidation (*Cpt1a*, *Acox*, and *Mcad*) significantly decreased due to PS exposure; additionally, changes in fatty acid oxidation induced by PS exposure can be genetically transmitted (Luo et al., 2019a; Luo et al., 2019b; Zhao et al., 2020).

The liver is a vital metabolic organ that mediates the absorption, digestion, synthesis, and storage of various biochemical components, and detoxification of environmental contaminants, and plays a key role in maintaining biological energy homeostasis (Purushotham et al., 2009). Metabolomics is dedicated to the comprehensive qualitative and quantitative metabolic analysis of organisms under pathophysiological or gene stimuli conditions, and it has been widely applied in environmental toxicology research (Nicholson et al., 1999). However, there is a knowledge-gap concerning comprehensive metabolic responses of the liver triggered by PS exposure. The marine medaka (*Oryzias melastigma*) was considered as a representative species for toxicological research, as it exhibits several advantages, such as wide adaptability to various temperature and salinity levels, small size, and high reproduction rate. Moreover, the species can be conveniently observed and manipulated for experimental processes. In this study, the marine medaka was exposed to 10-μm and 200-μm PS (PS-10 and PS-200), respectively, for 60 d to induce metabolic disturbances in its liver. Subsequently, an untargeted metabolomics approach based on gas chromatography–mass spectrometry was used to assess the comprehensive metabolic responses of the marine medaka liver to PS exposure.

2. Materials and methods

2.1. Materials

Ultrapure water was produced from a Milli-Q water system (Millipore Co., USA). High-performance liquid chromatography-grade methanol was ordered from Honeywell Burdick & Jackson (Muskegon, USA). *N*-methyl-N-(trimethylsilyl)-trifluoro-acetamide (≥98.5%), pyridine (anhydrous, 99.8%), and methoxyamine hydrochloride (98%) were purchased from Sigma-Aldrich (Shanghai, China). PS-10 and PS-200 were obtained from Shanghai Guanbu Electromechanical Technology Co., Ltd. (Shanghai, China). The salt used in the experiment was ordered from China Salt Engineering Technology Research Institute Co., Ltd. (China). Polycarbonate membrane with a 0.22-μm pore size was obtained from Millipore Co. (USA).

2.2. Animal experiments

The salt was dissolved in the ultrapure water to form artificial seawater with a salinity of 30‰, pH of 8.0–8.4, KH of 7–10 and over 40 types of ions. Eight-month-old adult marine medaka (male/female = 1:1) were cultured in the 30‰ artificial seawater, which was filtered successively using three layers of filter paper and a 0.22-μm filter membrane to remove bacteria. The concentration of microplastics in freshwater and drinking water ranges from 1×10^{-2} to 10^{8} particles/m^3, and that the particle size of microplastics in fishes from the North Sea is typically between 0.04 and 4.8 mm (Foekema et al., 2013; Koelmans et al., 2019). Additionally, toxicological studies have demonstrated that exposure to micron- and nano-scale plastic particles at a concentration between 2 μg/L and 40 mg/L can induce biological toxicity, such as microplastic accumulation in the liver, gill and intestine, and relevant inflammation, microbiota dysbiosis, oxidative stress and metabolic disturbances (Lu et al., 2016; Yu et al., 2018; Qiao et al., 2019b; Wang et al., 2019). Considering the abovementioned findings, PS-10 and PS-200 were added into separate seawater, samples with a final PS concentration of 10 mg/L (1.82×10^{10} particles/m^3 and 2.27×10^{6} particles/m^3 for PS-10 and PS-200, respectively). There were six marine medakas (one marine medaka per replicate) in each 2-L fish tank. On the 30, 45, and 60 d of the experiment, one marine medaka was removed from the PS-10 and PS-200 exposure groups, respectively, to observe if there were any toxic effects due to PS exposure. Healthy marine medaka of the same age (not in the control group) were also used to evaluate the toxicity.

Continuous aeration was performed to evenly disperse the microplastics in the water for their free intake by the marine medaka. Moreover, the exposure solution was renewed daily for 60 d. An ingestion test revealed that the maximum concentrations of ingested PS, after 24-h exposure, for non-feeding and feeding marine medaka were

33.0 ± 4.2 particles/individual and 19.6 ± 6.0 particles/individual, respectively, which indicated that the daily provided particles in this study were abundant for free intake by the marine medaka, and that the effects of ingestion on the microplastic concentration were negligible (Cong et al., 2019). Another independent experiment was conducted to determine the PS-induced changes in the hepatic phenotypes and PS accumulation under the same exposure conditions ($n = 6$ per group).

2.3. Sample preparation for metabolomics analysis

After being accurately weighed, the fresh cold marine medaka liver was put in a centrifuge tube. A zirconia ball and ice-cold methanol (80%, v/v) were successively added into the tube, and then the sample was homogenized at 30 Hz for 1.5 min using a grinding and mixing apparatus (MM400, Retsch, Germany). The homogenized sample was centrifuged at 13,000 rpm for 15 min at 4 °C, and 700 μL of the supernatant was absorbed and then vacuum-dried using a SpeedVac concentrator (Thermo Scientific, USA). Subsequently, 50 μL of methoxyamine solution (20 mg/mL in pyridine) was added into the dried sample and vortexed for 30 s. Following the oxidation reaction with methoxyamine for 1.5 h in a 37 °C water bath, 40 μL of *N*-methyl-N-(trimethylsilyl)-trifluoroacetamide was added to the sample for a 1-h silylation reaction in a 37 °C water bath. After the derivatized sample was centrifuged at 13,000 rpm for 15 min at 4 °C, the supernatant was used for instrumental analysis.

To monitor the reproducibility and stability of the metabolomics approach, the remaining supernatant of each centrifuged sample was collected and thoroughly mixed to produce the quality control sample, which was then processed according to the same parameters as those of other analytical samples regarding vacuum drying, derivatization, instrumental analysis, and data processing.

2.4. Instrumental analysis for metabolomics analysis

An untargeted metabolomics approach based on gas chromatography–mass spectrometry (GCMS-QP 2010 plus, Shimadzu, Japan) was used to obtain the metabolic profiling of the marine medaka liver. The experiment injection volume was 1 μL. The instrumental parameters were determined based on those employed in our previous work (Ye et al., 2012, 2014). The inlet temperature was 300 °C. High-purity helium was used as the carrier gas, and the split ratio and constant linear velocity were 5:1 and 40.0 cm/s, respectively. The metabolites were separated using a DB-5 MS capillary column (30 m × 250 μm × 0.25 μm, J&W Scientifc Inc., USA). The initial oven temperature was set to 70 °C for 3 min, raised to 300 °C at a heating rate of 5 °C per minute, that was held for 10 min. The interface and ion source temperatures were maintained at 280 and 230 °C, respectively. Metabolites were ionized in electron impact mode, and the ionization voltage was 70 eV. Mass signals were acquired from 33 to 600 m/z in full scan mode. The solvent delay and event times were 5.3 min and 0.2 s, respectively. The detector voltage was consistent with that of the tuning result. To obtain the retention indices of each metabolite, a light diesel sample was analyzed with the same parameters as those of the other analytical samples.

2.5. Data preprocessing for metabolomics analysis

GCMS solution 4.2 software (Shimadzu, Japan) was used to export the raw mass data in NetCDF format; the data were employed for peak alignment, matching and identification using the XCMS method (Smith et al., 2006). The data processing software ChromaTOF 4.43 (LECO Corporation, USA) was utilized for peak deconvolution to produce the feature ion of each metabolite. The metabolites were identified primarily according to the search results of the commercial mass spectra libraries, manual comparisons of the mass spectra, and mass spectra verification using the available reference standards, as well as according to the retention time, and retention index. The raw ion peaks of metabolites were normalized to the total ion current and then multiplied by 1×10^7, and the data were employed for subsequent statistical analysis.

2.6. Detection of triglycerides

In an additional experiment, liver tissue was collected, immediate frozen, and then stored at −80 °C to detect triglycerides using the triglyceride assay kit E1003-2 (Applygen, China). Two steel balls and 500 μL of T-PER™ Tissue Protein Extraction Reagent (Thermo, China) were added to the tissue sample, following which the sample was homogenized using a grinding and mixing apparatus (Retsch, Germany). One-hundred microliters of the lysate was transferred to a new 1.5-mL centrifuge tube and then heated at 70 °C for 10 min, followed by centrifugation at 2000 rpm at room temperature for 5 min. The supernatant was used to detect triglycerides. The protein concentration was measured using a bicinchoninic acid reagent (Thermo, China) to correct the triglyceride content.

2.7. Detection of hepatic PS accumulation

Liver tissue collected from the additional experiment described above, was used to detect hepatic PS accumulation. A sample of the tissue was placed in a glass tube, and added with 2-mL 65% HNO_3 (Sinopharm, China). Next, the sample was heated in a water bath at 70 °C for 2 h. Afterward, the digestive juice was diluted to 100 mL with water, followed by filtration with a 0.2-μm filter membrane. Finally, the PS on the filter membrane were observed and counted using an upright microscope (Olympus CX33, Japan).

2.8. Statistical analysis

Pathway analysis and principal component analysis were conducted using MetaboAnalyst 4.0 software (Chong et al., 2019). The Mann-Whitney *U* test (two-tailed) was used to evaluate the significant level of the differences in the metabolite contents and indicators related to the hepatic phenotypes among the groups by employing MultiExperiment Viewer 4.9.0 (Saeed et al., 2006). The statistically significant level was 0.05. MultiExperiment Viewer 4.9.0 was employed to plot the hierarchical clustering.

3. Results

3.1. PS exposure induces significant metabolic changes in marine medaka liver

The score plot of the principal component analysis demonstrated that the three quality control samples were clustered closely (Fig. 1A). Of the 8252 detected ions, there were 6029, 6601 and 7245 ions, accounting for 73.06%, 79.99% and 87.8% of the total ions, with relative standard deviations lower than 15%, 20% and 30%, respectively (Fig. 1B). These data demonstrated that the metabolomics approach was highly stable and repeatable (Begley et al., 2009).

According to the score plot of principal component analysis, the liver metabolic profiles of the PS-10 and PS-200 groups differed greatly from that of the control group (Fig. 1C). Subsequently, 83 differential metabolites were identified, of which 59 metabolites were further verified using reference standards based on the retention time, retention index and mass spectra ($P < 0.05$, Table S1). The hierarchical clustering illustrated that among the metabolites, 30 involved in carbohydrate metabolism, 15 involved in amino acid metabolism, 30 involved in lipid metabolism, 4 involved in nucleotide metabolism, and 4 involved in other metabolic pathways were significantly altered in response to PS-10 and PS-200 exposure (Fig. 2A). Additional pathway analysis revealed

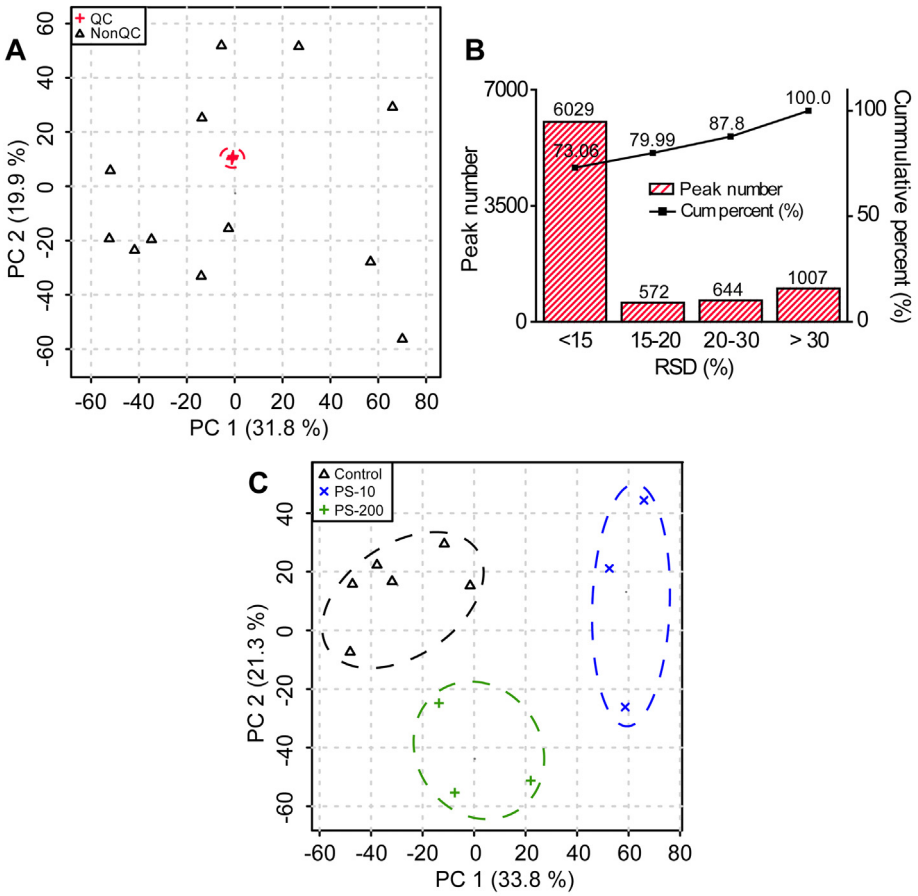

Fig. 1. Stability and reproducibility of the metabolomics approach and alterations in the metabolic profiling of marine medaka liver induced by PS exposure. (A) Quality control (QC) sample distribution in the score plot of principal component analysis. (B) Relative standard deviation (RSD) distribution of detected ion peaks. (C) PS exposure-induced alterations in the metabolic profiling of the marine medaka liver. $n = 6$, 3 and 3 in the control, PS-10 and PS-200 exposure groups, respectively.

that 33 and 28 metabolic pathways were significantly altered in response to PS-10 and PS-200 exposure, respectively. These altered metabolic pathways included glycerolipid metabolism, biosynthesis of unsaturated fatty acids, fatty acid elongation, glycolysis/gluconeogenesis, fructose and mannose metabolism, glycine, serine and threonine metabolism, arginine and proline metabolism, purine and pyrimidine metabolism (Fig. 2A and B). Most monosaccharide and organic acid levels were significantly reduced, while levels of most fatty acids, and fatty acid methyl and ethyl esters significantly increased in response to PS exposure. Moreover, most amino acid levels decreased in the PS-10 group, while those of disaccharides and trisaccharides significantly increased in the PS-200 group compared to those of the control group. The metabolic changes induced by PS exposure are described and discussed in detail in the following sections.

3.2. PS exposure induces significant changes in carbohydrate metabolism in marine medaka liver

Significant changes in carbohydrate metabolism were induced by PS exposure (Fig. 3). Levels of monosaccharides, such as glucose, mannose, fructose, and tagatose, significantly decreased in response to PS-10 exposure; galactose was an exception to the trend. Additionally, glucose, mannose, galacturonate, *N*-acetyl-D-glucosamine, and myo-inositol levels were also significantly reduced in response to PS-10 exposure. Levels of most organic acids, such as glycerate, lactate, fumarate and malate were significantly reduced in response to both PS-10 and PS-200 exposure, which indicated that PS exposure triggered the inhibition of glycolysis/gluconeogenesis and the tricarboxylic acid cycle. Moreover, the floridoside level significantly increased after both PS-10 and PS-200 exposure, which suggested that carbohydrate storage, transport, and assimilation disorders in marine medaka were induced by PS exposure. Notably, we observed that trisaccharides and disaccharides (including maltotriose, maltose, lactose and mannobiose) accumulated in PS-200-treated marine medaka liver but not in the PS-10-treated group.

3.3. PS exposure induces significant changes in amino acid and nucleotide metabolism in marine medaka liver

Significant changes in amino acid metabolism induced by PS exposure were observed (Fig. 4A). Levels of most amino acids, such as glutamate, proline, leucine, 4-aminobutyrate and β-alanine, involved in alanine, asparate and glutamate metabolism, valine, leucine and isoleucine metabolism, β-alanine metabolism, and arginine and proline metabolism, significantly decreased in response to PS exposure, especially to PS-10 exposure. However, the level of quinolinate, a product of β-alanine, significantly increased after PS-10 and PS-200 exposure. These data indicated that PS exposure triggered increased amino acid degradation and/or decreased amino acid synthesis. Moreover, significantly increased levels of hypotaurine and mimosine in the marine medaka treated with PS-10 suggested increased oxidative stress and inhibition of DNA replication and/or elongation, respectively, due to PS-10 exposure (Mosca et al., 1995; Shiota et al., 2018).

We also observed significant changes in nucleotide metabolism in response to PS exposure (Fig. 4B). Levels of uridine and adenosine-5′-

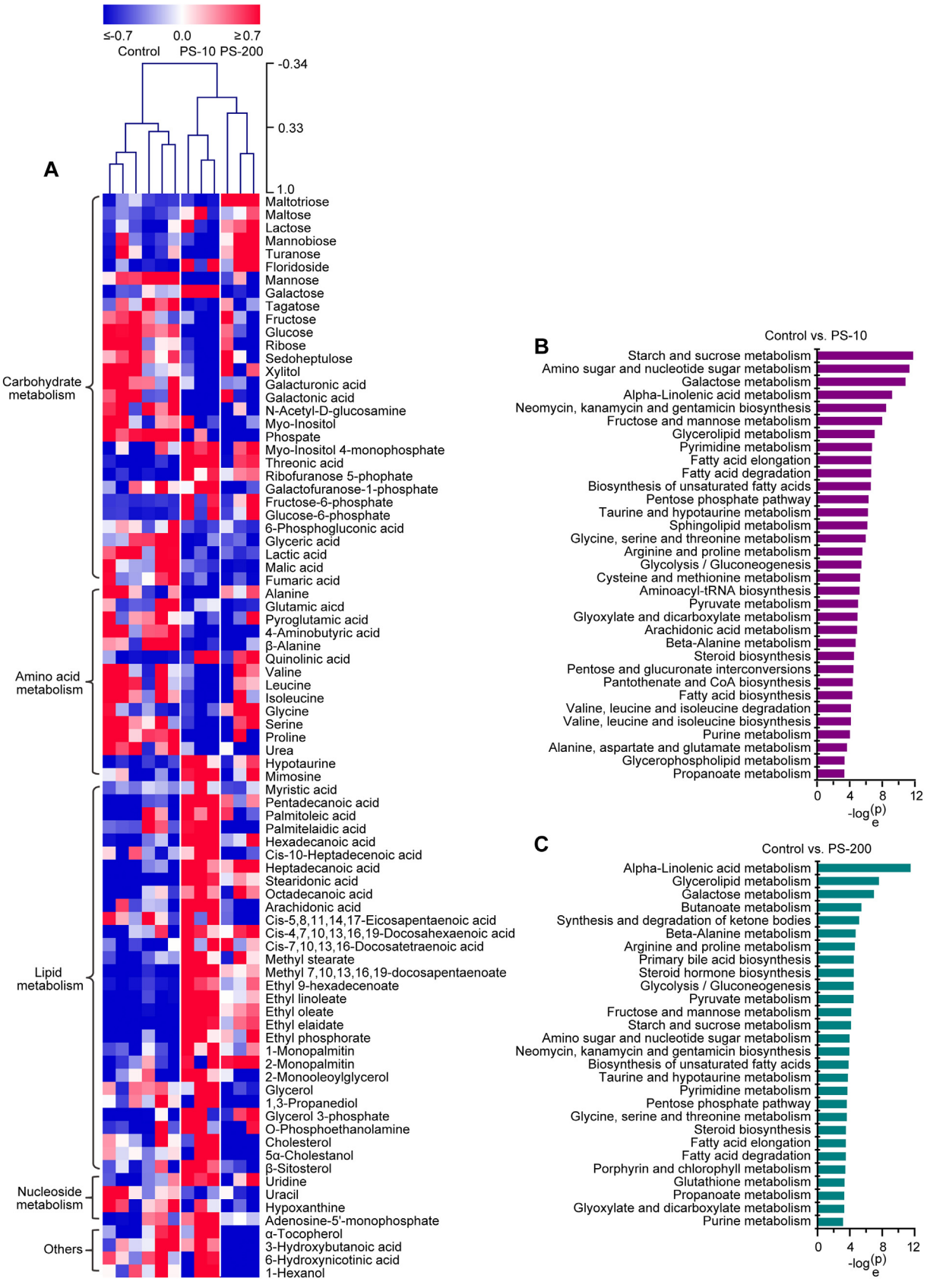

Fig. 2. PS exposure induces significant metabolic changes in marine medaka liver. (A) Hierarchical cluster analysis of metabolic changes in marine medaka liver. Levels of metabolites were normalized to unit variance and then used for hierarchical clustering. (B) Pathway analysis of metabolic changes induced by PS-10 exposure. (C) Pathway analysis of metabolic changes induced by PS-200 exposure. n = 6, 3 and 3 in the control, PS-10 and PS-200 exposure groups, respectively.

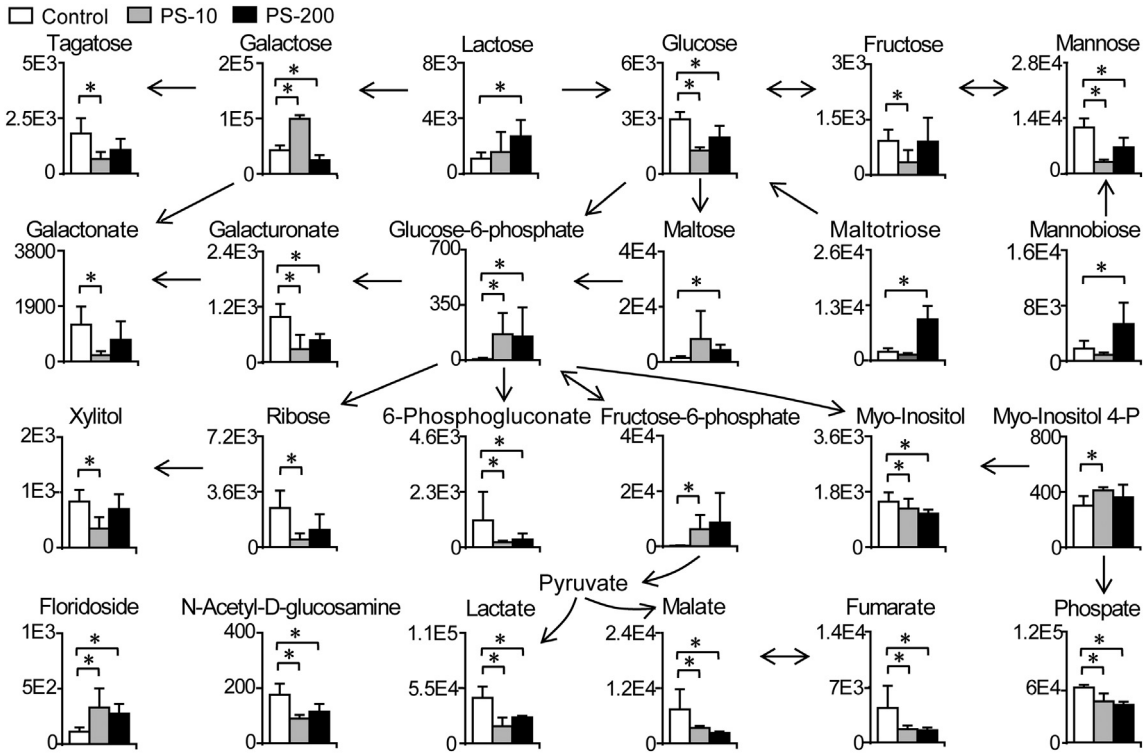

Fig. 3. PS exposure induces significant changes in carbohydrate metabolism in marine medaka liver. The column is expressed as the mean + SD. n = 6, 3 and 3 in the control, PS-10 and PS-200 exposure groups, respectively. *$P < 0.05$, two-tailed Mann-Whitney *U* test.

monophosphate significantly increased, while the uracil level significantly decreased after PS-10 exposure. In contrast, uracil and hypoxanthine levels both significantly decreased in response to PS-200 exposure. These data demonstrated disturbed nucleic acid metabolism and/or energy production due to PS exposure.

3.4. PS exposure induces significant changes in lipid metabolism in marine medaka liver

Significant changes in marine medaka liver lipid metabolism induced by PS exposure were apparent (Fig. 5). There were 12 and 6

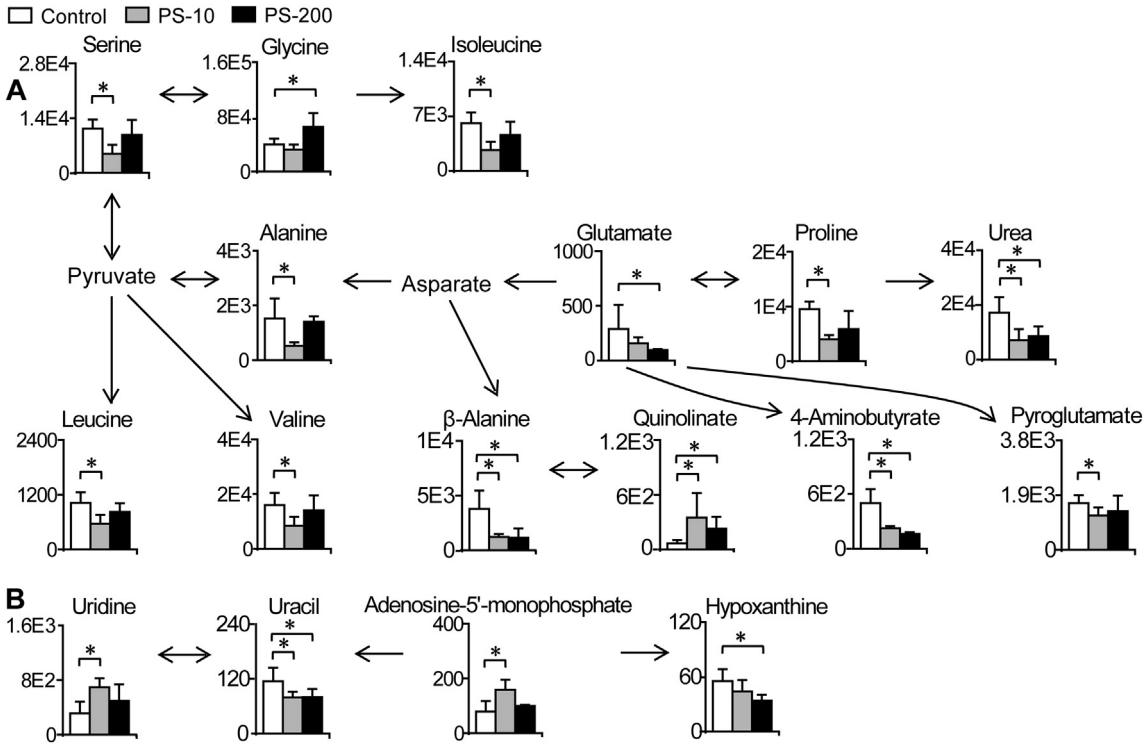

Fig. 4. PS exposure induces significant changes in amino acid (A) and nucleotide (B) metabolism in marine medaka liver. The column is expressed as the mean + SD. n = 6, 3 and 3 in the control, PS-10 and PS-200 exposure groups, respectively. *$P < 0.05$, two-tailed Mann-Whitney *U* test.

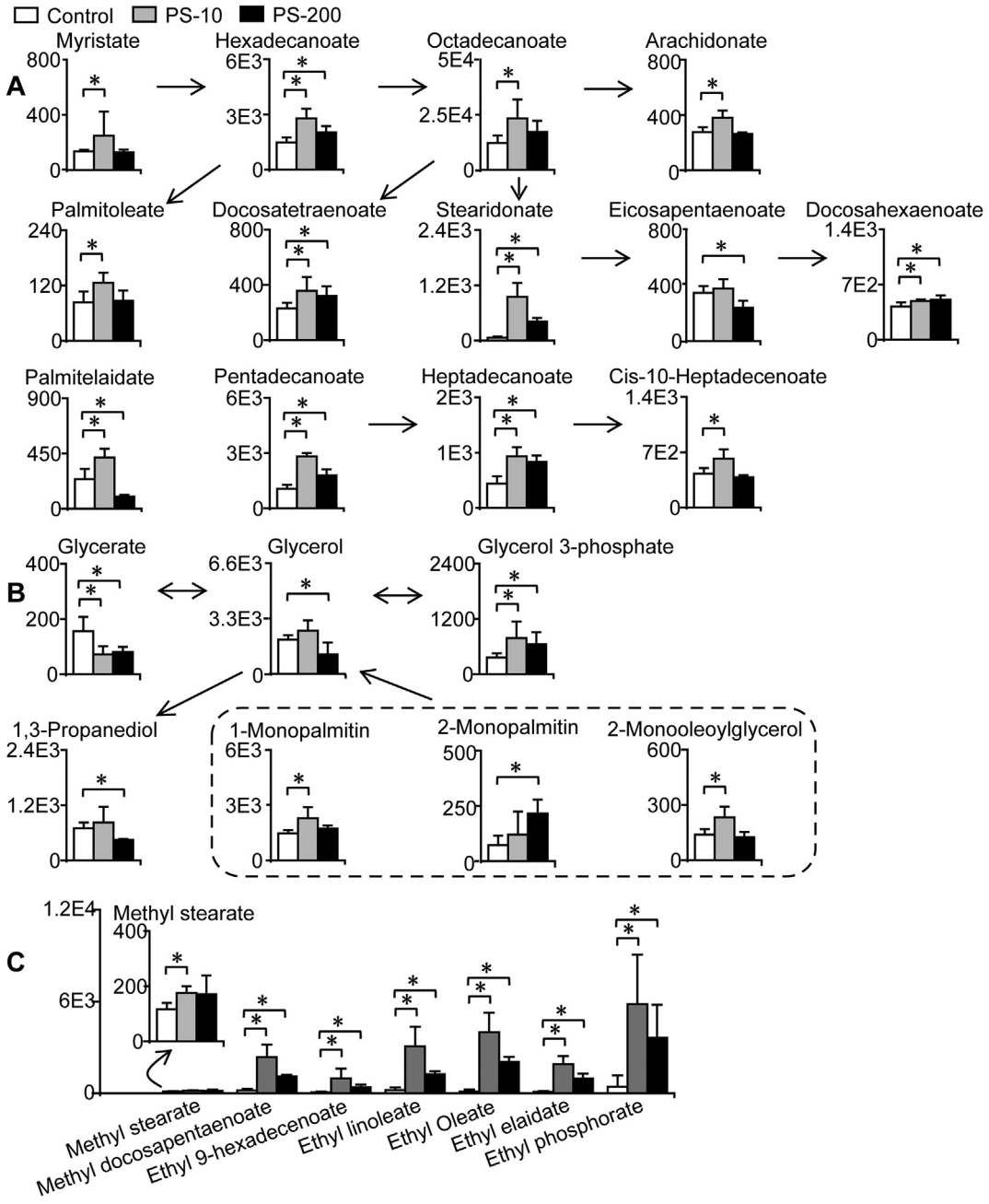

Fig. 5. PS exposure induces significant changes in lipid metabolism in marine medaka liver. The column is expressed as the mean + SD. n = 6, 3 and 3 in the control, PS-10 and PS-200 exposure groups, respectively. *$P < 0.05$, two-tailed Mann-Whitney *U* test. (A) Fatty acid accumulation. (B) Changes in glycolipid metabolism. (C) Methyl and ethyl ester accumulation.

fatty acids accumulating in the liver of marine medaka treated with PS-10 and PS-200, respectively. These fatty acids included pentadecanoate, hexadecanoate, heptadecanoate, stearidonate, cis-4,7,10,13,16,19-docosahexaenoate, and cis-7,10,13,16-docosatetraenoate (Fig. 5A). Furthermore, levels of 1-monopalmitin and 2-monooleoylglycerol were significantly increased by PS-10 exposure, and the level of 2-monopalmitin significantly increased in marine medaka liver due to PS-200 exposure, which indicated that monoglyceride accumulated in marine medaka liver in response to PS exposure (Fig. 5B). Additionally, levels of glycerate, 1,3-propanediol and glycerol significantly decreased, while the level of glycerol 3-phosphate significantly increased in response to either PS-10 or PS-200 exposure (Fig. 5B). Changes in the glycerolipid metabolism suggested increased triglyceride synthesis and decreased triglyceride degradation in marine medaka liver due to PS exposure. Moreover, methyl and ethyl esters, primarily fatty acid methyl and ethyl esters, such as methyl 7,10,13,16,19-docosapentaenoate, ethyl 9-hexadecenoate, ethyl linoleate, ethyl oleate, and ethyl elaidate, also accumulated in marine medaka liver in response to both PS-10 and PS-200 exposure (Fig. 5C). These data demonstrated lipid accumulation in marine medaka liver induced by PS exposure.

3.5. Effects of PS exposure on hepatic phenotypes in marine medaka

Another experiment was performed to further determine whether PS exposure induced significant changes in the hepatic phenotypes in marine medaka. No significant changes occurred in the hepatic triglycerides or the ratio of the liver weight to entire body weight in the marine medaka exposed to PS; however, there was an increasing trend in

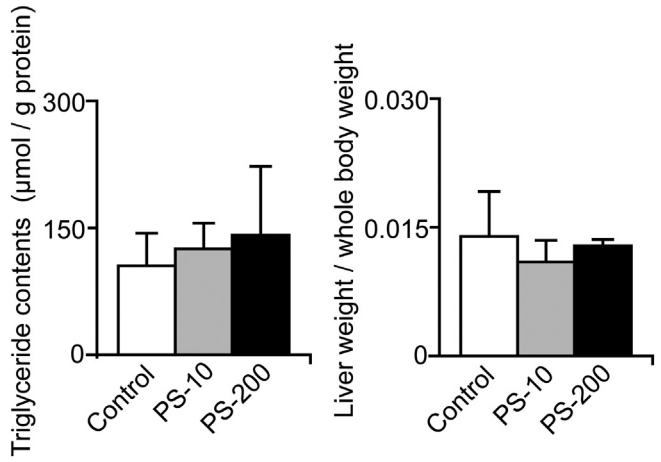

Fig. 6. Effects of PS exposure on hepatic phenotypes (A) and the accumulation (B) in marine medaka. The column is expressed as the mean + SD. n = 6 per group. Samples were from another independent experiment.

hepatic triglycerides in response to PS exposure (Fig. 6). The data indicated that PS-induced changes in the hepatic metabolome were more sensitive than those in relevant hepatic phenotypes, and were more suitable for assessing the toxic effects of microplastics.

3.6. PS size effects on hepatic metabolism in marine medaka

It was observed that PS size had certain effects on the hepatic metabolism in marine medaka. Although most changes induced by PS-10 were similar to those induced by PS-200, they were greater than the latter. For example, the levels of certain monosaccharides (e.g., fructose, tagatose, galactonate, xylitol, and ribose), and amino acids (e.g., serine, isoleucine, leucine, valine, and proline) significantly decreased in the PS-10-exposed group, while the corresponding decreases in the PS-200-exposed group were not significant. Additionally, most disaccharides and trisaccharides, including lactose, maltose, mannobiose and maltotriose, accumulated in the PS-200-exposed group, while this accumulation did not occur in the PS-10-exposed group. To test the effect of PS size on PS accumulation in the liver, the number of hepatic PS was counted. We observed that over 30 spherical PS with a diameter ≤3 μm accumulated in the PS-10-exposed group. However, accumulation was not observed in the PS-200-exposed group (Fig. 7). These results indicated that hepatic accumulation of PS was size-dependent, and may affect their toxic effects on hepatic metabolism.

4. Discussion

Accumulated data indicate that PS are widespread in global waterbodies and aquatic organisms, and that metabolic disorders of the liver are closely associated with the pathophysiology of diseases and toxicology of contaminants. However, the comprehensive metabolic responses of the liver to PS exposure have remained undefined. Therefore, an untargeted metabolomics approach was employed to reveal metabolic disorders of the marine medaka liver induced by PS exposure.

In this study, significant decreases in the levels of most monosaccharides and organic acids (including lactate, fumarate, and malate) were found in marine medaka liver, indicating that the monosaccharide metabolism, tricarboxylic acid cycle and glycolysis were inhibited, and that energy production from these metabolic pathways was reduced in response to both PS-10 and PS-200 exposure. Hepatic transcriptomic

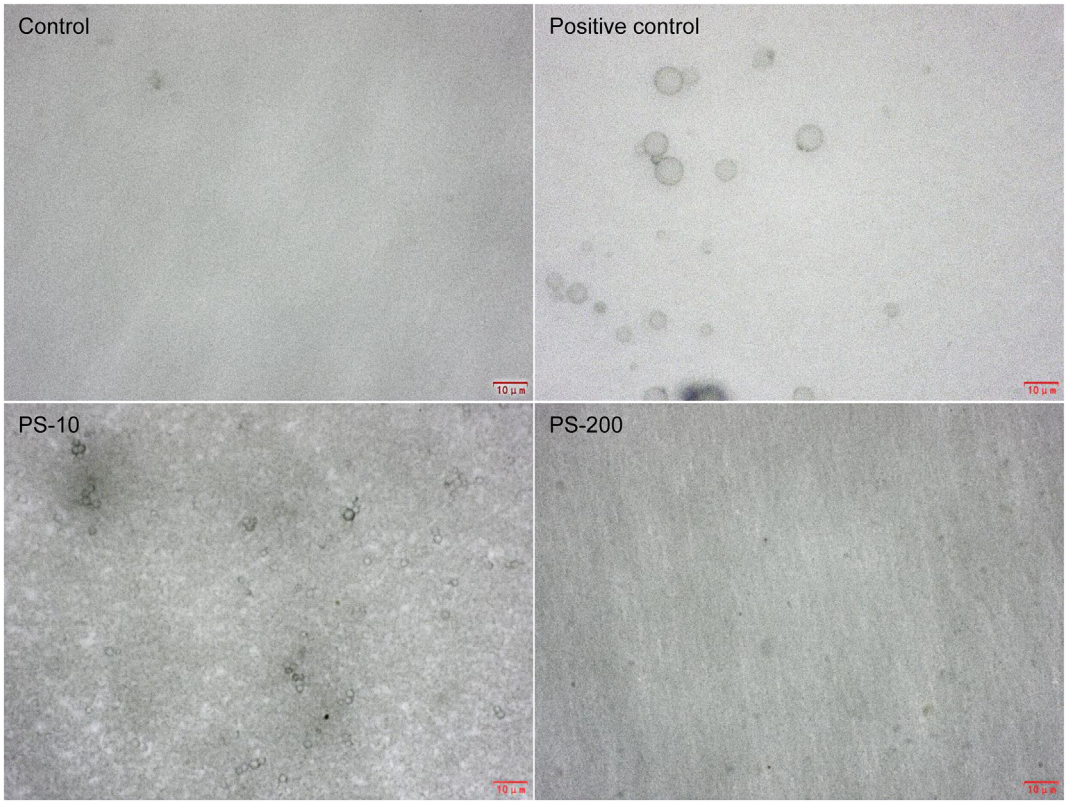

Fig. 7. PS accumulation in marine medaka liver. Representative micrographs of PS in each group. PS in the positive control group were from the exposure solution, and not due to ingestion by marine medaka; they were primarily used to compare the size (but not concentration) of PS in each group. Samples were from another independent experiment.

and biochemical analysis revealed that glucose, α-ketoglutarate, isocitrate dehydrogenase, *GK*, *HK*, *PK* and *PEPCKC* levels all significantly decreased in adult zebrafish due to PS exposure, which confirmed the inhibition of the hepatic monosaccharide metabolism, tricarboxylic acid cycle and glycolysis by PS exposure in this study (Zhao et al., 2020). PS exposure can also trigger significant disturbances in the tricarboxylic acid cycle and glycolysis in other tissues (e.g., intestine, testis and muscle), thus affecting PS toxicity by disrupting various physiological processes, such as energy supply, biomass synthesis and bioactive metabolite production (Antao Barboza et al., 2018; Qiao et al., 2019a; Xie et al., 2020). Additionally, we observed an increased level of floridoside, which is involved in carbohydrate storage, transport, and assimilation, in the marine medaka induced by both PS-10 and PS-200 exposure, which was consistent with the accumulation of trisaccharides and disaccharides due to PS-200 exposure. Moreover, significantly decreased levels of 6-phosphogluconate and ribose in the marine medaka liver induced by PS exposure in this study indicated the inhibition of the pentose phosphate pathway, revealing disturbed nucleotide synthesis and NADPH production. These data demonstrated the critical role of carbohydrate metabolism in PS-induced toxicology.

Fatty acids, and fatty acid methyl and ethyl esters accumulated in marine medaka liver in response to PS exposure in this study. Maternal mice exposure to PS during gestation and/or lactation triggered significant decreases in the expression levels of genes related to the oxidation of fatty acids in the liver of their dams and offspring, including *Acox*, *Cpt1a* and *Mcad*, which indicated that hepatic fatty acid oxidation decreased, and that energy and acetyl-CoA production from fatty acid oxidation were probably reduced in response to PS exposure (Luo et al., 2019a; Luo et al., 2019b). The disturbed fatty acid metabolism, monosaccharide metabolism, glycolysis, tricarboxylic acid cycle and amino acid metabolism in this study suggested energy deficits in the marine medaka liver due to PS exposure. Moreover, in obese and/or diabetic organisms, fatty acid clearance and storage are often impaired, leading to accumulation of fatty acids in the form of monoglycerides, diacylglycerols, triglycerides, cholesteryl esters, ceramides, and/or fatty acyl-CoAs; fatty acids accumulate in the circulation and various tissues, such as the liver, muscle, and arterial tissues, thereby promoting insulin resistance and atherosclerosis (Fisher and Gertow, 2005).

We also found significantly decreased levels of most amino acids in marine medaka liver, which indicated increased amino acid degradation, decreased synthesis and/or amino acid import due to PS-10 exposure. Amino acids can be converted into alpha-keto acids, carbon dioxide, ammonia, and proteins, while alpha-keto acids can be used to synthesize sugars, other nonessential amino acids, and lipids. Decreased levels of amino acids implied that fewer amino acids were available to synthesize proteins, organic acids, sugars, and/or lipids in marine medaka liver due to PS-10 exposure. The mRNA expression levels of three genes involved in carbohydrate metabolism, fatty acid metabolism, and amino acid metabolism, *aldh9a1a*, *ehhadh*, and *aldh2.2*, significantly decreased in the liver of zebrafish exposed to PS, which agreed with the decreases in most monosaccharides, organic acids, and amino acids in the liver of PS-exposed marine medaka in this study, indicating a potential decrease in the interconversions among the carbohydrates, amino acids and lipids in the liver after PS exposure (Zhao et al., 2020).

The increased level of mimosine indicated reduced DNA replication and/or elongation, which agreed with the results of disturbed nucleotide metabolism and reduced pentose phosphate pathway for nucleate synthesis in marine medaka liver exposed to PS in this study (Mosca et al., 1995). PS exposure reduced the hatchability of marine medaka embryos, increased the hatching time, and decreased the body length and weight of the larvae (Li et al., 2020). Further investigations revealed that exposure to environmental microplastics induced ethoxyresorufin-O-deethylase activity and DNA stand breaks in Japanese medaka in the early life stages (Pannetier et al., 2019).

Differences in the toxic effects of PS-10 and PS-200 on the hepatic metabolism may be because PS-10 is smaller and more easily accessible to the circulatory system and liver. These factors consequently allow PS-10 to cause more severe damage to the hepatic metabolism in comparison to that caused by PS-200. We found that PS accumulated in the PS-10-exposed group, but the accumulation did not occur in the PS-200-exposed group in this study. The levels of hepatic reactive oxygen species and antioxidases, including superoxide dismutase, catalase, and glutathione S-transferase, significantly increased in the nanoplastic-exposed group compared to those of the microplastic-exposed group, indicating stronger oxidative stress and higher antioxidant system activation in the liver in the nanoplastic-exposed group (Kang et al., 2021). Nonetheless, more severe intestinal damage (i.e., increased mucus ratio) and flora disorders were observed in the microplastic-exposed group compared to those of the nanoplastic-exposed group (Kang et al., 2021). Additionally, the accumulation of microplastics in target organs (e.g., intestine and liver) and their toxic effects are affected by many factors, such as: the feeding preference of test organisms for microplastics, digestion, transport and degradation of microplastics in test organisms, exposure concentration and time, and physical and chemical properties of microplastics. Moreover, the methods and technologies used also affect the observability of the toxic effects of microplastics, especially the application of various high-throughput omics technologies.

5. Conclusions

We found that many metabolic pathways were disturbed after PS exposure. The levels of most monosaccharides, organic acids and amino acids were significantly reduced, while those of most fatty acids, and fatty acid methyl and ethyl esters were significantly increased due to PS exposure. These data indicated the PS-triggered inhibition of the monosaccharide metabolism, tricarboxylic acid cycle, glycolysis, pentose phosphate pathway, and amino acid metabolism, and the accumulation of fatty acids, and fatty acid methyl and ethyl esters in marine medaka liver. Moreover, PS-10 exhibited stronger effects on the hepatic metabolism than did PS-200, which may be related to their hepatic accumulation. This study provides novel insights into microplastic ecotoxicology, and suggests the tricarboxylic acid cycle, glycolysis, pentose phosphate pathway, amino acid metabolism, and fatty acid metabolism as potential targets for intervening the toxic effects of microplastics and related diseases.

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CRediT authorship contribution statement

Guozhu Ye, Xu Zhang, Yi Lin and Qiansheng Huang conceived and designed this study. Guozhu Ye carried out the metabolomics analysis and data analysis, interpreted the data, wrote, and revised the manuscript. Xu Zhang conducted the animal experiment. Xinyu Liu deconvoluted the mass data. Xu Liao and Han Zhang initiated the instrumental analysis. Changzhou Yan checked the manuscript.

Declaration of competing interest

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

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