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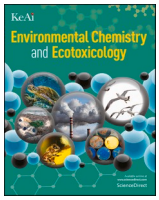

Research Paper

Emerging microplastic and nanoplastic threats: Decoding winter survival mechanisms in hybrid groupers through hepatic metabolic disruption☆

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ABSTRACT

This study explores the impact of microplastics (MPs) and nanoplastics (NPs) on hepatic lipid metabolism in *pearl gentian grouper* (*Epinephelus fuscoguttatus*♀ × *Epinephelus lanceolatus*♂) during overwintering and elucidates the underlying mechanisms. Fish were exposed to polystyrene (PS) MPs and NPs of varying sizes (5 μm, 500 nm, and 50 nm) for a 15-day exposure period. Histopathological analysis, oxidative stress assessment, and gene expression profiling related to lipid metabolism revealed significant toxic effects on the liver. Results showed that NPs preferentially accumulated in the liver, causing hepatocyte swelling, inflammation, and lipid metabolism disorders. Smaller particle sizes intensified oxidative stress, reduced triglyceride (TG) content, and elevated low-density lipoprotein cholesterol (LDL-C) and total cholesterol (T-CHO) levels. Transcriptomic analysis indicated that MPs and NPs altered the expression of lipid metabolism genes, particularly those in glyceride metabolism and lipolysis pathways, with significant upregulation of *PNPLA2* and *LIPG* ($p < 0.05$) under cold stress. This led to excessive energy reserve depletion and hepatic lipid metabolism dysfunction. This study establishes a "environmental stress-gene-metabolism" response model and provides novel insights into the molecular mechanisms by which NPs disrupt lipid homeostasis in aquatic organisms, offering a theoretical basis for understanding the toxicological effects of emerging contaminants.

1. Introduction

Plastics are widely used due to their exceptional material properties [1]; however, they present significant environmental challenges. In 2023, global plastic production reached 400 million metric tons [2], with forecasts predicting an increase to 1.48 billion metric tons by 2050 [3]. Over 90 % of plastic waste is not adequately recycled, and approximately 10 % of terrestrial plastic debris enters marine ecosystems, constituting about 85 % of total marine litter [4]. Within marine environments, plastics undergo degradation through physical, chemical, and biological processes [5,6], resulting in the formation of microplastics (MPs, <5 mm) and nanoplastics (NPs, <100 nm) [7,8]. These particles have been identified in seawater, sediments, and marine organisms [9,10]. MPs and NPs can release harmful additives, such as phthalates and bisphenol A [3], and adsorb heavy metals and persistent organic pollutants [11,12]. Extensive research has demonstrated that MPs and NPs exhibit bioaccumulative potential and toxicological effects, presenting substantial risks to ecosystems and human health through food chain transfer [13].

MPs and NPs can accumulate in organisms, triggering toxic effects

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such as gastrointestinal obstruction, metabolic dysregulation, reproductive system abnormalities, and changes in biochemical parameters [14]. The liver, as the central metabolic and secretory organ of the digestive system, plays a critical role in protein synthesis, nutrient metabolism, xenobiotic detoxification, and immune regulation [15]. Upon internalization of MPs and NPs into organisms, these particles elicit a compensatory response, marked by the upregulation of genes such as the sodium/potassium-transporting ATPase subunit alpha-4 (*Atp1a4*) and the calcium-activated chloride channel regulator 3 A-1 (*Clca3a1*). This response enhances cellular energy metabolism and chloride homeostasis to mitigate the disruptions caused by MPs [16]. A substantial body of experimental evidence demonstrates that MPs and NPs exert significant toxic effects on the liver of marine organisms. Research has shown that exposure to MPs and NPs can induce pathological changes in liver tissue, including hepatocyte swelling, inflammatory cell infiltration, and structural damage [17]. At the molecular level, temporary exposure to MPs or NPs can activate the unfolded protein response, specifically through the PERK-Eif2a signaling pathway, leading to oxidative stress in the liver. This, in turn, results in lipid accumulation and hepatocyte degeneration [18]. Additionally, animal studies have confirmed that exposure to MPs and NPs significantly alters the expression of genes involved in lipid metabolism, including key regulatory factors such as PPAR, SREBP1, and AMPK [19].

Compared to MPs, NPs exhibit significantly higher bioaccumulation potential in hepatic tissue. Quantitative analyses in both fish and mammalian models consistently show a greater accumulation of NPs in the liver than MPs, likely due to their smaller size and increased cellular uptake efficiency [20]. This heightened accumulation and greater "availability" of NPs result in more severe hepatic injury, including inflammatory responses, oxidative stress, dysregulation of lipid metabolism (such as hepatic steatosis), and, in some cases, progression to cirrhosis and fibrosis [18,21]. These differences underscore the critical role of particle size in determining the toxicity of MPs and NPs [22,23]. Compared to MPs, NPs possess a smaller size and a larger specific surface area, which enable them to more readily penetrate biological barriers, such as the gaps between intestinal epithelial cells or gill mucosa, and reach the liver via the bloodstream [24]. The increased bioavailability of NPs enhances their interaction with cellular membranes, inducing endoplasmic reticulum stress and mitochondrial dysfunction. Consequently, this results in increased oxidative stress and inflammation [25]. These properties account for the typically greater toxicity of NPs compared to MPs at the same exposure levels [26].

Current toxicological research predominantly focuses on model organisms (such as *zebrafish* and *tilapia*), with a notable scarcity of studies on fish species of high economic importance. Taking the *pearl gentian grouper* as an example, this species accounts for over 70 % of China's total grouper aquaculture production [27]. In 2022, China's grouper farming output reached 205,800 tons, ranking it as the third largest marine aquaculture fish species and establishing it as a vital economic species in the southeastern coastal regions. Given the widespread presence and ingestion of MPs and NPs in marine environments [28], the *pearl gentian grouper* inevitably becomes a carrier of these contaminants [29,30]. The toxicity study of MPs and NPs for this species has not been reported in a systematic study, and its key scientific issues such as bioaccumulation patterns and molecular toxicological mechanisms need to be carried out urgently. Accordingly, the aim of this study is to investigate the transfer patterns of MPs and NPs in the *pearl gentian grouper*, and focus on the mechanism of NPs on hepatic lipid metabolism in *pearl gentian grouper*. Combined with histopathological analysis, oxidative stress indicators, biochemical parameters, and transcriptome analysis of lipid metabolism related genes, we systematically evaluate the interfering effects of NPs on hepatic lipid metabolism and its potential molecular mechanisms.

2. Methods

2.1. Materials and chemicals

In this study, spherical PS MPs and NPs with particle sizes of 5 μm, 500 nm, and 50 nm were utilized. These samples were procured from Jiangsu Zhichuan Technology Co., Ltd. (Jiangsu, China). The stock solutions of PS MPs and NPs were stored at 4 °C and subjected to ultrasonication for 30 min prior to use to ensure uniform dispersion of the particles. The morphological characteristics and dimensions of PS MPs and NPs were analyzed using a desktop scanning electron microscope (SEM, JSM-7800F, TESCAN, Czech) and a transmission electron microscope (TEM, TF30, JEOL Ltd., Japan). Particle size distribution is quantified by laser diffraction for MPs (Microtrac S3500, Malvern Instruments, England) and dynamic light scattering (DLS, Malvern Zetasizer Nano ZS90, Malvern Instruments, England) for NPs. Surface charge characteristics are determined via zeta potential measurements (DLS, Malvern Zetasizer Nano ZS90, Malvern Instruments, England).

2.2. Fishing rearing and sampling

The *pearl gentian grouper* used in this experiment were obtained from Daqiuzhuang Aquaculture Co., Ltd. (Guangdong, China). The initial body weight of the fish was 54.79 ± 8.11 g, and the body length was 15.38 ± 0.96 cm. The experimental conditions were natural winter conditions with a temperature range of 10–15 °C. After two weeks of acclimation, the fish were randomly assigned to four groups (n = 25 per group): a control group and three treatment groups exposed to PS microbeads with particle sizes of 5 μm, 500 nm, and 50 nm, respectively. Based on the sampling results, we chose 1 mg/L as the exposure concentration for all groups (Fig.S1), and there is a 15-day exposure period. Prior to exposure, the PS MPs and NPs were pre-treated to prepare suspensions following the method described by Zhang et al. [31] (Fig. S2). At the end of the experiment, the fish were euthanized using tricaine. Subsequently, samples of liver, gill, intestine, stomach, and muscle tissues were collected, and immediately frozen in liquid nitrogen, and then stored at −80 °C in an ultra-low temperature freezer for further analysis. Ethical approval was obtained from the Ethical Committees of the South China Sea Institute of Oceanology, Chinese Academy of Science.

2.3. Organ distribution analysis

The pyrolysis method in combination with gas chromatography–mass spectrometry (Py-GC/MS) established by Zhou et al. [32] was used for quantification of MPs and NPs in the main tissue organs (gills, intestine, liver, muscle and stomach) of the *pearl gentian grouper*. The detailed procedure was as follows: 1 mL of 25 % tetramethylammonium hydroxide (Shanghai Aladdin Biochemical Co., Ltd., Shanghai, China) solution was added to the tissue samples, followed by digestion on a constant temperature shaker (300 rpm, 25 °C) for 24 h. Subsequently, 10 mL of anhydrous ethanol was added, and the mixture was incubated in a water bath at 80 °C for 30 min. After centrifugation at 4000 rpm for 5 min, the supernatant was discarded. The pellet was then resuspended in 0.50 mL of ultrapure water, and 200 μL of the suspension was transferred to a pyrolysis target in three aliquots, dried at 70 °C, and prepared for analysis. For Py-GC/MS analysis, styrene trimer (m/z = 91) was used as the characteristic indicator for polystyrene quantification (pyrolyzer: Frontier EGA/PY-3030D, Frontier Laboratories Ltd., Fukushima, Japan; gas chromatograph: Agilent 5977B, Agilent Technologies, Inc., Santa Clara, USA; mass spectrometer: Agilent 8890, Agilent Technologies, Inc., Santa Clara, USA).

2.4. Pathological examination

Liver tissue samples were fixed in 4 % paraformaldehyde for 24 h,

followed by embedding in paraffin (for hematoxylin-eosin staining, H&E) and optimal cutting temperature compound (for Oil Red O staining). Serial sections with a thickness of 5–8 μm were prepared using a microtome. H&E sections were used to examine histopathological changes in the liver tissue, while Oil Red O sections were employed to assess lipid droplet accumulation within hepatocytes. All sections were observed under a light microscope, and quantitative analysis of histopathological alterations and lipid droplet accumulation was performed using ImageJ image analysis software (National Institutes of Health, USA).

2.5. Biochemical analysis

Precisely 100 mg of liver tissue from *pearl gentian grouper* was weighed and placed into a 2 mL sterile grinding tube. Pre-chilled phosphate-buffered saline (PBS, pH 7.40) was added at a 1:9 (w/v) ratio. The samples were then ground in a cryogenic grinder at −30 °C, followed by centrifugation at 4 °C and 15000 rpm for 10 min. The supernatant was collected, and commercial kits were used to measure the levels of superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), and glutathione (GSH) (Beijing MREDA Technology Co., Ltd., Beijing, China), as well as total cholesterol (T-CHO), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C) (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.6. RNA-sequencing analysis and quantitative real-time PCR (q-PCR)

After the exposure period, three *pearl gentian groupers* were randomly selected from each group, and their liver tissues were collected for transcriptomic analysis. Following established protocols, total RNA extraction, RNA integrity assessment, library construction, and high-throughput sequencing were performed [33]. Based on normalized gene expression levels, differentially expressed genes (DEGs) were identified between the control and treatment groups using a threshold of greater than 1.5-fold change ($|\log2foldchange| > 0.585$) and a significance level of $p < 0.05$. Functional annotation and enrichment analysis of DEGs were conducted using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses. Additionally, KEGG enrichment analysis was employed to elucidate lipid metabolism pathways associated with the DEGs. Furthermore, the expression levels of 12 selected genes were validated using real-time quantitative polymerase chain reaction. Detailed information on gene names and primer sequences was provided in the supplementary materials (Table.S1).

2.7. Data analysis

Data visualization and graphical representation were performed using Origin 2023 (OriginLab Corporation, USA), SPSS (International Business Machines Corporation, USA), and GraphPad Prism 10 (GraphPad Software Corporation, USA). All data are presented as mean ± standard deviation. Statistical differences between the control and exposure groups were evaluated using one-way analysis of variance. p value less than 0.05 was considered indicative of a statistically significant difference between the exposure and control groups. Different numbers of asterisks represent different p values: * was for $p < 0.05$, ** was for $p < 0.01$, *** was for $p < 0.001$, **** was for $p < 0.0001$.

3. Results and discussion

3.1. Characterization of PS MPs and NPs

The purchased MPs and NPs of three different particle sizes were characterized using SEM, TEM, DLS, and Zeta potential measurements (Fig.S3). The SEM and TEM results revealed that the PS MPs and NPs of all three sizes used in this study were spherical in shape. The average particle sizes for $PS_{5\ \mu m}$, $PS_{500\ nm}$, and $PS_{50\ nm}$ were determined to be 4.66 ± 0.26 μm, 524.84 ± 22.26 nm, and 54.06 ± 2.38 nm, respectively. Zeta potential analysis indicated that the PS particles were predominantly negatively charged, with values of −14.90 ± 1.59 mV, −40.03 ± 11.77 mV, and − 1.75 ± 1.62 mV for the respective sizes. Notably, the 500 nm PS particles exhibited the highest Zeta potential value. We redispersed 50 nm NPs and measured their Zeta potential to be −28.75 ± 2.06 mV, indicating that under this method, NPs can be well dispersed (Fig.S2).

3.2. PS MPs and NPs have extensive absorption and distribution in various tissues of fish

In this study, the distribution characteristics of PS MPs and NPs in various tissues of *pearl gentian grouper* after a 15-day exposure at a concentration of 1 mg/L were determined using Py-GC/MS. The experimental results demonstrated that the polystyrene trimer, with a retention time of 24–26 min and a mass to charge ratio of 91, could serve as a characteristic marker for PS quantification without significant interference from the protein extraction process [32] (Fig. 1a, Fig. 1b, Fig. 1c, Fig.S4). Tissue distribution analysis revealed that $PS_{5\ \mu m}$ particles exhibited the highest accumulation in the digestive system, specifically in the stomach and intestine, with concentrations of 3.61 μg/g and 5.60 μg/g wet tissue, respectively, which were approximately 2–6 times higher than those of NPs. In respiratory organs, the accumulation of $PS_{50\ nm}$ particles in gill tissue was significantly greater than that of other sizes, reaching 5.60 μg/g wet tissue, compared to 0.56 μg/g and 0.94 μg/g wet tissue for the other particle sizes. In muscle tissue, PS content showed a particle size-dependent pattern, decreasing with smaller particle sizes (ranging from 2.67 ± 0.62 to 1.49 ± 0.71 μg/g wet tissue). The liver tissue displayed a distinct distribution profile, with the highest content of $PS_{50\ nm}$ (22.94 ± 6.46 μg/g wet tissue) and a notable increasing trend as particle size decreased (Fig. 1d, Fig. 1e, Fig. 1f, Fig. 1g, Fig. 1 h, Table.S2).

Particle size is a critical factor influencing the uptake and distribution of MPs. Due to their higher specific surface area and lipophilic properties, NPs tend to aggregate on the intestinal surface, whereas MPs are primarily excreted through feces [34]. Notably, regardless of size, both MPs and NPs can impair intestinal barrier function, thereby enabling their penetration through intestinal epithelial cells into the internal environment [35]. Once inside the body, MPs and NPs can bypass the protective barriers of the circulatory and lymphatic systems, resulting in widespread systemic distribution [36]. The liver, as a central organ for metabolism and detoxification, is the primary site for the accumulation of exogenous PS, with concentrations significantly higher than in other tissues, making it a primary target for the toxic effects of MPs and NPs. Furthermore, the presence of MPs and NPs has also been detected in other organs, such as the brain and kidneys [37,38]. In the respiratory system, NPs are easily absorbed through gill tissues and transported to various internal organs via the bloodstream, while MPs, although retained, are less prone to accumulation [39]. Despite extensive research on the impact of particle size on the tissue accumulation of MPs and NPs, variables such as exposure duration, exposure method, tissue type, and detection techniques can significantly influence experimental outcomes. Consequently, the widespread distribution and accumulation patterns of MPs and NPs in *pearl gentian grouper* underscore their potential risk to food safety, warranting further attention and investigation. In addition to this, the presence of MPs has been detected in other economically important species, such as *large yellow croaker* and *eel* [40]. Studies have shown that *eels*, due to their benthic habits, are more likely to come into contact with MPs in sediments. Consequently, the MPs detected in their intestines are often associated with higher concentrations of adsorbed pollutants [41,42]. With the continuous development of the aquaculture industry, food safety issues have increasingly garnered public attention. Therefore, reducing biological contamination and enhancing food safety at the table have become key areas of research.

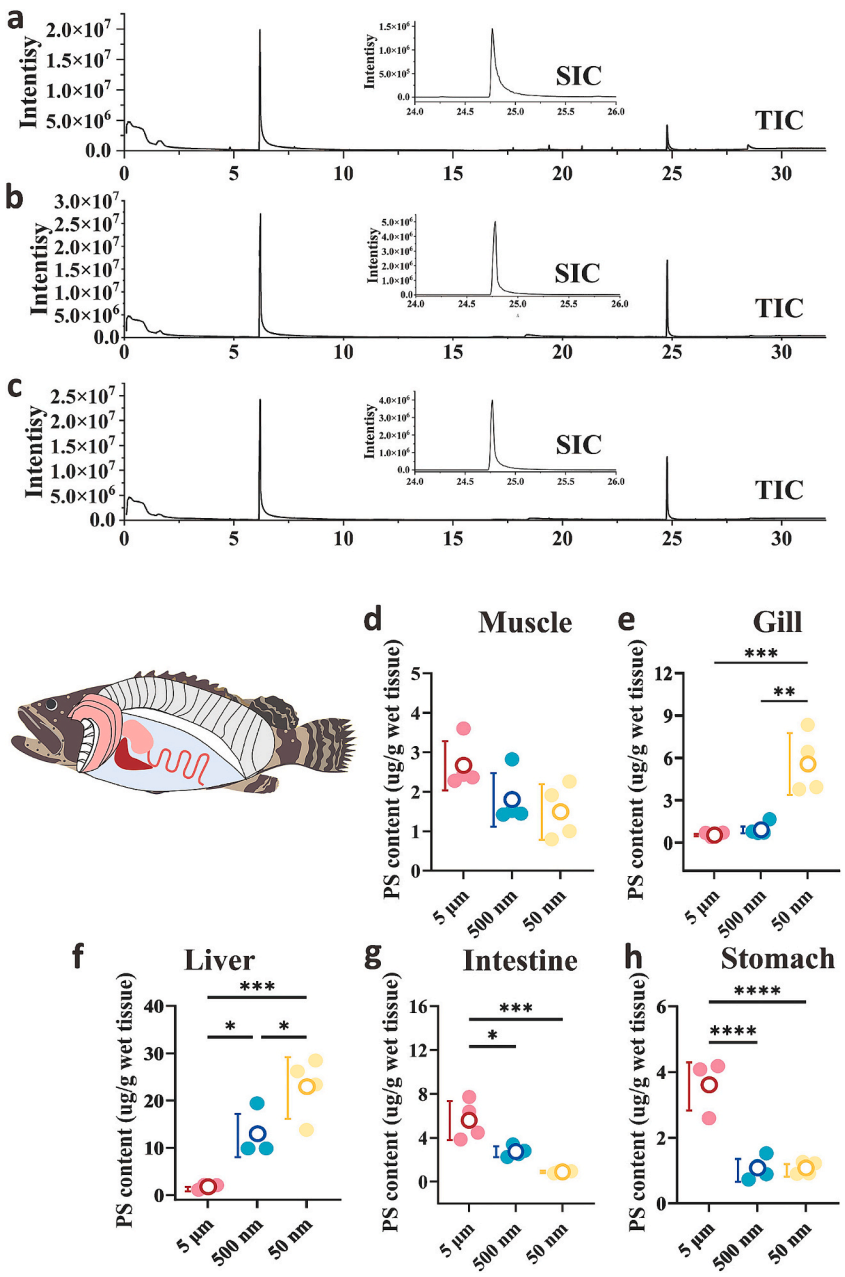

Fig. 1. (a-c) Py-GC/MS chromatogram of $PS_{5\ \mu m}$, $PS_{500\ nm}$ and $PS_{50\ nm}$. The total ion chromatogram (TIC), selected indicator ion chromatogram (SIC, m/z 91). (d-h) Determination of MPs and NPs content in major organs of *pearl gentian grouper* after exposure to MPs and NPs of different particle sizes, including (d) Muscle; (e) Gill; (f) Liver; (g) Intestine; (h) Stomach. Data are expressed as mean ± standard deviation. With four independent tests in every treatment.

3.3. Main pathological changes in liver following MPs and NPs exposure of different size

Following a 15-day exposure experiment, MPs and NPs caused significant pathological damage to liver tissue compared to the control group. Histopathological analysis revealed characteristic damage, including hepatocyte swelling, indistinct cell boundaries, vacuolar degeneration, inflammatory cell infiltration, fatty degeneration, and localized fibrous tissue proliferation (Fig. 2a, Fig.S5). Quantitative assessment using a liver injury scoring system indicated a clear particle size-dependent pattern in these pathological changes (Fig. 2c). Specifically, as the particle size of MPs and NPs decreased, their ability to penetrate the intestinal epithelial barrier and directly impact the liver via the circulatory system was significantly enhanced [43]. This finding aligns with previous studies [44], smaller NPs exhibited a broader tissue distribution within organisms and exerted stronger toxic effects when compared with MPs. It is noteworthy that the liver exhibits a degree of tissue damage closely associated with liver dysfunction [45]. This study revealed that MPs and NPs exposure not only caused typical hepatocyte damage but also triggered a distinctive disruption in lipid metabolism. Through Oil Red O staining analysis, we observed a significant reduction in lipid deposition area in the liver tissue of the exposure group (Fig. 2b, Fig. 2d, Fig.S6), a phenomenon that differs from previous reports. This finding suggests that MPs and NPs may exacerbate liver injury by interfering with lipid metabolism pathways in the liver [46]. Therefore, MPs and NPs exposure can lead to liver dysfunction through multiple mechanisms, including the induction of hepatic inflammation, promotion of fatty degeneration, and disruption of lipid metabolism.

The experimental results demonstrated that MPs and NPs exposure exerted a significant particle size-dependent effect on the levels of TG, T-

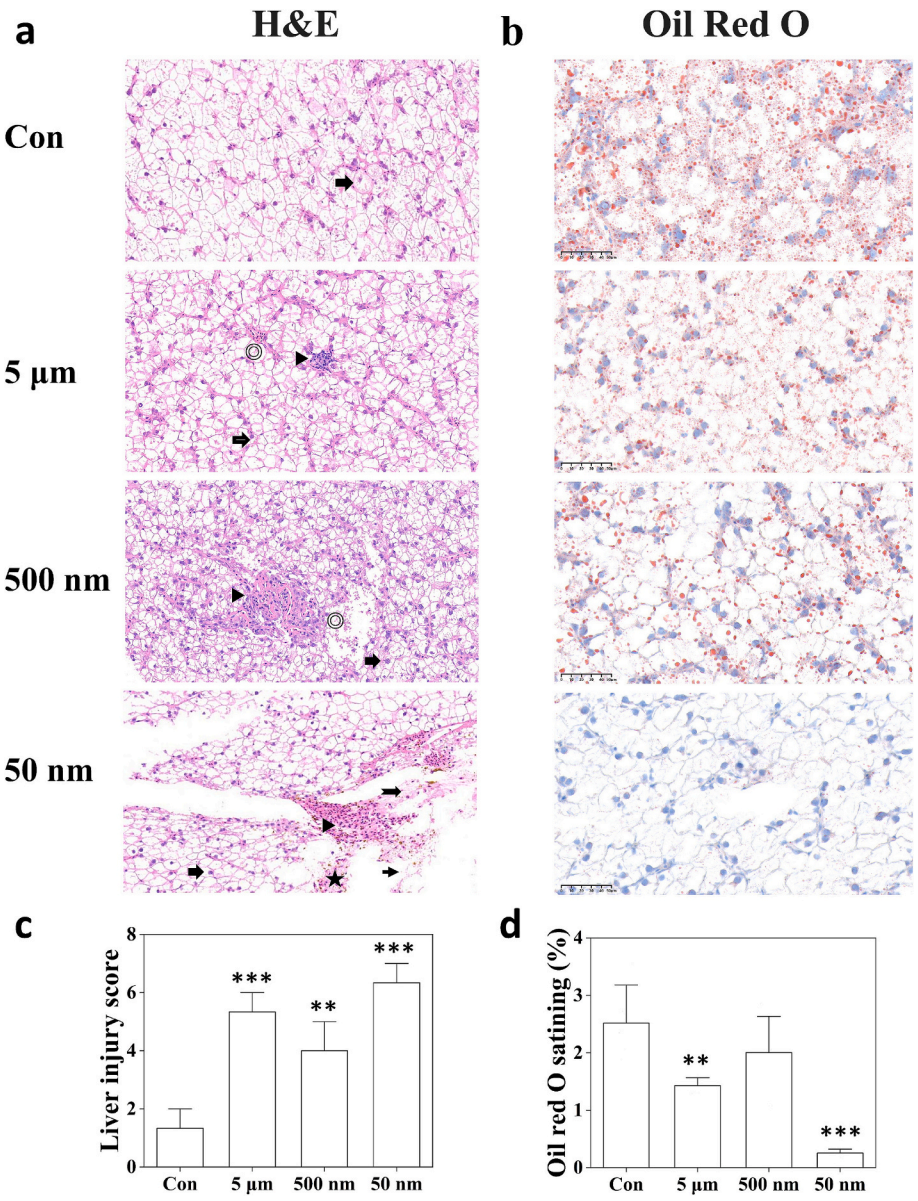

Fig. 2. Pathological changes in the liver after exposure to PS MPs and NPs of different particle sizes. (a) Hematoxylin-eosin staining images of liver. (Magnification:×20) (b) Quantitative analysis of liver damage. (c) Oil red o staining image of liver. (Magnification:×20) (d) Oil red o staining area percentage. Different symbols represent different types of damage. (▶: Inflammatory cell infiltration; ➧: Hepatocyte swelling; ➠: Fibrosis; →: Tissue damage; ◎: Liver lobule destruction; ★: Pigmentation). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

CHO, and LDL-C in liver tissue. As particle size decreased, the TG content in the liver tissue exhibited a gradual declining trend (Fig. 3a), while the levels of T-CHO and LDL-C showed a marked increase (Fig. 3b, Fig. 3c), with the most pronounced changes observed in the 50 nm exposure group ($p < 0.05$). TG, as a critical regulator of energy homeostasis and lipid metabolism [47], is primarily stored in adipocytes in the form of lipid droplet, providing energy reserves for the organism [48]. The reduction in TG content suggests that MPs and NPs exposure may accelerate the breakdown of TG to meet energy demands. This observation is in accordance with the findings of Chen et al. [49], who reported that MPs exposure led to a decrease in TG levels in mouse liver, subsequently triggering liver dysfunction, hepatocyte damage, and lipid metabolism disorders.

LDL-C functions as a primary mediator of lipid transport and plays a pivotal role in shuttling lipids from the liver to peripheral tissues [19]. The elevated LDL-C levels observed in this study suggest a compensatory mechanism to alleviate metabolic stress induced by MPs and NPs exposure, though this adaptive response may concurrently disrupt lipid homeostasis and energy metabolism. As a critical regulator of fish metabolism, growth, and health, cholesterol is instrumental in governing lipid catabolism and energy allocation, while also modulating various physiological processes, including cellular signaling cascades [50]. Research by Lee et al. [51] has demonstrated that the accumulation of MPs and NPs in the body can induce liver damage accompanied by elevated cholesterol levels. This may represent an adaptive defense mechanism, whereby the organism provides additional energy to mitigate the toxic effects of MPs and NPs. Furthermore, exposure to MPs and NPs may also promote the development of atherosclerosis by triggering inflammatory responses. Specifically, the inflammatory environment can lead to the oxidation of LDL-C, which is subsequently engulfed by macrophages and transformed into cholesterol and lipid rich foam cells [52]. By comprehensively analyzing the changes in TG, LDL-C, and T-CHO levels, it can be inferred that MPs and NPs exposure significantly accelerates the organism's energy metabolism processes, mobilizing

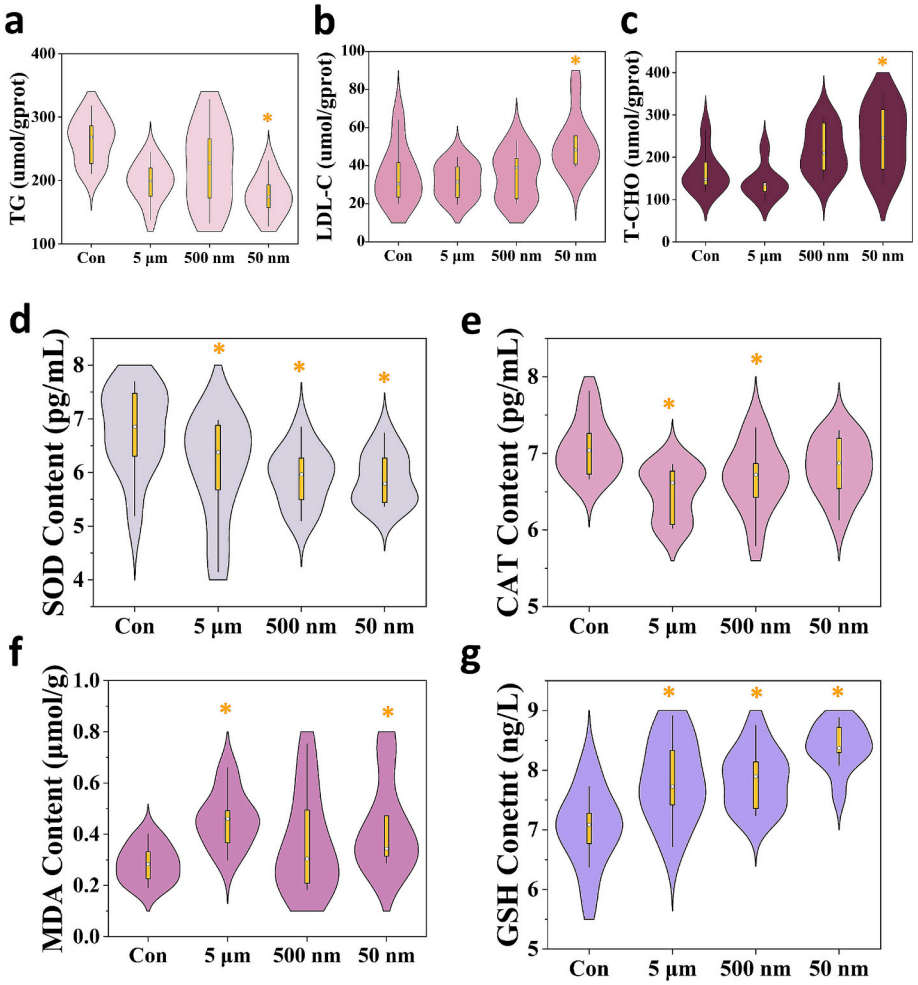

Fig. 3. Analysis of biochemical parameters and oxidative stress. (a) TG content; (b) LDL-C content; (c) T-CHO content; (d) SOD content; (e) CAT content; (f) MDA content; (g) GSH content.

lipid reserves to provide energy support in response to exogenous contaminants. This finding offers a crucial metabolic perspective for further elucidating the toxicity mechanisms of MPs and NPs.

3.4. *Effect of different particle size PS MPs and NPs on oxidative stress in liver tissues*

Exposure to MPs and NPs can trigger oxidative stress responses in organisms, with a pronounced particle size-dependent effect: as particle size decreases, the intensity of oxidative stress significantly increases [53]. This heightened oxidative stress prompts cells to release numerous inflammatory mediators, which activate the organism's immune system, leading to inflammatory responses and ultimately disrupting the homeostasis of hepatic lipid metabolism. Among these processes, the excessive production of reactive oxygen species (ROS) is regarded as a central mechanism contributing to hepatotoxicity and lipotoxicity.

In the *pearl gentian grouper*, we observed that MPs and NPs exposure significantly disrupted the balance of the antioxidant system. Specifically, the levels of SOD and CAT were markedly reduced (Fig. 3d, Fig. 3e), while the levels of MDA and GSH were significantly elevated (Fig. 3f, Fig. 3g). SOD and CAT, as the first line of defense in the antioxidant system, are responsible for converting superoxide radicals into hydrogen peroxide and further decomposing it into water and oxygen, respectively [54,55]. The decline in their activities indicates a significant impairment in the organism's ability to scavenge ROS. Under these conditions, GSH plays a vital role in mitigating MPs and NPs induced oxidative damage by converting peroxides into less toxic hydroxyl compounds [56].

It is noteworthy that MDA, a specific marker of lipid peroxidation in cell membranes [57], exhibited a significant increase in content, directly reflecting the extent of cell membrane damage caused by exposure to MPs and NPs. Previous studies have shown that MDA not only acts as a biomarker of oxidative stress but also induces apoptosis in various cell types, including hepatocytes [58]. Furthermore, due to their ability to directly penetrate cell membranes, NPs can more effectively disrupt the cellular microenvironment and metabolic processes, while the toxic additives they release further intensify oxidative stress levels. The findings of this study are highly consistent with those reported by Tao et al. [59], confirming that exposure to MPs and NPs significantly impairs the liver's antioxidant system function and leads to abnormal elevations in MDA and GSH levels.

3.5. *Overview of changes in hepatic transcriptome*

This study employed RNA-seq technology to systematically investigate the molecular regulatory mechanisms of lipid metabolism in the liver of the pearl gentian grouper under MPs and NPs exposure. Transcriptomic analysis revealed that 441, 397, and 499 DEGs were identified in the respective exposure groups, with 23 genes exhibiting a consistent differential expression pattern across all three groups (Fig. 4a, Fig. 4b, Fig. 4c). To gain deeper insights into the biological roles of these DEGs, we conducted comprehensive functional enrichment analyses

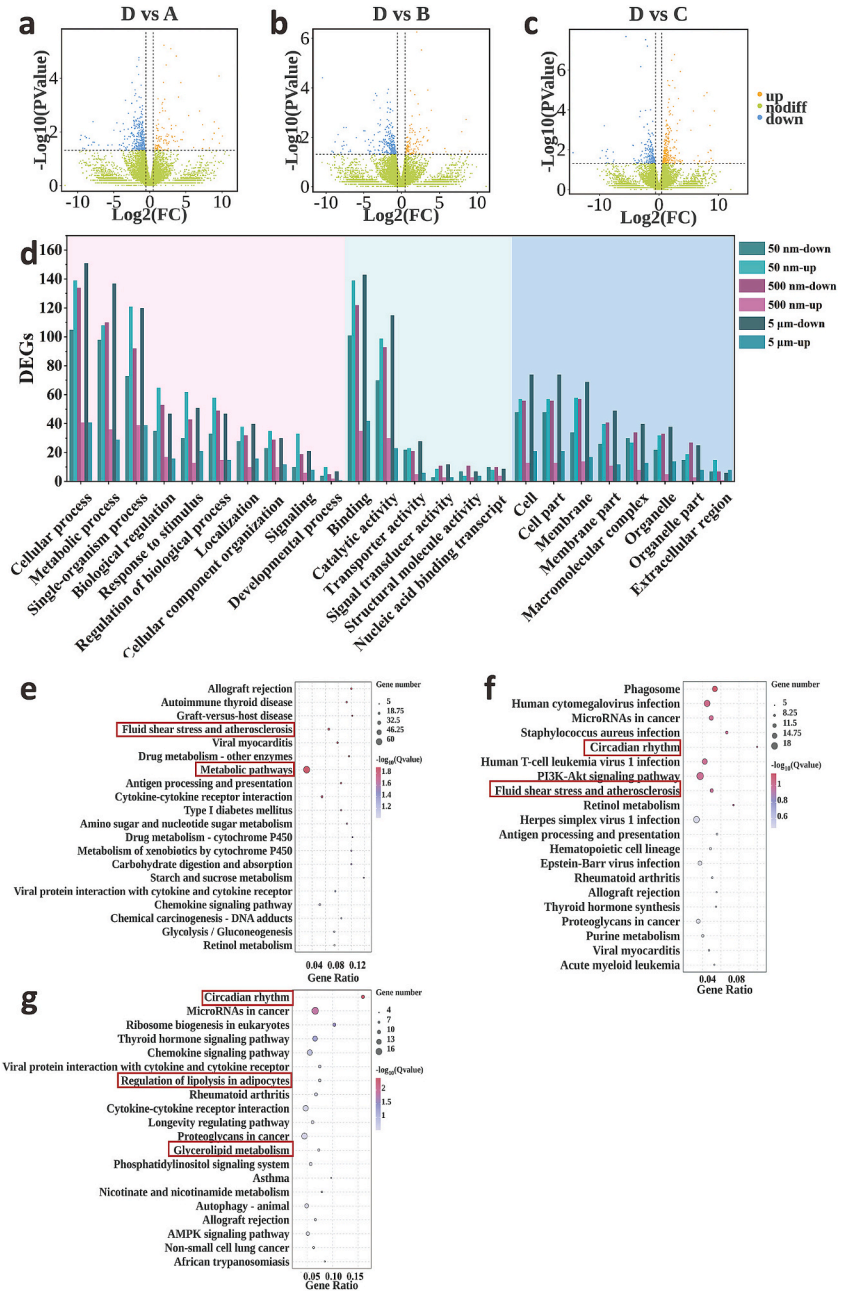

Fig. 4. Transcriptome analysis results of MPs of different particle sizes. (a-c) Volcano plot of expression differences between different particle size exposure groups and control groups. (a) 5 μm. (b) 500 nm. (c) 50 nm. (d) GO functional annotation analysis. (e-f) KEGG pathway enrichment analysis after exposure to MPs and NPs of different particle sizes. (e) 5 μm. (f) 500 nm. (g) 50 nm.

using GO and KEGG databases. GO annotation classified the DEGs into three primary categories: Biological Process, Cellular Component, and Molecular Function (Fig. 4d). The analysis revealed that significantly enriched DEGs were primarily associated with biological functions such as “cellular processes,” “metabolic processes,” “single-organism responses,” “binding,” and “catalytic activity.” Notably, the pronounced enrichment in “metabolic processes” suggests that MPs and NPs exposure may induce widespread disruptions in cellular metabolic homeostasis [60]. This finding aligns with the observed changes in TG, T-CHO, and LDL-C levels, as well as the results of Oil Red O staining, collectively confirming the substantial interference of MPs and NPs exposure on hepatic lipid metabolism. Furthermore, the significant enrichment in the “catalytic activity” category indicates that MPs and NPs exposure might influence the organism’s antioxidant defense system by affecting enzyme activity [61]. This hypothesis is supported by the observed variations in antioxidant enzyme activity in the present study. Additionally, research by Dar et al. [62] has demonstrated that the expression and activity of antioxidant enzymes exhibit a characteristic “biphasic response”: they are positively regulated under low stress conditions but significantly suppressed under high stress conditions. This phenomenon may account for the observed dysfunction in the antioxidant system in this study.

Based on transcriptomic data analysis, we categorized the 934 identified DEGs into 10 distinct gene clusters with varying expression patterns. Among these, the expression profiles of genes in Clusters 3, 4, 6, and 7 were significantly associated with disruptions in hepatic lipid metabolism (Fig.S7). KEGG pathway enrichment analysis revealed that these DEGs were predominantly involved in metabolic pathways such as triglyceride metabolism, adipocyte lipolysis regulation, glycerophospholipid metabolism, steroid biosynthesis, and pathological

processes like atherosclerosis. Notably, DEGs in other clusters were primarily linked to biological processes including apoptosis, immune response, and signal transduction. Further analysis indicated significant variations in the hepatic lipid metabolism pathways affected by MPs and NPs exposure. These particle size-dependent pathway differences are likely to reflect a close relationship between the toxicity mechanisms of MPs and NPs and their physicochemical properties. Specifically, MPs may primarily disrupt cellular functions through physical stimulation and surface adsorption, whereas NPs can penetrate cells directly, exerting toxic effects by interfering with organelle functions and molecular signaling pathways. This finding provides crucial molecular-level evidence for understanding the particle size-dependent toxicity mechanisms of MPs and NPs.

Transcriptomic analysis revealed that although PS MPs and NPs of varying particle sizes exhibited significant enrichment in lipid metabolism-related pathways, the specific metabolic pathways affected differed markedly (Fig. 4e, Fig. 4f, Fig. 4g). Specifically, $PS_{5\ \mu m}$ was predominantly enriched in pathways such as "atherosclerosis" and "regulation of lipolysis in adipocytes"; $PS_{500\ nm}$ showed significant enrichment in pathways related to "atherosclerosis" and "circadian rhythm"; while $PS_{50\ nm}$ primarily influenced pathways including "circadian rhythm," "regulation of lipolysis in adipocytes," and "triglyceride metabolism." These particle size-dependent pathway variations highlight fundamental differences in the toxicity mechanisms of MPs and NPs [63]. Notably, although MPs and NPs may induce similar toxic effects, the intensity and underlying mechanisms of their impact vary considerably. Due to their higher bioavailability, NPs are more readily absorbed and accumulated in tissues, leading to stronger oxidative stress and inflammatory responses [64]. In contrast, MPs primarily exert their effects by disrupting gut function and altering gut microbiota homeostasis [65]. This finding is consistent with the results reported by Choi et al. [66] and Jeong et al. [67]. However, existing studies are limited to indicate that both MPs and NPs can cause gut microbiota dysbiosis, the underlying mechanisms of these effects require further investigation.

To validate the reliability of the RNA-seq results, we performed q-PCR analysis on key genes involved in the regulation of adipocyte lipolysis and triglyceride metabolism (such as *PNPLA2*, *MGLL*, *LIPC*, and *LPIN1*) as well as other genes associated with lipid metabolism (*C1QL4* and *FABP3*) (Fig. 5a, Fig. 5b). Since the $PS_{500\ nm}$ exposure group showed limited enrichment in lipid metabolism pathways, q-PCR validation was conducted on the control group and other two treatment groups. The results demonstrated that the expression patterns of these genes were highly consistent with the Illumina sequencing data, exhibiting similar

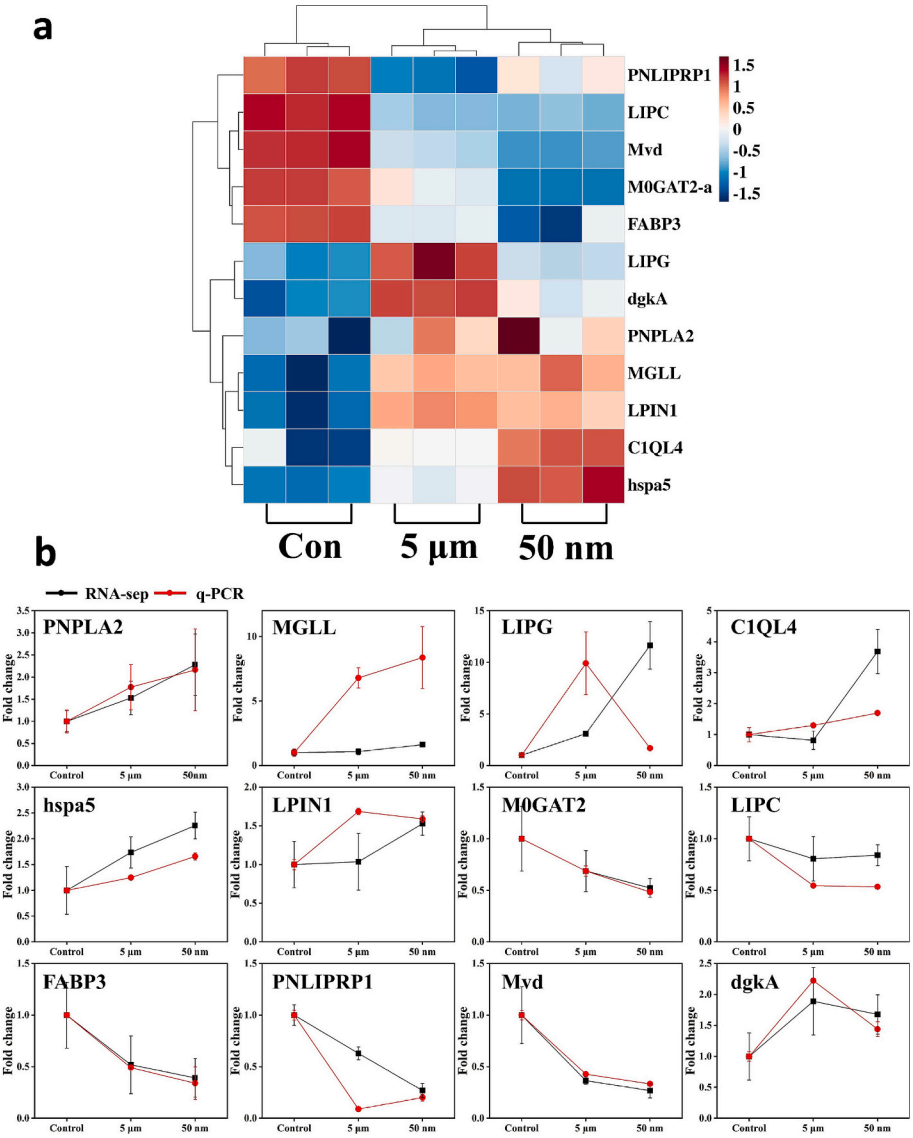

Fig. 5. (a) Heatmap of q-PCR gene expression; (b) q-PCR validation of RNA-sep. Up and down represent up-regulated genes and down-regulated genes, respectively.

upregulation and downregulation trends, thereby confirming the reliability of the DEGs analysis.

3.6. Potential mechanism of hepatic lipid metabolism disruption by small particle size PS MPs and NPs

Based on these findings, we propose the hypothesis of multiple mechanisms of action of MPs and NPs exposure leading to disorders of hepatic lipid metabolism (Fig. 6).

(1) MPs and NPs exposure during the overwintering period interferes with glyceride metabolism and adipocyte lipolytic regulatory processes. Research by Wang et al. [68] demonstrated that under cold stress, *perch* adapt to the demands of lipid metabolism by downregulating gene expression in the mTOR signaling pathway while upregulating *PPAR-a* expression. Notably, *pearl gentian grouper* exhibits a significant reduction in food intake when temperatures drop below 24 °C, and feeding ceases entirely at 13 °C [69]. Combining the Oil Red O staining and transcriptomic analysis results from this study, we found that MPs and NPs exposure accelerates lipid breakdown in adipose tissue, leading to premature depletion of energy reserves and thereby impairing the fish's ability to cope with cold stress. This observation aligns with findings by Biro et al. [70], who reported that small *oncorhynchus mykiss* experience significantly increased mortality rates due to early exhaustion of lipid reserves during winter. It is noteworthy that the smaller the particle size of MPs, the more pronounced the depletion of hepatic lipid reserves. Although this study did not directly measure fatty acid content, considering that fatty acids are central components of lipid metabolism, future research should prioritize examining changes in fatty acid profiles. This will contribute to a more comprehensive understanding of the molecular mechanisms underlying MPs and NPs induced lipid metabolism disorders, particularly by elucidating their toxic effects from the perspective of fatty acid metabolism.

(2) MPs and NPs exposure leads to energy metabolism imbalance. To cope with the physiological stress induced by MPs and NPs exposure, fish must increase their metabolic demands to maintain essential physiological functions, resulting in a significant reduction of energy allocated for growth and development, thereby causing an imbalance in energy distribution [71]. The reduction in lipid content is not only attributed to the lack of nutritional value in MPs and NPs but may also be linked to the increased energy demand caused by the inflammatory responses they trigger [72]. This finding is corroborated by the results of hematoxylin-eosin staining. Transcriptomic analysis further revealed significant enrichment in the IL-17 signaling pathway and autophagy-related pathways, which are energy-dependent biological processes that exacerbate energy consumption [73]. This phenomenon is consistent with the findings of Watts et al. [74], who reported that MPs exposure leads to a notable reduction in energy reserves and lipid depletion in crabs. Additionally, studies suggest that certain organisms may activate anaerobic metabolic pathways to cope with chemical stress induced by MPs and NPs exposure [75]. Collectively, these findings jointly explained that MPs and NPs exposure disrupts energy metabolism balance, resulting in reduced hepatic lipid storage and subsequently impairing the normal growth and development of organisms. Given that numerous studies have demonstrated a significant increase in the levels of inflammatory mediators (e.g., TNF-α, IL-1β) and their gene expression under exposure to MPs and NPs as well as cold stress conditions, we did not directly measure the concentrations of these inflammatory mediators or autophagy markers (e.g., LC3-II) to further validate the transcriptomic analysis results. Future studies could employ real-time sample collection and multi-omics approaches to further substantiate the related hypotheses [76–81].

(3) Disruption of TG metabolism and abnormal transport. TG plays a critical role in maintaining energy homeostasis and serves as the primary form of energy storage in adipose tissue. Under conditions of increased energy demand, such as fasting, TG can be

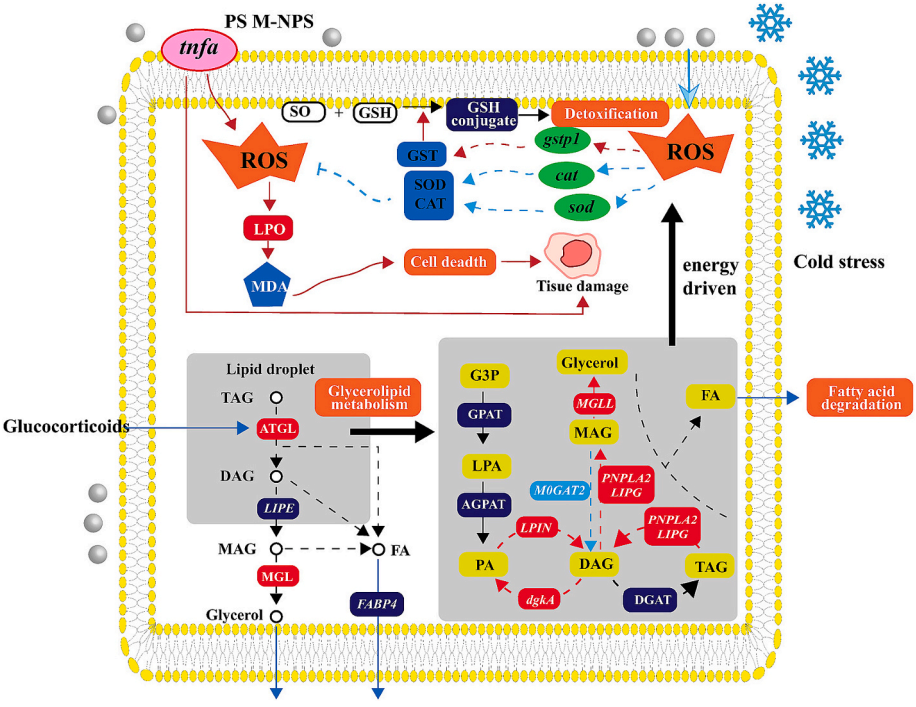

Fig. 6. Diagram of glycerol metabolism and lipid decomposition regulation in *pearl gentian grouper* exposed to 50 nm PS NPs. Red rectangles indicate up-regulated genes, and blue rectangles indicate down-regulated genes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

hydrolyzed to release fatty acids, providing energy for the organism [82]. Adipose triglyceride lipase (ATGL) acts as the rate-limiting enzyme in TG breakdown, primarily catalyzing the initial hydrolysis step of TG to produce diacylglycerol, which serves as a substrate for hormone-sensitive lipase (HSL), thereby supplying essential energy to the organism [83]. Following MPs and NPs exposure, the expression level of ATGL is significantly upregulated, which subsequently promotes the catabolism of TG in the liver. This process results in a reduction of hepatic TG content, while TG is transported from the liver to peripheral tissues in the form of LDL-C via apolipoprotein-mediated mechanisms to meet the energy demands of other tissues. This abnormal metabolism and transport of TG may represent a key mechanism underlying the disruption of hepatic lipid metabolism induced by MPs and NPs exposure.

3.7. Mitigation measure

To mitigate the adverse effects of MPs and low temperatures, we propose the following measures from the perspectives of the aquaculture environment and dietary interventions, taking into account both economic costs and practical benefits. Firstly, to address the issue of MPs pollution, we recommend implementing a dual-filtration system at the water inlets and outlets of aquaculture facilities. Specifically, a primary large-pore filter can be used to remove larger debris, followed by a secondary small-pore filter to effectively capture MPs. This approach can significantly reduce the entry and re-circulation of MPs within aquaculture systems. Additionally, we suggest the adoption of bacteria-microalgae combined bioremediation technology to improve water quality and yield in closed-loop aquaculture systems while specifically targeting the removal of MPs to prevent their re-entry into the environment [84,85].

Furthermore, as increased membrane lipid unsaturation and oxidative stress exacerbate cellular damage under low-temperature conditions [86], we recommend the use of functional feed additives to alleviate cold stress in fish. Specifically, diets enriched with antioxidants such as vitamin E and vitamin C, as well as other bioactive compounds including propolis, phycocyanin, highly unsaturated fatty acids, inositol, and phospholipids, can enhance fish resilience to cold stress [87]. Studies have shown that pre-winter feeding with high-oil diets improves the health status of *gilthead sea bream*, while winter diets supplemented with the aforementioned compounds significantly increase the survival rate of *gilthead sea bream* during cold periods [88]. These cost-effective and feasible dietary interventions can be readily adopted by aquaculture practitioners to improve the physiological adaptation of fish to temperature fluctuations.

4. Conclusion

This study shows that MPs and NPs enter systemic circulation via the respiratory and digestive systems, accumulating in the liver of *pearl gentian grouper*. This accumulation causes pathological damage, oxidative stress, and abnormal lipid metabolism, with effects intensifying as particle size decreases. During the overwintering phase, MPs and NPs exposure accelerates lipid degradation, likely as a compensatory mechanism to maintain energy homeostasis under thermal stress. Two potential mechanisms underlie this disruption: (1) immune-mediated energy demand, where the immune response to MPs and NPs increases energy needs, enhancing lipid reserve mobilization; and (2) direct modulation of lipid metabolism, with MPs and NPs upregulating hepatic TG catabolism through altered expression of genes like *PNPLA2*, *MGLL*, and *LIPG*, reducing hepatic TG levels while increasing circulating LDL-C. These findings offer novel insights into the mechanisms of hepatic lipid metabolism disruption by MPs and NPs during overwintering.

CRediT authorship contribution statement

Julong Wang: Writing – review & editing, Writing – original draft, Visualization, Formal analysis, Data curation. **Yan Gao:** Validation, Supervision. **Shanshan Yao:** Validation, Supervision. **Lang Lin:** Writing – review & editing, Supervision, Funding acquisition, Data curation, Conceptualization. **Hengxiang Li:** Validation, Supervision. **Shan Liu:** Validation, Supervision. **Rui Hou:** Validation, Supervision. **Zhijian Jiang:** Validation, Supervision. **Xiangrong Xu:** Validation, Supervision.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.enceco.2025.07.021.

Data availability

Data will be made available on request.

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