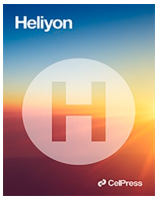
Heliyon 10 (2024) e30858

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Different recovery patterns of the surviving bivalve *Mytilus galloprovincialis* based on transcriptome profiling exposed to spherical or fibrous polyethylene microplastics

Boobal Rangaswamy [a], Jinsung An [b], Ihn-Sil Kwak [c,*]

[a] *Department of Biotechnology, PSG College of Arts & Science, Coimbatore, Tamil Nadu 641014, India*
[b] *Department of Civil and Environmental Engineering, Hanyang University, Ansan, 15588, Republic of Korea*
[c] *Department of Ocean Integrated Science, Chonnam National University, Yeosu, 59626, Republic of Korea*

ARTICLE INFO

Keywords:
Microplastics
Environmental pollution
RNA-Seq
Transcriptomics
Ecotoxicity

ABSTRACT

Microplastics (MPs) are pervasive pollutants exuded from anthropogenic activities and ingested by animals in different ecosystems. This transcriptomic profiling study aimed to explore the impact of polyethylene MPs on *Mytilus galloprovincialis*, an ecologically significant bivalve species. The toxicity of two MPs types was found to result in increased cellular stress when exposed up to 14 days. Moreover, recovery mechanisms were also observed in progress. Mussels exhibited different gene expression patterns and molecular regulation in response to cellular reactive oxygen species (ROS) stress. The transcriptome analysis demonstrated a notable hindrance in cilia movement as MPs ingested through gills. Subsequent entry resulted in a significant disruption in the cytoskeletal organization, cellular projection, and cilia beat frequency. On day 4 (D4), signal transduction and activation of apoptosis evidenced the signs of toxic consequences. Mussels exposed to spherical MPs shown significant recovery on day 14 (D14), characterized by the upregulation of anti-apoptotic genes and antioxidant genes. The expression of P53 and BCL2 genes was pivotal in controlling the apoptotic process and promoting cell survival. Mussels exposed to fibrous MPs displayed a delayed cell survival effect. However, the elevated physiological stress due to fibrous MPs resulted in energy transfer by compensatory regulation of metabolic processes to expedite cellular recovery. These observations highlighted the intricate and varied reaction of cell survival mechanisms in mussels to recover toxicity. This study provides critical evidence of the ecotoxicological impacts of two different MPs and emphasizes the environmental risks they pose to aquatic ecosystems. Our conclusion highlights the detrimental effects of MPs on *M. galloprovincialis* and the need for more stringent regulations to protect marine ecosystems.

1. Introduction

Microplastics (MPs) are the smaller unit components from the fragmentation of plastics used in various commercial and domestic applications causing a significant environmental threat [1]. Animals can easily ingest these MPs, which are less than 5 mm in size and quickly transfer among ecosystems, leading to hazardous effects [2]. The accumulation of MPs in aquatic habitats persists for many

* Corresponding author.
E-mail address: iskwak@jnu.ac.kr (I.-S. Kwak).

https://doi.org/10.1016/j.heliyon.2024.e30858
Received 3 September 2023; Received in revised form 1 May 2024; Accepted 7 May 2024
Available online 10 May 2024

years, resulting a serious risk to marine and freshwater environments [3]. MPs deposited in these environments affect the biota, from surface waters to sediments [4]. The bioavailability of MPs in aquatic ecosystems makes them susceptible to various organisms such as mammals, fish, crustaceans, invertebrates, and zooplankton [5–8]. Furthermore, MPs have the potential to inflict physical harm on organisms through direct interaction, as well as through chemical effects mediated by MP monomers and leached additives [9]. More research is needed to mitigate the damage and deleterious risks to organisms caused by MPs.

Microplastic pollution comprises various chemical components, such as polyvinyl chloride (PVC), polyethylene terephthalate (PET), polypropylene (PP), polyamide (PA), and polystyrene (PS) [10]. Among these, polyethylene (PE) is particularly prevalent in different forms of debris discovered in water, sediments, and sludge in aquatic environments [11]. The escalating levels of PE significantly influence the nutrient cycle [12] in aquatic systems, which can ultimately result in tissue damage in animals [13]. Ingestion of microplastics causes a significant change in the expression of immune genes [14] and can cause severe consequences when attached to tissues [15], including the retardation of fundamental functions such as nutrition, development, growth, reproduction, and survival. Recent breakthroughs in transcriptome sequencing technology have provided researchers with a more efficient way to delineate the fate of toxicants, antagonistic effects, and biological responses, thereby enabling them to better understand and address the issue of MPs pollution [16].

In *Danio rerio,* MPs lead to functional redundancy, damage to cells and tissues, developmental defects, cytological problems, and apoptosis [17–19]. However, aquatic organisms, such as microalgae, zooplankton, brine shrimp, and copepods, have also exhibited signs of adverse reactions to MPs [20–23]. These adverse effects include reduced reproduction, survival, and growth retardation.

Mussels are known for their suspension feeding mechanisms, which let the contaminants in the water accumulate, making them a critical medium for transfer into humans on consumption [24]. Research has shown that the effects of polyethylene MPs exposure in blue mussels (*Mytilus edulis*) have influenced gut microbiota, immune response, and energy metabolism [25,26]. *M. galloprovincialis*, the dominant group of the *Mytilus* genus along the Korean coast, is widely consumed and an excellent pollution bioindicator [27]. Hence, conducting a comprehensive investigation of MPs toxicity (mostly PE and PS) in bivalves is imperative by considering the appropriate concentration of toxicants and retention time based on the mussel's condition and age [26]. Studies have shown that MPs can harm the cell viability and digestion in mussels, *M. galloprovincialis,* while also increasing the levels of heat shock proteins, antioxidants, and glutathione-related enzymes [24,28,29]. Similarly, transcriptomics results of polyethylene microbeads treated mussels (*M. galloprovincialis)* have indicated the activation of the apoptotic process and stress response-related proteins [30].

MPs toxicity was studied to highlight the significant impacts on mussels, *M. galloprovincialis*. To achieve this, the transcriptome of treatment groups exposed to MPs (spherical and fibrous) was compared with control groups. In addition, our analysis unravel the principal biological responses at different time points (Day 4 - D4; Day 14 - D14) by assessing the molecular reactions to the physical and chemical effects of MPs. The study's objectives included the functional annotation of the transcriptome, analysis, and scrutiny of the response to the exposed MPs of increasing concentration over time.

2. Materials and methods

2.1. MP treatment test organism maintenance

Bivalves, *M. galloprovincialis* (shell length, 6.1 ± 1.0 cm, weight, 24.8 ± 2.0 g), were purchased from local aquatic fish markets at Gwangyang Bay, Korea. The mussels were transported in stabilized, aerated tanks to the laboratory within 1 h. Then set to acclimate in a 4 L water tank (3 mussels/L) for 3 days. Artificial seawater (Instant Ocean, VA, USA) was used in the tanks by adjusting salinity to 25.0 ± 1.0 %. The tanks were maintained with controlled supply of aeration, water temperature (19 ± 1 °C), pH (7.5 ± 0.5), and dissolved oxygen (DO) of 60 %. During the acclimatization period, Shellfish Diet 1800TM and Nanno 3600 (Reed Mariculture, CA, USA) 2:1 was diluted and supplemented as feed.

2.2. Experimental setup and exposure conditions

The MPs toxicity induction was performed using two distinct types of polyethylene MPs: spherical and fibrous. The spherical MPs (Cospheric, Santa Barbara, CA, US) were 27–32 μm in length and 1 g/cc in density, and the fibrous MPs (KITECH, Korea Institute of Industrial Technology, Cheonan, Republic of Korea) were 200–400 μm in length and 1.4 g/cc in density. To assess the impact on mussels, considerably high MP concentrations were used [31]. The experiment was carried out for 14 days. The mussels maintained in the acclimatized condition were exposed to MPs of 100 mg/L concentration. The experimental setup in the laboratory was supported by consistent temperature (19 ± 1 °C) and shifting of the light-dark cycle for 12 h. The water quality was maintained by removing settled waste in the tanks with appropriate water exchange once every 7 days. The exposure setup consists of triplicates with 10 mussels in 4 L water each. At each time point, gill samples representing control and treatment groups were collected from the mussels (n = 3) for RNA sequencing analysis. Sampled gill tissues were washed in phosphate buffered saline and kept at −80 °C. The sample groups treated with spherical MPs were D4 sphere, D14 sphere, and control, whereas samples treated with fibrous MPs were named D4 fiber, and D14 fiber, with respective control.

2.3. RNA extraction and illumina sequencing

The total RNA was extracted from triplicate mussel samples using RNAeasy kit (Qiagen, Düsseldorf, Germany) and the RNA concentration was calculated using Quant-IT RiboGreen (Invitrogen Corp., Waltham, MA, US). Finally, the integrity of the total RNA

was assessed using TapeStation RNA screen tape (Agilent Technologies, Santa Clara, CA, US). RNA samples with RNA Integrity Number (RIN) > 7.0 were utilized for RNA library construction.

0.5 μg of total RNA was used to prepare a library independently for each sample using Illumina TruSeq stranded Total RNA Library Prep Gold Kit (Illumina Inc., San Diego, CA, USA). The workflow involves the removal of rRNA from the total RNA followed by mRNA fragmentation into smaller units using divalent cations under elevated temperatures. Further, first-strand cDNA was synthesized using SuperScript II reverse transcriptase (Invitrogen pCorp, Waltham, MA, US) and random primers, followed by second-strand cDNA synthesis using DNA Polymerase I, RNase H, and dUTP. These cDNA fragments were then processed at the ends, adding a single 'A' base and ligating the adapters. The products were then purified and enriched with PCR to create the final cDNA library.

The libraries were quantified using the Kapa library quantification kit (Kapa Biosystems, Cape Town, South Africa). The quality assessment used the TapeStation D1000 ScreenTape (Agilent Technologies, Santa Clara, CA, US). Indexed libraries were submitted to Illumina NovaSeq (Illumina, Inc., San Diego, CA, USA). Macrogen Inc. (Gangnam-gu, Seoul, Republic of Korea) performed the paired-end sequencing.

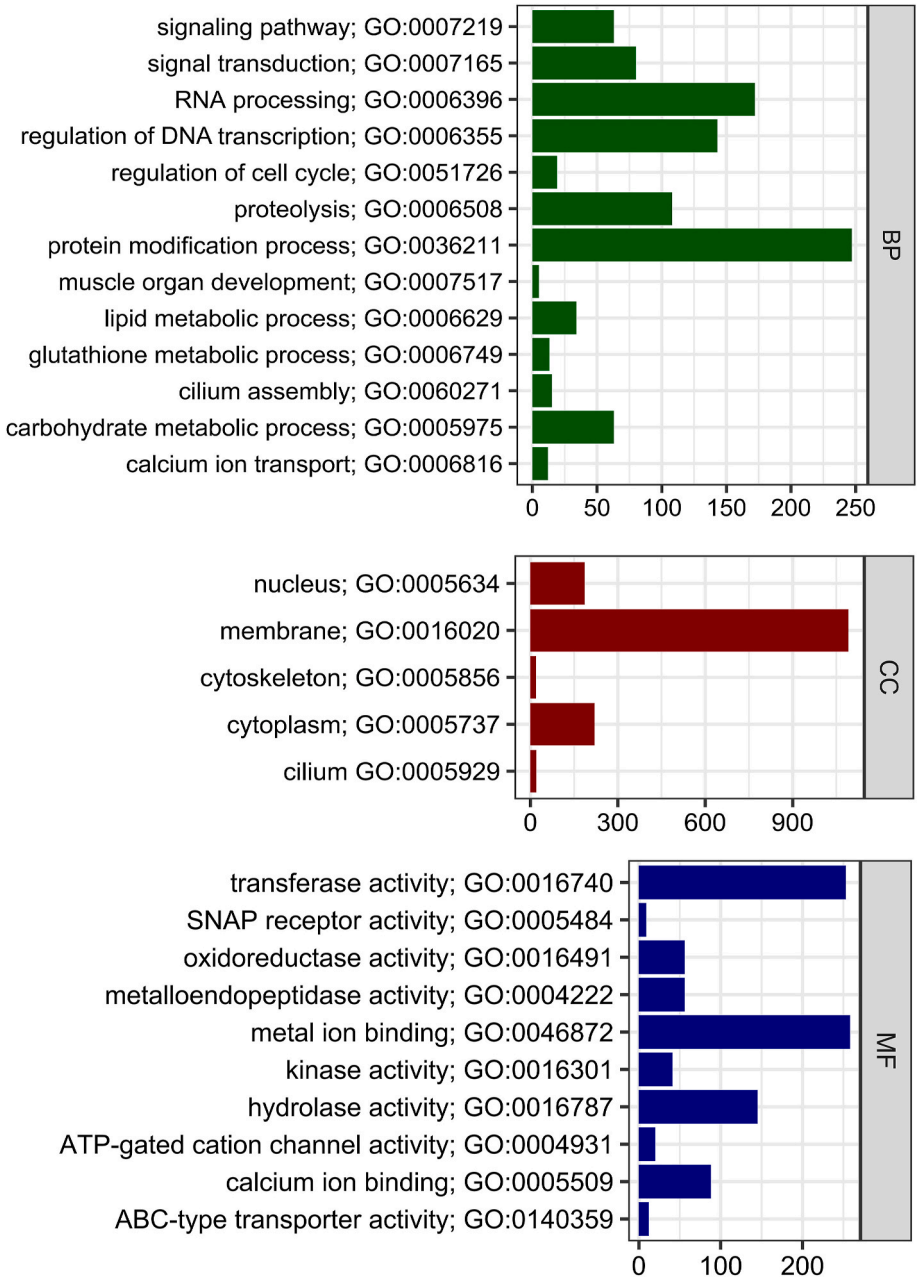


Fig. 1. Transcriptome DEGs of the MPs treatment group represents the GO categories and overall functional annotation.

2.4. Bioinformatics and statistical analyses

Low-quality reads (QC value < 30) were trimmed using the Trimmomatic program [32] to assure accuracy for subsequent bioinformatics analysis. Clean reads were mapped and aligned to the reference genome through the bowtie aligner [33] using the HISAT2 program [34]. The transcript assembly was performed using the StringTie program [35]. Transcript quantification of the samples yielded read count, transcript length, and depth of expression. The differential expression of samples was determined using the Bioconductor package [36] in R Statistical Software [37]. DESeq2 [38] was performed to generate significant differentially expressed genes (DEGs). The statistical analyses included a Wald test and p-value correction based on the false discovery rate (FDR). Only transcripts with fold changes >2 and FDR-corrected p-values <0.05 were used for further analyses. Functional enrichment analysis was performed using the Bioconductor package, clusterprofiler 4.0 [39]. The outputs were visualized using Enrichplot and the ggplot2 package. The outputs were visualized using Enrichplot and ggplot2 package. Hierarchical clustering of relative expression values according to Euclidean distance was conducted to create the heatmap, which allowed us to visualize expressional differences and cluster the genes. Venn diagrams were made for further comparisons against each control to determine the number of significantly expressed genes shared or unique among the two MPs treated groups. Unique genes from each experimental step were identified, and their involvement in biological processes was investigated using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database.

The transcripts were further interpreted using KEGG database [40] to retrieve additional information. Gene ontology (GO) terms were assigned to the DEGs and categorized into "Biological process (BP)," "Cellular component (CC)," or "Molecular function (MF)." GO terms of pvalue <0.05 were considered significantly enriched.

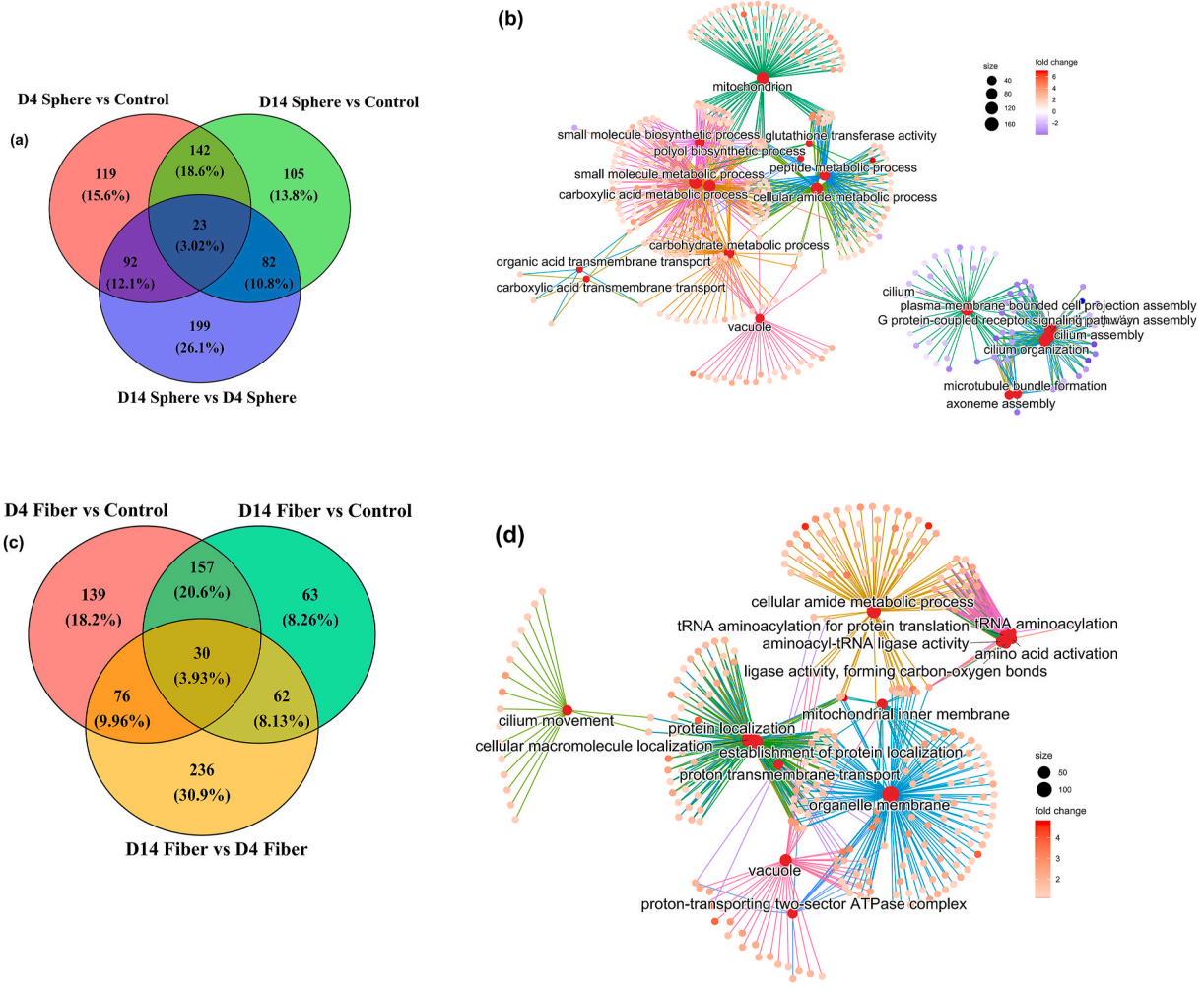


Fig. 2. Venn diagram showing the significant unique and shared DEGs between compared treatment groups and control. Regulated gene sets related (a) within sphere treatment groups vs. control. (b) A cnet plot of gene sets (P < 0.05, FDR <0.05) networking the biological processes. (c) within fiber treatment groups vs. control. (d) A cnet plot of gene sets (P < 0.05, FDR <0.05) networking the biological processes.

3. Results

3.1. Transcriptome assembly and differential gene expression

RNA-seq experiments were conducted to investigate the effect of MPs exposure on gene expression in *M. galloprovincialis*. 21 libraries were constructed from cDNA fragments. The raw data were processed for quality and accuracy, resulting in approximately 116.9–128.5 million reads per set, with over 75 million clean reads in each data set. The D4 sphere samples had 4793 upregulated genes and 3399 downregulated genes, while the D14 set had 3048 upregulated genes and 2669 downregulated genes. For the fiber MPs treatment groups, we identified 3870 upregulated and 2838 downregulated DEGs in the D4 set, and 3459 upregulated and 2937 downregulated DEGs in the D14 set. Furthermore, 4181 upregulated and 4951 downregulated DEGs in the sphere MPs exposure set and 3117 upregulated and 3780 downregulated DEGs in the fiber MPs exposure group were identified by comparison of D14 vs. D4 treatment groups (Fig. S1).

3.2. Gene ontology and functional annotation of DEGs

The GO analysis of the whole transcriptome showed that the different gene expression in response to MPs exposure has notable effect on how the organism works, as shown in Fig. 1. The CC category classified 45 % of the genes. Over 1000 DEGs were classified as membrane components in CC, followed by cytoplasm and nucleus components with 30 % genes. The remaining genes were assigned to BP and MF, with 28 % and 27 %, respectively. Further analysis under BP showed >100 DEGs related to protein modification, transcriptional regulation, proteolysis, and RNA processing. Approximately 50–100 genes were related to metabolic function, signal transduction, and signaling pathways. Regarding MF, the highest number of DEGs represented metal ion binding, transferase, and hydrolase activity. Additionally, 50–100 genes were assigned to calcium ion binding, oxidoreductase, and kinase activity.

3.3. Comparison between different time points and overlap of DEGs

Sphere and fiber MPs had a more significant impact on unique gene expression in the D4 samples compared to the D14 and control groups (Fig. 2). Specifically, spherical MPs affected 15.6 % of unique genes in D4 and 13.8 % in D14. On the other hand, fiber MPs affected 18.2 % of DEGs in D4 and 8.26 % in D14. The study found that the D4 and D14 sphere samples shared 142 genes. These genes were linked to metabolic processes and membrane-based cell components. Moreover, the exposure to fiber MPs found around 157 common genes that regulate metabolic processes.

The variation of gene regulation in D14 compared to D4 as a lasting effect revealed that sphere MPs affected 199 unique genes, while fiber MPs affected 236 unique genes in the D14 sample. The comparison of combinations consolidated 23 significant genes in sphere MPs and 30 in the fiber MPs treatment samples. The assigned GO as a network revealed that these significant genes involved metabolic processes and mitochondrion component functions. Furthermore, the findings showed a variation of DEGs between the sphere and fiber MPs effects regarding signaling effects, cilium, cytoskeletal components, and metabolic processes. Finally, 9–10 % of genes were shared DEGs between time points D4 and D14, contributing to the expression variation for a prolonged duration.

3.4. GO analysis imparting the effectual response

The annotated terms were grouped into functional units based on GO classes. The regulated DEGs caused significant changes in the organism, as shown in Fig. 3. Exposure to MPs led to substantial alterations in molecular regulation, protein modification, DNA

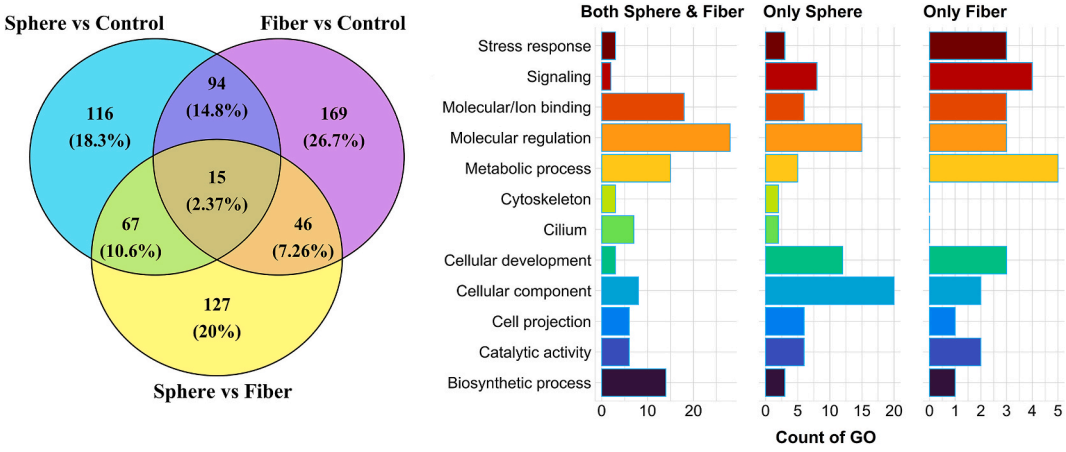


Fig. 3. Venn diagram shows the number of significant DEGs on MPs exposure for up to 14 days. Gene sets related to treatment groups vs. control and between treatment groups; the bar diagram represents the biological processes corresponding to different GO classes.

transcription, and RNA processing. Positive and negative regulation were evident in 24 % of common GOs and >10 % of unique GOs. Under the metabolic process, significant GO classes included function at cellular and biomolecular levels (DNA, RNA, protein, and peptide). The biosynthetic processes of organic cyclic compounds, cellular nitrogen compounds, nucleobase-containing compounds, and lipid biosynthesis were also identified as clusters, accounting for 12 % of common GO terms and over 3 % of unique GOs. The molecular binding category comprised 15 % of the GO classes, including ion binding, signal receptor binding, tubulin binding, purine nucleotides, metal ions, and ATPs. These observations revealed that GO categories, such as stress response, signaling, and cellular components, significantly impacted a sequential process. GO classes categorized under cellular projection, cytoskeleton, cilium components, and cellular development accounted for >3 % of the total GOs.

3.5. Functional GO enrichment analysis of DEGs

Gene set enrichment analysis (GSEA) explored the toxic effects and stress that impacted biological regulations (Fig. 4). In the spherical MPs treatment group compared to the control , enriched genes showed that they slowed down energy production, methyl transferase, lysosomes, and controlling development. In contrast, cytoskeletal function (BP), ion transporter activity (MF), cilium component organization (CC), and movement were activated. The initial exposure (D4) significantly activated metabolic processes and catalytic activity, while suppressing cilium and cell projection. However, an increased intake of MPs in D14 revealed activated cilium assembly, cell projection assembly, and axonemal assembly.

In organisms exposed to fiber MPs, they activated inorganic ion homeostasis, signaling, glutamine, and phosphorus metabolic processes (BP), and suppressed the cell cycle process, cellular response to damage stimulus, DNA repair (BP), and catalytic activity (MF). During initial exposure, terms related to heterocyclic compound biosynthesis, RNA metabolic processes, and regulation of cellular metabolic processes (BP) were suppressed. Simultaneously, the activation of cilium movement (CC) and membrane transport (MF) occurred. Continuous exposure suppressed the organization and assembly of cilia, the activation of mitochondrial parts (the inner membrane and envelope), and the activity of glutathione transferase.

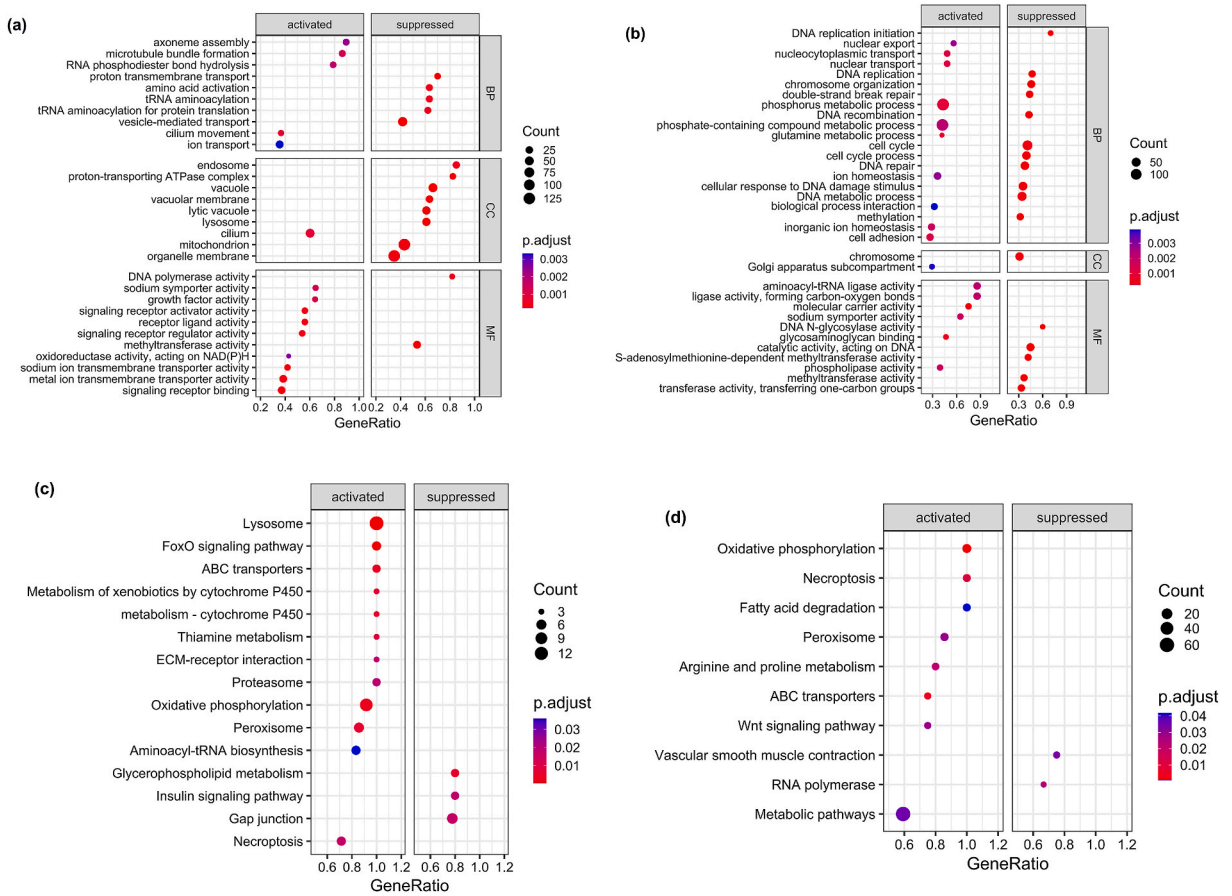


Fig. 4. (a) GO analysis of genes differentially expressed indicates the activated and suppressed biological processes in mussels exposed to spherical MPs. (b) GO analysis of genes differentially expressed shows the activated and suppressed biological processes in mussels exposed to fiber MPs. (c) KEGG analysis of DEGs indicates the activated and suppressed biological processes in the spherical MPs treatment group. (d) KEGG analysis of DEGs shows the activated and suppressed biological processes in the fiber MPs treatment group.

3.6. Functional KEGG enrichment analysis of DEGs

Mapping the DEGs to the KEGG database allowed us to identify enriched functional pathways (Fig. 4). The exposure to sphere MPs had a significant impact on several pathways, including the lysosome, oxidative phosphorylation, FoxO signaling pathway, ABC transporters, necroptosis, and cytochrome metabolism. Interestingly, the MPs suppressed the insulin pathway and Gap junction. On the other hand, exposure to fiber MPs activated metabolic pathways, oxidative phosphorylation, necroptosis, ABC transporters, peroxisome, and the Wnt signaling pathway. However, the comparatively low gene ratio significantly suppressed vascular muscle contraction.

3.7. Clustering of DEGs resembling efficient regulation

The gene expression pattern of the DEGs was analyzed to understand their protein interaction function and ability to respond to the toxic effects of MPs. Fig. 5 depicts the resulting clustering analysis. Notably, DEGs expressed in the protein function of the signaling response included neuronal calcium sensor 1 (NCS1), calcium/calmodulin dependent protein kinase (CAMK), regulator of g-protein signaling (RGS), NOTCH1, mitogen-activated protein kinases (MAPK), and STATs (signal transducers and activators of transcription).

The clustering of heat shock protein 70 (HSP70), heat shock protein 90 (HSP90), and cysteine-rich peptides (Myticin) indicated the stress response prevailed in mussels. Additionally, the significant genes involved in the induction and regulation of apoptotic function were identified. The former category genes are apoptosis-inducing factor (AIF), cysteine-aspartic acid protease (Caspase), and programmed cell death protein 1 (PDCD1). And the regulating genes are B-cell lymphoma 2 (BCL-2), BCL-2 associated X (BAX), P53, and bifunctional apoptosis regulator (BAR). The significant antioxidant response elements (ARE) due to MP toxicity represented the functions of cytochrome P450 (CYP), glutathione S-transferase (GST), glutathione peroxidase (GPX), superoxide dismutase (SOD), and catalase (CAT).

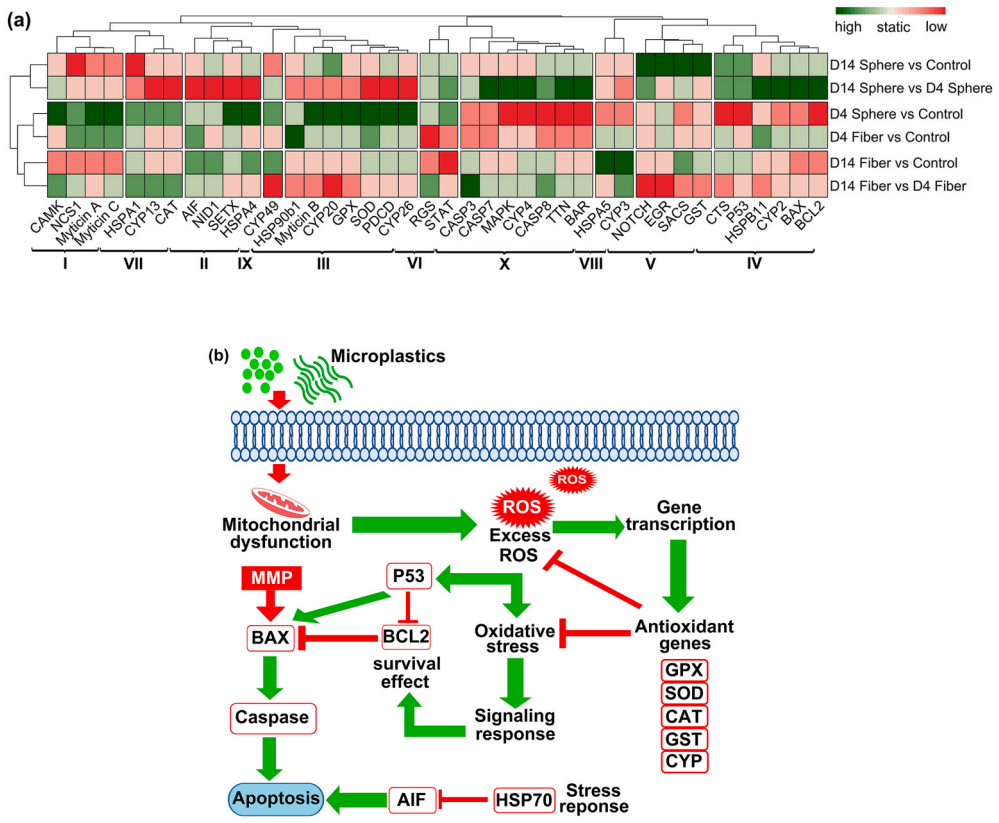


Fig. 5. (a) Heatmap shows the relative expression of the significant genes in the treatment groups; (b) Schematic representation of biological responses in the treatment groups exposed to varying spherical and fibrous MPs concentrations. Mitochondrial impairment results in the ROS generation and regulation of caspase-dependent and caspase-independent apoptosis; accumulated ROS increases oxidative damages and down-regulated cytoprotective genes; molecular regulation of stress response proteins controls apoptosis, DNA repair and regulate gene transcription to initiate survival responses and recovery.

4. Discussion

The study revealed that MPs have a significant impact on various biological responses. The research showed that spherical and fibrous MPs have distinctive regulatory effects and recovery. The transcriptomic analysis of *M. galloprovincialis* highlighted that the toxicity and bioavailability of MPs depend on their properties, including size and shape. The gradual increases in MPs in the system affected DEGs, highlighting the negative impact of MPs on biological systems. The DEGs indicated regulations related to cellular processes such as ciliary assembly, signal transduction, ion homeostasis, stress response, antioxidant mechanisms, and cellular recovery.

4.1. Ecological relevance to the exposed MPs concentration

The study's use of MPs at toxic levels in aquatic habitats, as noted by Alfaro-Núñez et al. [41], Phuong et al. [42] posed a threat to the survival of mussels. The observed gene expression was in line with ecologically plausible bioaccumulation, as suggested by Lim [43]. The gradual increase in MP concentration reflected the natural environment. Kotta et al. [44] noted that this approach is ideal for obtaining precise transcriptomic profiles with varying regulations to recover from lethal effects.

4.2. Cilia function related to exposed MPs content

The impact of MPs on the mussels was significant, especially during initial exposure, when the synergy of mediated stress effects was at its highest. The study confirmed that DEGs played a crucial role in counteracting the toxic effects of MPs. Ingested MPs affect animals' growth and development, energy cycles, oxidative stress, immune responses, and genotoxicity [45,46]. Continuous exposure suppressed the cytoskeletal assembly (axoneme, microtubule) and cilium-related development processes.

The cilia beat frequency and movement in mussels were indicators of physical particle penetration and stress. According to Meseck et al. [47], signs of stress are created by the interruption of function and abnormal effects of ingestion through the gills. The stress impulses from this point were majorly responsive to oxidative stress, signaling, and apoptotic activation in *M. galloprovincialis*, as Wang et al. [48] reported. The difference in activated ion transport, signal transduction, and suppressed homeostasis is the cognitive stress impact due to accumulated levels of MPs through gills interfering in the nervous system's control [49]. In addition, MPs increase oxidative stress in mussels through adhesion to the soft tissues [15,50]. Indeed, the maximal stress levels and declined activities due to fiber MPs were observed.

4.3. Signaling and immune response in MPs stress

The initial stages of MP exposure in the organisms demonstrated an increase in the levels of CAMK genes. CAMK controls neurotransmitter synthesis and regulates cellular processes, the cell cycle, and differentiation [51,52]. The Ca^{2+} binding proteins, members of the neuronal calcium sensor protein family, have anti-apoptotic signaling response functions involving the MAP-K signaling pathway [53,54]. The gene expression of three variant cysteine-rich antimicrobial peptides (myticin A, myticin B, and myticin C) in mussels are known for chemotactic regulation [55]. Mussels have a highly complex immune process that helps them defend against stress. Venier et al. [52] have reported that controlled levels of myticin protein variants during initial exposure are highly protective against pathogenic intervention that many other organisms fail at increased stress levels. The level of myticin C in fibrous MP treated groups was a crucial indicator withstanding the stress caused by effective ROS during the delay in recovery, as Domeneghetti et al. [56] suggested.

4.4. ROS-mediated induction of apoptosis and stress proteins in mussels

As the concentration of MPs increased, the generation of ROS led to apoptosis through caspase-dependent and caspase-independent effects [57,58]. This type of apoptosis occurs typically due to continual stress, which helps the mussels maintain internal stability and adapt to variations. It was observed that fiber MPs-treated samples followed the AIF pathway, caspase-independent apoptotic pathway in addition to caspase-dependent apoptosis involving caspase-3 and caspase-7 genes recorded in both spherical and fibrous MPs-treated mussels. ROS-mediated apoptotic pathways via P53 and BAX genes promote the activation of caspase/CASP3, causing oxidative stress and stress protein regulation [10,19].

HSP90 proteins have a significant role in regulating various cellular signaling network activities. The presence of the P53 binding site in the 5′ regulatory region is crucial in determining apoptosis regulation [59]. In the case of mussels, Heat Shock Proteins (HSPs) were the monitoring factors since P53 proteins are also involved in essential cellular processes. P53-associated transcription factors directly impact critical cellular processes like the cell cycle, DNA repair, senescence, and apoptosis [60], corroborating our observations.

The level of stress-related proteins (HSPs) is a crucial indicator of stress levels in mussels (*M. galloprovincialis*). Quan et al. [61] validated the molecular efficacy of HSPs in controlling the mechanical damage resulting from the accumulation of oxidative stress and generated ROS. Furthermore, Genest et al. [62] highlighted a close link between the proliferation of HSP70 and molecular repair mechanisms that control the cell cycle and DNA damage. HSP70 regulation controls apoptosis and DNA damage in cells, while HSP90 regulates signaling network regulation. Therefore, the interruption of signal transduction was a definitive cause due to suppressed regulation of HSP90 genes. Saleh et al. [63] have mentioned that low levels of HSP90 can adversely affect growth and developmental

processes, particularly signal transduction networks. It demonstrated that mussels that ingested spherical MPs had an active HSP90 expression, while those exposed to fiber MPs had a delayed effect due to the low expression rate.

4.5. Metabolic function and energetics in association with MPs stress

The spontaneous upregulation in cellular projection, cilia movement, and cytoskeletal organization demonstrated a paramount factor in the sequential process of energy demand. The metabolic activities, i.e., the peptide and carbohydrate metabolic processes associated with development regulation, profoundly influenced mussels when exposed to spherical MPs. On the other hand, the metabolic process was activated by the regulated gene expression for cellular, macromolecular, and biosynthetic processes. These findings suggest that MPs can significantly impact the metabolic activities of mussels. As a result, mussels experienced a high level of energetic shift utilized towards counteracting physiological and oxidative damages instead of development systems. Specific observations on energy trade-offs in mussels under stress have been reported by Laura E. Petes [64]. As a fitness consequence, activation and significant enrichment of metabolic pathways are crucial for energy synthesis, metabolic activities, developmental processes, and cellular regulation to survive prolonged stress [65].

Our observations indicate a relationship between the activated metabolic processes in mussels and the effects of DNA damage and apoptotic activation. Mussels exposed to spherical MPs showed an immediate signaling effect that triggered metabolic pathways to regulate recovery. The higher stress threshold in fiber MP samples delayed a similar response and compensated for it later to counteract the relatively increasing ROS damages. The rate of metabolic functioning is highly related to the frequency of behavioral reactions in mussels exposed to continuous stress [66]. The response, however, differs depending on the type of stress and tolerance potential [67].

4.6. DEGs of antioxidant response element

Based on our analysis, the cluster-based relationship was predicted between various regulatory DEGs and their molecular function in MP toxicity. Our findings showed that the enriched genes in each cluster (shown in Fig. 5) were closely associated with signaling response, stress response, and molecular regulation, which positively affected cell survival. The upregulation of cytoprotective genes such as SOD, CAT, GPX, and GST played a crucial role in this mechanism. In addition, the proliferation of ARE is an essential biomarker of the oxidative stress response. This mechanism protects cells from toxic pollutants [68].

The expression variation of antioxidant-related genes indicated oxidative damage due to MPs toxicity. Treatment groups significantly altered the SOD, CAT, GPX, and GST genes, with CAT and SOD showing upregulation throughout the experiment period. In contrast, these gene regulations were stable with an elevated response to GST due to spherical MPs. This observation corroborates the findings of Détrée and Gallardo-Escárate [30] and Liu et al. [69]. The antioxidant responses were used as biomarkers in assessing the effects of pollutants in *M. galloprovincialis* [70]. Oxidative stress is also associated with inflammatory processes such as epithelial disruption and necroptosis, a regulated mode of inflammatory cell death with features of apoptosis and necrosis [71]. Both treatment groups significantly enriched necroptosis, highlighting the significance of upregulating cytoprotective genes.

4.7. Molecular regulation variability and functional reversibility

The process of molecular transformations is crucial in downregulating the growth and development process when there is an increase in stress intensity. The physical effects of detoxification were an apparent response to the differing concentrations affected by the two MPs. The stress effects of fiber MPs resulted in compensation strategies, such as metabolic pathways to regulate metabolism and energetics due to severe stress damage. The bifunctional apoptosis regulator (BAR) was responsible for the MP treatment groups' adaptation and cell survival response. The BAR genes induced caspase8/CASP8 and inhibited CASP3 [72]. Certainly, the expression of anti-apoptotic BCL-2 proteins and mitogen-activated protein kinase (MAPK) mediated the control of apoptosis [73]. These genes regulated deformities in the cellular process and were responsible for the adaptation and cell survival responses in the MPs treatment groups.

The physiological function of mussels was disturbed and damaged, which disrupted signaling networks, development, and homeostasis. Cilial impairment, metabolic imbalances, oxidative damage, and high energy demand were common issues among the affected mussels. However, it was interesting to note the differences between the two groups regarding molecular regulation, the immediacy of survival, and recovery patterns. Stress response genes and immune genes played a crucial role in enhancing the adaptive changes in these mussels, while antioxidant genes helped regulate the potential for survival. The mussels exposed to sphere MPs recovered due to impulsive metabolic and energy factors that mediated the survival effect. On the other hand, the mussels exposed to fiber MPs had a delayed defense mechanism, necessitating additional energy compensation strategies to reconcile metabolic pathways and functional reversibility.

5. Conclusions

Transcriptomic analyses of *M. galloprovincialis* have demonstrated the biological responses and molecular changes in response to toxic contaminants like MPs. This study focused on the toxicity of MPs in mussels, as polyethylene is a prevalent polymer found in their food. The severity of cellular stress causing cellular damage was higher in the fibrous MP treatment groups. Furthermore, the physical impact of MPs significantly impacted the axoneme assembly and movement of cilia, leading to a direct effect on the function of the nervous system.

The cell survival effect in mussels exposed to spherical MPs was effective in overcoming oxidative damage. However, the counteract was insufficient to overcome the physiological stress induced by fiber MP particles, and the recovery was delayed due to prolonged stimulus and suppressed metabolism in mussels. Regulatory metabolic pathways attempted a compensatory mechanism, which delayed functional reversibility. In conclusion, the study highlights the importance of investigating the toxic effects of MPs on aquatic habitats and their impact on the marine life that relies on them. The results provide crucial insights into the molecular changes and biological responses of mussels to toxic contaminants and emphasize the need for effective management of pollutants in aquatic habitats.

Funding

This study was supported by the National Research Foundation of Korea, South Korea, which the Korean Government funds [NRF-2018-R1A6A1A-03024314], by Korea Environment Industry & Technology Institute (KEITI) through Aquatic Ecosystem Conservation Research Program, funded by Korea Ministry of Environment (MOE) (2021003050001), and "Risk assessment to prepare standards for protecting marine ecosystem" of Korea Institute of Marine Science & Technology Promotion (KIMST) funded by the Ministry of Oceans and Fisheries (KIMST-20220383).

Institutional review board statement

The study was conducted according to the Chonnam National University Institutional Animal Care and Use Committee guidelines.

Informed consent statement

Not applicable.

Consent to publish

All authors approved the manuscript and give their consent for submission and publication.

Data availability statement

There is no research related data stored in publicly available repositories, and the data will be made available on request.

CRediT authorship contribution statement

Boobal Rangaswamy: Writing – review & editing, Writing – original draft, Visualization, Data curation. **Jinsung An:** Methodology, Investigation, Formal analysis, Data curation. **Ihn-Sil Kwak:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, 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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e30858.

References

[1] S.S. Sana, L.K. Dogiparthi, L. Gangadhar, A. Chakravorty, N. Abhishek, Effects of microplastics and nanoplastics on marine environment and human health, Environ. Sci. Pollut. Res. 27 (2020) 44743–44756, https://doi.org/10.1007/s11356-020-10573-x.

[2] M. González-Pleiter, C. Edo, Á. Aguilera, D. Viúdez-Moreiras, G. Pulido-Reyes, E. González-Toril, S. Osuna, G. de Diego-Castilla, F. Leganés, F. Fernández-Piñas, R. Rosal, Occurrence and transport of microplastics sampled within and above the planetary boundary layer, Sci. Total Environ. 761 (2021) 143213, https://doi.org/10.1016/j.scitotenv.2020.143213.

[3] S. Rezania, J. Park, M.F. Md Din, S. Mat Taib, A. Talaiekhozani, K. Kumar Yadav, H. Kamyab, Microplastics pollution in different aquatic environments and biota: a review of recent studies, Mar. Pollut. Bull. 133 (2018) 191–208, https://doi.org/10.1016/j.marpolbul.2018.05.022.

[4] S. Rist, A. Vianello, M.H.S. Winding, T.G. Nielsen, R. Almeda, R.R. Torres, J. Vollertsen, Quantification of plankton-sized microplastics in a productive coastal Arctic marine ecosystem, Environ. Pollut. 266 (2020) 115248, https://doi.org/10.1016/j.envpol.2020.115248.

[5] S.E. Nelms, J. Barnett, A. Brownlow, N.J. Davison, R. Deaville, T.S. Galloway, P.K. Lindeque, D. Santillo, B.J. Godley, Microplastics in marine mammals stranded around the British coast: ubiquitous but transitory? Sci. Rep. 9 (2019) 1–8, https://doi.org/10.1038/s41598-018-37428-3.

[6] C.J. Thiele, M.D. Hudson, A.E. Russell, M. Saluveer, G. Sidaoui-Haddad, Microplastics in fish and fishmeal: an emerging environmental challenge? Sci. Rep. 11 (2021) 1–12, https://doi.org/10.1038/s41598-021-81499-8.

[7] N.A.C. Welden, P.R. Cowie, Environment and gut morphology influence microplastic retention in langoustine, Nephrops norvegicus, Environ. Pollut. 214 (2016) 859–865, https://doi.org/10.1016/j.envpol.2016.03.067.

[8] M. Cole, P. Lindeque, E. Fileman, C. Halsband, R. Goodhead, J. Moger, T.S. Galloway, Microplastic ingestion by zooplankton, Environ. Sci. Technol. 47 (2013) 6646–6655, https://doi.org/10.1021/es400663f.

[9] S.L. Wright, R.C. Thompson, T.S. Galloway, The physical impacts of microplastics on marine organisms: a review, Environ. Pollut. 178 (2013) 483–492, https://doi.org/10.1016/j.envpol.2013.02.031.

[10] G. Limonta, A. Mancia, A. Benkhalqui, C. Bertolucci, L. Abelli, M.C. Fossi, C. Panti, Microplastics induce transcriptional changes, immune response and behavioral alterations in adult zebrafish, Sci. Rep. 9 (2019) 1–11, https://doi.org/10.1038/s41598-019-52292-5.

[11] G. Balakrishnan, M. Déniel, T. Nicolai, C. Chassenieux, F. Lagarde, Towards more realistic reference microplastics and nanoplastics: preparation of polyethylene micro/nanoparticles with a biosurfactant, Environ. Sci.: Nano 6 (2019) 315–324, https://doi.org/10.1039/C8EN01005F.

[12] H. Yu, M. Liu, D. Gang, J. Peng, C. Hu, J. Qu, Polyethylene microplastics interfere with the nutrient cycle in water-plant-sediment systems, Water Res. 214 (2022) 118191, https://doi.org/10.1016/j.watres.2022.118191.

[13] R. Gautam, J.H. Jo, M. Acharya, A. Maharjan, D.E. Lee, P.B. Pramod, C.Y. Kim, K.S. Kim, H.A. Kim, Y. Heo, Evaluation of potential toxicity of polyethylene microplastics on human derived cell lines, Sci. Total Environ. 838 (2022) 156089, https://doi.org/10.1016/j.scitotenv.2022.156089.

[14] C. Zhang, L. Zhang, L. Li, M. Mohsen, F. Su, X. Wang, C. Lin, RNA sequencing provides insights into the effect of dietary ingestion of microplastics and cadmium in the sea cucumber Apostichopus japonicus, Front. Mar. Sci. 10 (2023) 1–16, https://doi.org/10.3389/fmars.2023.1109691.

[15] P. Kolandhasamy, L. Su, J. Li, X. Qu, K. Jabeen, H. Shi, Adherence of microplastics to soft tissue of mussels: a novel way to uptake microplastics beyond ingestion, Sci. Total Environ. 610–611 (2018) 635–640, https://doi.org/10.1016/j.scitotenv.2017.08.053.

[16] M. Hong, S. Tao, L. Zhang, L.T. Diao, X. Huang, S. Huang, S.J. Xie, Z.D. Xiao, H. Zhang, RNA sequencing: new technologies and applications in cancer research, J. Hematol. Oncol. 13 (2020) 1–16, https://doi.org/10.1186/s13045-020-01005-x.

[17] N. Gao, Z. Huang, J. Xing, S. Zhang, J. Hou, Impact and molecular mechanism of microplastics on zebrafish in the presence and absence of copper nanoparticles, Front. Mar. Sci. 8 (2021) 1–13, https://doi.org/10.3389/fmars.2021.762530.

[18] G. De Marco, G.O. Conti, A. Giannetto, T. Cappello, M. Galati, C. Iaria, E. Pulvirenti, F. Capparucci, A. Mauceri, M. Ferrante, M. Maisano, Embryotoxicity of polystyrene microplastics in zebrafish Danio rerio, Environ. Res. 208 (2022) 112552, https://doi.org/10.1016/j.envres.2021.112552.

[19] S. Umamaheswari, S. Priyadarshinee, K. Kadirvelu, M. Ramesh, Polystyrene microplastics induce apoptosis via ROS-mediated p53 signaling pathway in zebrafish, Chem. Biol. Interact. 345 (2021) 109550, https://doi.org/10.1016/j.cbi.2021.109550.

[20] G. Liu, R. Jiang, J. You, D.C.G. Muir, E.Y. Zeng, Microplastic impacts on microalgae growth: effects of size and humic acid, Environ. Sci. Technol. 54 (2020) 1782–1789, https://doi.org/10.1021/acs.est.9b06187.

[21] M. He, M. Yan, X. Chen, X. Wang, H. Gong, W. Wang, J. Wang, Bioavailability and toxicity of microplastics to zooplankton, Gondwana Res. 108 (2022) 120–126, https://doi.org/10.1016/j.gr.2021.07.021.

[22] Y. Wang, D. Zhang, M. Zhang, J. Mu, G. Ding, Z. Mao, Y. Cao, F. Jin, Y. Cong, L. Wang, W. Zhang, J. Wang, Effects of ingested polystyrene microplastics on brine shrimp, Artemia parthenogenetica, Environ. Pollut. 244 (2019) 715–722, https://doi.org/10.1016/j.envpol.2018.10.024.

[23] Z. Bai, N. Wang, M. Wang, Effects of microplastics on marine copepods, Ecotoxicol. Environ. Saf. 217 (2021) 112243, https://doi.org/10.1016/j.ecoenv.2021.112243.

[24] I. Paul-Pont, C. Lacroix, C. González Fernández, H. Hégaret, C. Lambert, N. Le Goïc, L. Frère, A.-L. Cassone, R. Sussarellu, C. Fabioux, J. Guyomarch, M. Albentosa, A. Huvet, P. Soudant, Exposure of marine mussels Mytilus spp. to polystyrene microplastics: toxicity and influence on fluoranthene bioaccumulation, Environ. Pollut. 216 (2016) 724–737, https://doi.org/10.1016/j.envpol.2016.06.039.

[25] L.L. Li, R. Amara, S. Souissi, A. Dehaut, G. Duflos, S. Monchy, Impacts of microplastics exposure on mussel (Mytilus edulis) gut microbiota, Sci. Total Environ. 745 (2020), https://doi.org/10.1016/j.scitotenv.2020.141018.

[26] L. Van Cauwenberghe, M. Claessens, M.B. Vandegehuchte, C.R. Janssen, Microplastics are taken up by mussels (Mytilus edulis) and lugworms (Arenicola marina) living in natural habitats, Environ. Pollut. 199 (2015) 10–17, https://doi.org/10.1016/j.envpol.2015.01.008.

[27] F. Provenza, D. Rampih, S. Pignattelli, P. Pastorino, D. Barceló, M. Prearo, A. Specchiulli, M. Renzi, Mussel watch program for microplastics in the Mediterranean sea: identification of biomarkers of exposure using Mytilus galloprovincialis, Ecol. Indic. 142 (2022), https://doi.org/10.1016/j.ecolind.2022.109212.

[28] C.J. O'Brien, H.C. Hong, E.E. Bryant, K.M. Connor, The observation of starch digestion in blue mussel Mytilus galloprovincialis exposed to microplastic particles under varied food conditions, PLoS One 16 (2021) 1–10, https://doi.org/10.1371/journal.pone.0253802.

[29] D. Pavičić-Hamer, I. Kovačić, T. Sović, M. Marelja, D.M. Lyons, Exposure to polymethylmethacrylate microplastics induces a particle size-dependent immune response in mediterranean mussel Mytilus galloprovincialis, Fishes 7 (2022), https://doi.org/10.3390/fishes7060307.

[30] C. Détrée, C. Gallardo-Escárate, Single and repetitive microplastics exposures induce immune system modulation and homeostasis alteration in the edible mussel Mytilus galloprovincialis, Fish Shellfish Immunol. 83 (2018) 52–60, https://doi.org/10.1016/j.fsi.2018.09.018.

[31] J.S. Choi, K. Kim, S.H. Hong, K. Il Park, J.W. Park, Impact of polyethylene terephthalate microfiber length on cellular responses in the Mediterranean mussel Mytilus galloprovincialis, Mar. Environ. Res. 168 (2021) 105320, https://doi.org/10.1016/j.marenvres.2021.105320.

[32] A.M. Bolger, M. Lohse, B. Usadel, Trimmomatic: a flexible trimmer for Illumina sequence data, Bioinformatics 30 (2014) 2114–2120, https://doi.org/10.1093/bioinformatics/btu170.

[33] B. Langmead, S.L. Salzberg, Fast gapped-read alignment with Bowtie 2, Nat. Methods 9 (2012) 357–359, https://doi.org/10.1038/nmeth.1923.

[34] D. Kim, B. Langmead, S.L. Salzberg, HISAT: a fast spliced aligner with low memory requirements Daehwan HHS Public Access, Nat. Methods 12 (2015) 357–360, https://doi.org/10.1038/nmeth.3317.HISAT.

[35] M. Pertea, G.M. Pertea, C.M. Antonescu, T.C. Chang, J.T. Mendell, S.L. Salzberg, StringTie enables improved reconstruction of a transcriptome from RNA-seq reads, Nat. Biotechnol. 33 (2015) 290–295, https://doi.org/10.1038/nbt.3122.

[36] M.I. Love, S. Anders, V. Kim, W. Huber, RNA-Seq workflow: gene-level exploratory analysis and differential expression, F1000Research 4 (2016), https://doi.org/10.12688/f1000research.7035.2.

[37] RCore Team, R: A Language and Environment for Statistical Computing, 2022. https://www.r-project.org/.

[38] M.I. Love, W. Huber, S. Anders, Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2, Genome Biol. 15 (2014) 1–21, https://doi.org/10.1186/s13059-014-0550-8.

[39] T. Wu, E. Hu, S. Xu, M. Chen, P. Guo, Z. Dai, T. Feng, L. Zhou, W. Tang, L. Zhan, X. Fu, S. Liu, X. Bo, G. Yu, clusterProfiler 4.0: a universal enrichment tool for interpreting omics data, Innovation 2 (2021) 100141, https://doi.org/10.1016/j.xinn.2021.100141.

[40] M. Kanehisa, KEGG: Kyoto Encyclopedia of genes and genomes, Nucleic Acids Res. 28 (2000) 27–30, https://doi.org/10.1093/nar/28.1.27.

[41] A. Alfaro-Núñez, D. Astorga, L. Cáceres-Farías, L. Bastidas, C. Soto Villegas, K. Macay, J.H. Christensen, Microplastic pollution in seawater and marine organisms across the tropical eastern pacific and galápagos, Sci. Rep. 11 (2021) 1–8, https://doi.org/10.1038/s41598-021-85939-3.

[42] N.N. Phuong, A. Zalouk-Vergnoux, L. Poirier, A. Kamari, A. Châtel, C. Mouneyrac, F. Lagarde, Is there any consistency between the microplastics found in the field and those used in laboratory experiments? Environ. Pollut. 211 (2016) 111–123, https://doi.org/10.1016/j.envpol.2015.12.035.

[43] X.Z. Lim, Microplastics are everywhere - but are they harmful? Nature 593 (2021) 22–25, https://doi.org/10.1038/d41586-021-01143-3.

[44] J. Kotta, M. Lenz, F.R. Barboza, H. Jänes, P.A.D. Grande, A. Beck, C. Van Colen, T. Hamm, J. Javidpour, A. Kaasik, G. Pantó, R. Szava-Kovats, H. Orav-Kotta, L. Lees, S. Loite, J. Canning-Clode, S.K.M. Gueroun, A. Kõivupuu, Blueprint for the ideal microplastic effect study: critical issues of current experimental approaches and envisioning a path forward, Sci. Total Environ. 838 (2022), https://doi.org/10.1016/j.scitotenv.2022.156610.

[45] M. Pang, Y. Wang, Y. Tang, J. Dai, J. Tong, G. Jin, Transcriptome sequencing and metabolite analysis reveal the toxic effects of nanoplastics on tilapia after exposure to polystyrene, Environ. Pollut. 277 (2021) 116860, https://doi.org/10.1016/j.envpol.2021.116860.

[46] X.W. Tang, R. Yu, M.H. Fan, Z. Yang, Z. Liao, Z.X. Yang, C.Y. Xie, Y.K. Xuan, J.X. Wang, X.L. Zhang, X.J. Yan, Physiological and transcriptome analysis of Mytilus coruscus in response to Prorocentrum lima and microplastics, Front. Mar. Sci. 9 (2022) 1–17, https://doi.org/10.3389/fmars.2022.1087667.
[47] S.L. Meseck, G. Sennefelder, M. Krisak, G.H. Wikfors, Physiological feeding rates and cilia suppression in blue mussels (Mytilus edulis) with increased levels of dissolved carbon dioxide, Ecol. Indic. 117 (2020) 106675, https://doi.org/10.1016/j.ecolind.2020.106675.
[48] J. Wang, R.M. Ren, C.L. Yao, Oxidative stress responses of Mytilus galloprovincialis to acute cold and heat during air exposure, J. Molluscan Stud. 84 (2018) 285–292, https://doi.org/10.1093/mollus/eyy027.
[49] M.A. Carroll, E.J. Catapane, The nervous system control of lateral ciliary activity of the gill of the bivalve mollusc, Crassostrea virginica, Comp. Biochem. Physiol. Mol. Integr. Physiol. 148 (2007) 445–450, https://doi.org/10.1016/j.cbpa.2007.06.003.
[50] E.T.F. Freitas, A.M.S. Moreira, R.S. de Paula, G.R. Andrade, M.D. de Carvalho, P.S. Assis, E.C. Jorge, A.V. Cardoso, Ultrastructure of the gill ciliary epithelium of Limnoperna fortunei (Dunker 1857), the invasive golden mussel, BMC Zool. 7 (2022) 1–14, https://doi.org/10.1186/s40850-022-00107-y.
[51] J.A.P. Rostas, K.A. Skelding, Calcium/calmodulin-stimulated protein kinase II (CaMKII): different functional outcomes from activation, depending on the cellular microenvironment, Cells 12 (2023), https://doi.org/10.3390/cells12030401.
[52] P. Venier, L. Varotto, U. Rosani, C. Millino, B. Celegato, F. Bernante, G. Lanfranchi, B. Novoa, P. Roch, A. Figueras, A. Pallavicini, Insights into the innate immunity of the Mediterranean mussel Mytilus galloprovincialis, BMC Genom. 12 (2011), https://doi.org/10.1186/1471-2164-12-69.
[53] K.I. Nagata, A. Puls, C. Futter, P. Aspenstrom, E. Schaefer, T. Nakata, N. Hirokawa, A. Hall, The MAP kinase kinase kinase MLK2 co-localizes with activated JNK along microtubules and associates with kinesin superfamily motor KIF3, EMBO J. 17 (1998) 149–158, https://doi.org/10.1093/emboj/17.1.149.
[54] R.D. Burgoyne, Neuronal calcium sensor proteins: generating diversity in neuronal Ca 2+ signalling, Nat. Rev. Neurosci. 8 (2007) 182–193, https://doi.org/10.1038/nrn2093.
[55] A. Padhi, B. Verghese, Molecular diversity and evolution of myticin-C antimicrobial peptide variants in the Mediterranean mussel, Mytilus galloprovincialis, Peptides 29 (2008) 1094–1101, https://doi.org/10.1016/j.peptides.2008.03.007.
[56] S. Domeneghetti, M. Franzoi, N. Damiano, R. Norante, N.M. El Halfawy, S. Mammi, O. Marin, M. Bellanda, P. Venier, Structural and antimicrobial features of peptides related to myticin C, a special defense molecule from the mediterranean mussel Mytilus galloprovincialis, J. Agric. Food Chem. 63 (2015) 9251–9259, https://doi.org/10.1021/acs.jafc.5b03491.
[57] T.J. Lee, E.J. Kim, S. Kim, E.M. Jung, J.W. Park, S.H. Jeong, S.E. Park, Y.H. Yoo, T.K. Kwon, Caspase-dependent and caspase-independent apoptosis induced by evodiamine in human leukemic U937 cells, Mol. Cancer Ther. 5 (2006) 2398–2407, https://doi.org/10.1158/1535-7163.MCT-06-0167.
[58] Y. Shi, Mechanisms of caspase activation and inhibition during apoptosis, Mol. Cell. 9 (2002) 459–470, https://doi.org/10.1016/S1097-2765(02)00482-3.
[59] C.N. Pantzartzi, A. Kourtidis, E. Drosopoulou, M. Yiangou, Z.G. Scouras, Isolation and characterization of two cytoplasmic hsp90s from Mytilus galloprovincialis (Mollusca: Bivalvia) that contain a complex promoter with a p53 binding site, Gene 431 (2009) 47–54, https://doi.org/10.1016/j.gene.2008.10.028.
[60] B.J. Aubrey, G.L. Kelly, A. Janic, M.J. Herold, A. Strasser, How does p53 induce apoptosis and how does this relate to p53-mediated tumour suppression? Cell Death Differ. 25 (2018) 104–113, https://doi.org/10.1038/cdd.2017.169.
[61] Y. Quan, Z. Wang, H. Wei, K. He, Transcription dynamics of heat shock proteins in response to thermal acclimation in Ostrinia furnacalis, Front. Physiol. 13 (2022) 1–10, https://doi.org/10.3389/fphys.2022.992293.
[62] O. Genest, S. Wickner, S.M. Doyle, Hsp90 and Hsp70 chaperones: collaborators in protein remodeling, J. Biol. Chem. 294 (2019) 2109–2120, https://doi.org/10.1074/jbc.REV118.002806.
[63] M. Saleh, A.-A.S. Abdel-Baki, M.A. Dkhil, M. El-Matbouli, S. Al-Quraishy, Silencing of heat shock protein 90 (hsp90): effect on development and infectivity of Ichthyophthirius multifiliis, BMC Vet. Res. 19 (2023) 1–13, https://doi.org/10.1186/s12917-023-03613-4.
[64] B.A.. M., A.L.H. Laura E. Petes, Intertidal mussels exhibit energetic trade-offs between reproduction and stress resistance, Ecol. Monogr. 78 (2016) 387–402. http://www.jstor.org/stable/27646141.
[65] K.P. Sebens, G. Sarà, E. Carrington, Estimation of fitness from energetics and life-history data: an example using mussels, Ecol. Evol. 8 (2018) 5279–5290, https://doi.org/10.1002/ece3.4004.
[66] E.A.M. Curley, R. Thomas, C.E. Adams, A. Stephen, Behavioural and metabolic responses of Unionida mussels to stress, Aquat. Conserv. Mar. Freshw. Ecosyst. 31 (2021) 3184–3200, https://doi.org/10.1002/aqc.3689.
[67] M. Huhn, G.S.I. Hattich, N.P. Zamani, K. von Juterzenka, M. Lenz, Tolerance to stress differs between Asian green mussels Perna viridis from the impacted Jakarta Bay and from natural habitats along the coast of West Java, Mar. Pollut. Bull. 110 (2016) 757–766, https://doi.org/10.1016/j.marpolbul.2016.02.020.
[68] J.S. Choi, Y.J. Jung, N.H. Hong, S.H. Hong, J.W. Park, Toxicological effects of irregularly shaped and spherical microplastics in a marine teleost, the sheepshead minnow (Cyprinodon variegatus), Mar. Pollut. Bull. 129 (2018) 231–240, https://doi.org/10.1016/j.marpolbul.2018.02.039.
[69] Z. Liu, R. Xu, W. Wei, P. Jing, X. Li, Q. Zhu, H. Sun, Y. Dong, G.S. Zakharova, Flexible H2V3O8 nanobelts/reduced graphene oxide electrodes with high mass loading for lithium ion batteries, Solid State Ionics 329 (2019) 74–81, https://doi.org/10.1016/j.ssi.2018.11.017.
[70] B. Fernández, J.A. Campillo, C. Martínez-Gómez, J. Benedicto, Antioxidant responses in gills of mussel (Mytilus galloprovincialis) as biomarkers of environmental stress along the Spanish Mediterranean coast, Aquat. Toxicol. 99 (2010) 186–197, https://doi.org/10.1016/j.aquatox.2010.04.013.
[71] Z. Yu, N. Jiang, W. Su, Y. Zhuo, Necroptosis: a novel pathway in neuroinflammation, Front. Pharmacol. 12 (2021) 1–13, https://doi.org/10.3389/fphar.2021.701564.
[72] W. Roth, P. Kermer, M. Krajewska, K. Welsh, S. Davis, S. Krajewski, J.C. Reed, Bifunctional apoptosis inhibitor (BAR) protects neurons from diverse cell death pathways, Cell Death Differ. 10 (2003) 1178–1187, https://doi.org/10.1038/sj.cdd.4401287.
[73] J. Yue, J.M. López, Understanding MAPK signaling pathways in apoptosis, Int. J. Mol. Sci. 21 (2020), https://doi.org/10.3390/ijms21072346.