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Gender differences in protein expression after polystyrene nanoplastics exposure in mussels *Mytilus galloprovincialis*

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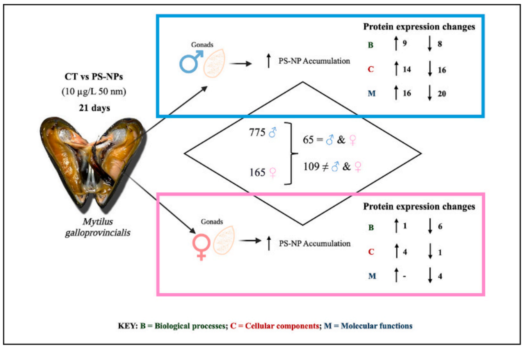
HIGHLIGHTS

- Polystyrene nanoplastics (PS-NPs) are accumulated in male and female mussel *Mytilus galloprovincialis* gonads.
- PS-NPs accumulation in male gonads alters protein expression more significantly than in females.
- In males, PS-NPs induce protein changes that can result in infertility.
- In females, pathways affected, lead to oogenesis and energy metabolism impairments.
- PS-NPs impact reproductive success and cellular homeostasis, leading to biodiversity loss.

GRAPHICAL ABSTRACT



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ABSTRACT

Plastic pollution is a significant issue that the scientific community has been actively studying due to its harmful effects on aquatic ecosystems. Nanoplastics can pass through cellular barriers and enter the mussel's bloodstream. More worryingly, they can also penetrate sperm cells and oocytes, potentially impacting their motility and resilience. Reproductive success drives a shift in population dynamics and plays a vital role in maintaining a healthy ecosystem. Proteomics helps detect protein changes caused by exposure to contaminants, such as nanoplastics, in marine organisms, providing deeper molecular-level insights into contamination-induced cellular pathways. Therefore, this study aimed to utilise a high-throughput proteomic approach to evaluate the impact of polystyrene nanoplastics (PS-NPs) on the gonads of male and female *M. galloprovincialis*, using a SWATH-MS analysis after 21 days of exposure to 10 µg/L of PS-NPs (50 nm). The accumulation of PS-NPs was also evaluated in male and female mussels. A comparison in protein expression of controls vs. those exposed in male and female mussel gonads and between males and females was evaluated. The findings indicate that PS-NP accumulation in male gonads alters protein expression more significantly than in females, interfering with protein synthesis, energy production, intracellular transport, and cellular homeostasis, and possibly impaired reproductive function. Female gonads exposed to PS-NPs revealed disruption in proteins associated with translation, RNA processing and signaling, ribosome biogenesis, cell cycle regulation, and stress response. Protein

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folding, lipid metabolism, and calcium signaling pathways were also affected, leading to oogenesis, meiotic progression, intracellular transport, and energy metabolism impairments. These disruptions ultimately impact reproductive success and cellular homeostasis, leading to a decline in biodiversity.

1. Introduction

Plastic pollution is a significant problem the scientific community has been assessing because of its toxicity in aquatic ecosystems. A rise in global plastic production (400.3 Mt) was reported in 2022 compared to previous years; however, in Europe, there was a decrease of 1 % compared to 2021 (Plastics Europe, 2023). Nevertheless, despite programmes such as Operation Clean Sweep®, which promotes the containment of plastic pellets along the entire plastic value chain (www.opcleansweep.eu), plastics still find their way into the ocean. A particular concern is nanoplastics (NPs; $<$ 100 nm), with concentrations ranging from 0.3 to 488 μg/L in seas, rivers, and nature reserves throughout Asia, Europe, Antarctica, and the Arctic Ocean (Shi et al., 2024). Their small size and large surface area confer different toxic properties than larger plastic particles. In marine biota, NP toxicity is associated with reduced growth rates, energy and movement, stress, inflammation, and abnormalities (Kögel et al., 2020). Energy storage for processes such as gametogenesis is significantly influenced by food availability, and nanoplastics are misidentified as low-nutritional food sources by mussels (Wegner et al., 2012). NPs can cross cellular boundaries and enter the bloodstream of mussels, but more concerningly, they can invade spermatozoa and oocytes, potentially affecting motility and robustness. In the case of spermatozoa, specifically, NP contamination can lead to a decrease in sperm release, inhibition of sperm motility, and DNA damage (Lewis and Ford, 2012). This DNA damage can impair the sperm's ability to fertilise oocytes. The accumulation of NPs is gender- and time-specific in the marine mussel *M. galloprovincialis, with* more detrimental effects observed in females (polystyrene NPs [PS-NPs]; 10 μg/L, 28 days; Gonçalves et al., 2025). A weight of evidence model confirms that NPs harm females under chronic exposure (Gonçalves et al., 2025), potentially causing oocyte malformation, reducing successful fecundity, and leading to total developmental arrest during embryo-larval development (PS-NPs; 50 nm; 1.5 h; *Crassostrea gigas;* Tallec et al., 2018). Furthermore, a decrease in sperm motility, a reduction in sperm velocity, and unsuccessful embryogenesis in *C. gigas* was notable after exposure to PS-NPs with amine or carboxyl functional groups (1 h; 0.1–25 μg/mL; Tallec et al., 2020).

The Mediterranean mussel *Mytilus galloprovincialis* is found in high densities and relatively stable populations with a wide geographical distribution at different latitudes and adapted to various environmental parameters and stressors. As sessile filter-feeding organisms, mussels are capable of accumulating contaminants present, and any shift in populational dynamics can be a sign of the hazard of the presence of these contaminants. Gamete production and the energy that goes into it are essential for the reproductive success of mussels, such as *M. galloprovincialis*, as well as for larval survival and the recruitment of juveniles (Kautsky, 1982). Lipids are crucial, especially for female gonad development (Martínez-Pita et al., 2012), and play a key role in regulating the folding, organisation and final structure of all membrane proteins. Any interference or detour of energy to counterbalance any toxic effects from contaminants in the marine environment can cause unbalance and jeopardise gametogenesis and reproductive success. When ripe, mussels release spermatozoa and oocytes into the water column, where fertilisation occurs. In this sense, contaminants can interfere with the gametogenesis process and have toxic effects on the gametes released into the water column and larval development. Chelyadina et al. (2018, 2022, 2024) highlighted that environmental and anthropogenic factors such as starvation, hypoxia, diesel fuel, synthetic surfactants, sex hormones and metals can influence the sexual structure of *M. galloprovincialis* on the Black Sea coast, resulting in sex inversion from females to males. Reproductive success dictates the changes in populational dynamics and is crucial for a healthy ecosystem. Every species has its importance, and removing any level within the food chain can cause a massive downfall of biodiversity in a whole ecosystem. Therefore, understanding the effects of contaminants on species reproduction is vital to ensure that there will be future generations. A proteomic approach is crucial to acknowledge the impact that emerging contaminants, such as NPs, have at a biological, cellular and molecular level and the target toxicity they pursue. This approach enables the identification of a more significant number of protein alterations due to exposure to contaminants, like NPs, in marine organisms, giving access to a more excellent knowledge at a molecular level of those contamination-induced cellular pathways (López-Pedrouso et al., 2020).

In *M. galloprovincialis,* a male-associated polypeptide, MAP-39, was identified (Mikhailov and Mendez, 1995). The expression of MAP-39 is associated with gonad development, maturation and spawning. The concentration of this polypeptide is 10 % of total soluble proteins in mature male gonads, whilst only traces are attainable in female gonads (Mikhailov and Mendez, 1995). In Zhong et al. (2020), an exposure to trichloropropyl phosphate (TCPP; 100 nmol/L; 42 days), a halogenated organophosphate ester widely used as flame retardants and plasticisers, 219 proteins were differentiated expressed between males and females *M. galloprovincialis*, where the gender-specific proteins were involved in protein synthesis and degradation, energy metabolism and the functions of the cytoskeleton and motor proteins. Proteomic responses after microplastic (MPs) exposure in the clam *Scrobicularia plana* caused cytoskeletal and cell structure alterations, disturbances in energy metabolism, oxidative stress, fatty acids, DNA binding and neurotransmission (polyethylene MPs [PE-MPs]; 11–13 μM; 1 mg L^{-1}; 14 days; Bebianno et al., 2022). With the adjacent BAP adsorbed onto MPs, a higher differentiation of protein expression was detected, where changes in glucose metabolism, RNA binding and apoptosis were also induced (Bebianno et al., 2022). *Mytilus edulis* exposed to high-density PE-MPs (25 μg/L; 0.5–330 μM) for 52 days caused alterations in the haemolymph proteome, and most proteins affected are involved in vital biological processes such as immune regulation, detoxification, metabolism and structural development (Green et al., 2019). Now, NPs induce more toxicity than MPs, causing more ROS production and higher total antioxidant capacity levels, with downregulation of specific genes involved in stress and immune response after transcriptomic sequencing in *Mytilus coruscus* (20 mg L^{-1}; PS-NPs 100 nm; PS-MPs 1 μM; Qi et al., 2023). As NPs are efficiently ingested by mussels (Qi et al., 2023), understanding the proteomic responses towards NP toxicity is of utmost importance. A SWATH-MS analysis enables a deep proteome analysis by combining targeted and shotgun data extraction techniques (Anjo et al., 2015).

Therefore, this study aimed to use a high throughput proteomic approach to assess the effects of polystyrene NPs on the gonads of male and female *M. galloprovincialis*. To our knowledge, this is the first report about the effects of PS-NPs on mussel reproduction fitness.

2. Materials and methods

2.1. Experimental design

Firstly, Polysciences, Inc. (Germany) supplied Fluoresbrite® Plain YG spherical polystyrene nanoplastics (PS-NPs, 50 nm, CAS 9003-53-6) was used in this experiment. The hydrodynamic diameter of PS-NPs in seawater increases (852 ± 103 nm) as the salt present triggers aggregation/agglomeration kinetics. The characterization analysis of PS-NPs in seawater revealed a − 0.068 ± 0.23 mV zeta-potential. Additional information regarding PS-NP characterization of these nanoparticles can be found in Gonçalves et al. (2022). Given the lack of known environmental concentrations of NPs for experimental purposes, a concentration of 10 μg/L was selected based on the acute toxicity effects of LC_{50} levels reported in Gonçalves et al. (2022), underscoring the importance of the research in this field. Abiotic parameters were analysed daily in filtered seawater (FSW) by measuring salinity (36.8 ± 2.2), temperature (19.0 ± 0.8 °C), pH (7.9 ± 0.2) and oxygen saturation (100 ± 1.7 %) with a multiparametric probe (Odeon, PONSEL, FR). Tanks were equipped with glass lids and aeration pipettes to prevent further plastic pollution, including airborne contamination. No gloves or plastic tools were used during tissue dissection.

Mussels, *M. galloprovincialis* (50 mm ± 5 mm), were collected from the Ria Formosa Lagoon in Southeast Portugal (37°00′30.6"N 7°59′39.6" W). They were cleaned and placed into 30 L tanks (25 L FSW) at two mussels per liter density in triplicate setups, with a 12 h/12 h light/dark cycle and kept under constant aeration. The experiment was done during the first reproductive season. Following a four-day acclimation, a group of mussels was not exposed, and another was exposed to 10 μg/L of PS-NPs for 21 days. Mussels were only fed with food existent in FSW. The exposure time selected was based on the most significant effects obtained on a previous experiment where NPs accumulation, oxidative stress and damage was assessed on male and female mussel gonads exposed to the same type and concentration of PS-NPs and published in Gonçalves et al. (2025). Contaminants and seawater were refreshed every two days, with concentrations reset and samples taken in the beginning and after 21 days of exposure for sex determination, PS-NP accumulation and proteomic analysis, where the mussels were dissected, gonads retrieved, followed by the determination of the sex for each sample. To achieve mussel sex determination, a smear of the gonad tissue of each mussel was examined under an optical microscope (Compound Light Microscopy) at 400× magnification to check for spermatozoa or oocytes. Sperm motility and the spherical morphology of oocytes were used as indicators of gamete quality. After sex identification, the samples were labelled appropriately and stored at −80 °C to preserve them for further investigation.

2.2. PS-NP accumulation

Using a fluorescence-based approach and the molecular rotor probe 9-(dicyanovinyl)-julolidine (DCVJ), the accumulation of PS-NPs was evaluated in mussel male and female gonads unexposed and subjected to 10 μg/L of PS-NPs for 21 days, following the Gagné (2019) method. First, using a VWR Star-Beater, the male and female mussels' gonads were homogenised separately at 20 % (*w/v*) in an ice-cold buffer solution (50 mM NaCl, 10 mM Hepes-NaOH [pH 7.4], 1 mM EDTA, and 1 mM DTT) for 5 min while shaking at 20 rpm and utilising grinding balls. Samples were centrifuged for 20 min at 2 °C and 15,000 *g* to isolate the cytosolic fraction. The supernatant was then promptly frozen at −80 °C until it could be examined further. Then, to 50 μL of each sample supernatant, a 200 μL of 10 μM of DCVJ probe was added in a dark 96 wells microplate and the fluorescence was measured. A Berthold Tristar 5 spectrofluorometric microplate reader was then used to analyse the samples, which had emission spectra from 400 to 800 nm and excitation at 450 nm. The wavelength of PS-NPs is 510 nm, and the outcomes with the units of PS-NPs expressed in relation to controls as μg/g wet weight.

2.3. Sample preparation

Male and female gonads ($n = 4$ per sex and time) were homogenised in a Laemli solution (10 % SDS, 30 % glycerol, 0.6 M DTT, 0.012 % bromophenol blue, and 0.5 M Tris HCl, pH 6.8). They were then sonicated slowly to prevent foam formation in a VWR Star-Beater (40 min, 5/s shaking, with grinding balls) and boiled at 95 °C for 10 mins. The samples were then placed into labelled Eppendorf's and frozen at −80 °C until further proteomic analysis.

2.4. LC-MS methodology

The total protein content of male and female gonads controls and PS-NP exposed was quantified using the Pierce™ 660 nm Protein Assay Kit (Thermo Scientific™). One pooled sample was prepared for each condition containing 70 μg of protein for the ion library generation. For relative protein quantification experiments (SWATH), 50 μg of protein from each male and female sample was used for the following steps. An internal standard solution was added to each sample (including the pooled samples) containing green fluorescent protein (GFP) and Maltose/maltodextrin-binding periplasmic protein (MALE). After the denaturation step at 95 °C for about 5 min, the protein content was separated by SDS-PAGE for about 17 min at 110 V (Short-GeLC Approach) (Anjo et al., 2015) and stained with Coomassie Brilliant Blue G-250. Each lane was divided into three separate gel fractions (5 fractions in pooled samples) for the destaining step using a 50 mM ammonium bicarbonate solution with 30 % acetonitrile, followed by overnight protein digestion with trypsin. Peptide extraction from the gel was performed by incubating solutions containing different percentages of acetonitrile (30, 50, and 98 %) with 1 % formic acid. For protein identification, the five fractions were evaporated in a vacuum centrifuge, resuspended in 25 μL of 2 % ACN with 0.1 % FA, and 10 μL were analysed by data-dependent acquisition (DDA). For relative protein quantification, the 3 fractions from each sample were joined, evaporated in a vacuum centrifuge, resuspended in 40 μL of 2 % ACN with 0.1 % FA, and 10 μL were analysed by data-independent acquisition (DIA/SWATH-MS). Samples were analysed on a NanoLC™ 425 System (Eksigent) coupled to a Triple TOF™ 6600 mass spectrometer (Sciex) and the ionisation source (ESI DuoSpray™ Source). The chromatographic separation was performed on a Triart C18 Capillary Column 1/32″ (12 nm, S-3 μm, 150 × 0.3 mm, YMC) and using a Triart C18 Capillary Guard Column (0.5 × 5 mm, 3 μm, 12 nm, YMC) at 50 °C. The flow rate was set to 5 μL/min, and mobile phases A and B were 5 % DMSO plus 0.1 % formic acid in water and acetonitrile, respectively. The LC program was performed as follows: 5–30 % of B (0–50 min), 30–98 % of B (50–52 min), 98 % of B (52–54 min), 98–5 % of B (54–56 min), and 5 % of B (56–65 min).

The ionisation source was operated in the positive mode set to an ion spray voltage of 5500 V, 25 psi for nebuliser gas 1 (GS1), 10 psi for nebuliser gas 2 (GS2), 25 psi for the curtain gas (CUR), and source temperature (TEM) at 100 °C. For data-dependent acquisition (DDA) experiments, the mass spectrometer was set to scanning full spectra (m/z 350–1500) for 250 ms, followed by up to 100 MS/MS scans (m/z 100–2000) with 30 ms for the accumulation time. Candidate ions with a charge state between +1 and + 5 and counts above a minimum threshold of 100 counts per second were isolated for fragmentation, and one MS/MS spectrum was collected before adding those ions to the exclusion list for 15 s (mass spectrometer-operated by Analyst® TF 1.8.1, Sciex®). The rolling collision energy was used with a collision energy spread of 5. For SWATH experiments, the mass spectrometer was operated in a looped product ion mode and precisely tuned to a set of 168 windows, covering the precursor mass range of 350–2250 m/z. A 50 ms survey scan (350–2250 m/z) was acquired at the beginning of each cycle, and SWATH-MS/MS spectra were collected from 100 to 2250 m/z for 19 ms, resulting in a cycle time of 3.29 s.

2.5. Ion-library from DDA information

A specific ion library of the precursor masses and fragment ions was created by combining all pool files in one protein identification search using the ProteinPilot™ software (v5.0, Sciex). The parameters of the paragon method were cysteine alkylation by acrylamide, digestion by trypsin, and gel-based ID. The protein identification search was conducted against the *Mytilus galloprovincialis* database (UniProt), downloaded in May 2024. An independent False Discovery Rate (FDR) analysis, using the target-decoy approach provided by Protein Pilot™, was used to assess the quality of the identifications.

2.6. Relative quantification of proteins (SWATH-MS)

SWATH data were processed by the SWATH™ processing plug-in for PeakView™ (v2.0.01, Sciex®). Protein relative quantification was performed in all samples using the information from the protein identification search. Peptide relative quantification was obtained considering the FDR < 1 % for at least three replicates in at least one of the conditions and by the sum of up to 5 fragments/peptide. Peptide relative peak areas were normalised for the total sum of fragment areas for the respective sample, and protein quantities were obtained by the sum of up to 15 peptides/protein.

2.7. Statistical analysis

The software program GraphPad Prism, version 9.4.1 (GraphPad Software, Inc. CA), was used for statistical analysis of PS-NPs accumulation. The significant differences between treatments, time, and sex were evaluated using parametric tests (ANOVA, followed by Tukey's posthoc test) as determined by data distribution and variance homogeneity (Shapiro-Wilk test). The results were significant if $p < 0.05$.

Statistical analysis of proteomic data was conducted in MetaboAnalyst 6.0 (www.metaboanalyst.ca). The two-group comparisons were performed using the non-parametric Wilcoxon rank-sum test to select proteins with *p*-values below 0.05. Heatmap representations were performed considering the Euclidean distance and the Ward clustering algorithm. Venn diagrams were generated in the SRplot (Tang et al., 2023) platform using the module "proportional Venn" and jvenn (Bardou et al., 2014) for more than three groups. The functional analysis was performed by DAVID (Sherman et al., 2022; Huang da et al., 2009) using the *Mytilus galloprovincialis* database as background. The enriched GO terms were represented for the number of genes by Heatmaps generated in the platform ImageGP (Tong Chen, 2022).

3. Results and discussion

3.1. PS-NP accumulation

After 21 days of exposure, the accumulation of PS-NPs in mussel female gonads is higher than in male gonads (*see* Table 1). As oocytes are larger than spermatozoa, this may facilitate the penetration and accumulation of PS-NPs, and this accumulation may weaken mussels' immune systems and cause additional prejudice towards gamete production, embryo-larval development and fertilisation (Tallec et al., 2018; Capolupo et al., 2021; Gonçalves et al., 2022, 2025). After internalisation of PS-NPs, a decrease in fertilisation rate was observed in oysters *C. gigas* (50 nm; 10 μg/L; 50 nm; 1.5 h; Tallec et al., 2018), as well as a reduction in sperm motility and the formation of PS-NPs agglomerates on jelly-coatings of oocytes as a result of PS- NPs exposure (PS-COOH and PS-NH_2; 0.1–100 mg L^{-1}; 1, 3, and 5 h; González-Fernández et al., 2018). Additionally, deformities in larval development have also been recorded in *M. galloprovincialis* (PS-NH_2; 50 and 100 nm; 10 μg/L; 48 h post fertilisation, Auguste et al., 2021), and the smaller PS-NPs have a more detrimental effect, whereby larvae exposed to PS-NH_2 nanoplastics demonstrate disruption of the molecular processes involved in shell formation (Auguste et al., 2021). PS-NP accumulation here is positively related to other outcomes such as the antioxidant (SOD) and biotransformation (GST) enzymes and lipid peroxidation (Gonçalves et al., 2025). Widespread cellular dysfunction brought on by PS-NP accumulation by male mussel gonads inhibits vital biological functions (protein synthesis, transport, energy metabolism, etc.). The harmful effects of oxidative stress, particularly on CAT, and NP buildup cause this chain reaction of disturbances, making it more difficult for cells to maintain homeostasis and finish vital functions like reproductive and gonadal health. The cell manages the damage by up-regulating stress adaptation systems, such as the antioxidant and biotransformation enzymes (SOD and GST). However, stress can result in cell death or inability to reproduce if it surpasses the cell's ability to heal itself, causing impairment of spermatozoa motility and ability to fecund oocytes (Tallec et al., 2018; Gallo et al., 2021).

Table 1
Accumulation of PS-NPs (μg/g wet weight) relative to controls in male and female gonads of *M. galloprovincialis* after 21 days of exposure (mean ± std). Significant differences between treatment/times for each gender is marked with '*', and significant differences between male and female mussels is marked with '**' ($p < 0.05$; Anova – Tukey HSD).

Time of exposure (days)	PS-NPs μg/g wet weight	
	♂	♀
0	1 ± 1*	1 ± 1*
21	1.78 ± 0.16**	4.45 ± 0.10**

Moreover, when female mussel gonads ingest PS-NPs, important cellular functions necessary for healthy cell function and reproduction are severely disrupted. Gonadal function and oocyte development are probably impacted when vesicle-mediated and intracellular protein transport is inhibited since this affects the distribution of vital proteins and organelles. Furthermore, genes essential for gonadal health, reproduction, and cell division are not properly expressed when mRNA processing and protein synthesis are disrupted. Also, spindle organisation and chromosome segregation, essential for meiosis and fertilisation, are hampered by microtubule malfunction, resulting in genomic instability and unsuccessful reproduction. Therefore, female mussels' ability to reproduce can be jeopardised once they need enough energy to produce and maintain the gonads by the combined impacts of PS-NPs on antioxidant and oxidative stress, protein degradation, and cellular dysfunction, leading to problems with oocyte viability and fertility. (Gallo et al., 2022; Zhang and Wu, 2023; Shang et al., 2025).

3.2. Protein relative quantification

Unexposed proteins of male and female gonads, as well as those differentially expressed because of exposure to PS-NPs, allowed the identification of 3865 proteins (considering 5 % FDR against the *Mytilus galloprovincialis* from Uniprot), and 2128 proteins were further quantified. The list of quantified proteins is found in the supplementary material, Table S1. The exposure of male and female gonads to PS-NPs significantly altered the proteome of both genders ($p < 0.05$; Figs. 1–4). The Venn diagram (Fig. 1) shows the proteins differentially expressed in control males and females, between control and PS-NPs exposed males, between control and PS-NPs exposed females, between control male and female gonads, and between PS-NPs exposed male and female gonads, as well as the protein common among treatments and sex gonads ($p < 0.05$). As can be seen from Fig. 1, although in general female mussels have more proteins (Shang et al., 2025), the number of differentially expressed proteins was more significant in male gonads than in females after exposure to PS-NPs ($p < 0.05$). The differentially expressed proteins in male and female controls exposed to PS-NPs were hierarchically clustered, and heat maps of both conditions showing differential proteins up and down-regulated are presented in Fig. 2. When mussels were exposed to PS-NPs, 775 proteins changed between controls and PS-NPs exposed male gonads ($p < 0.05$; Figs. 1–2A), while only 156 proteins were differentially expressed between controls and PS-NPs female gonads. ($p < 0.05$; Figs. 1–2B). Among these proteins, 65 were similar between male and female gonads ($p < 0.05$). However, when proteins were compared between control male and female gonads, 109 proteins were different ($p < 0.05$; Figs. 1–3A), but when exposed to PS-NPs, 118 proteins were differently expressed between male and female gonads ($p < 0.05$; Fig. 3B) with eight of these proteins similar between controls

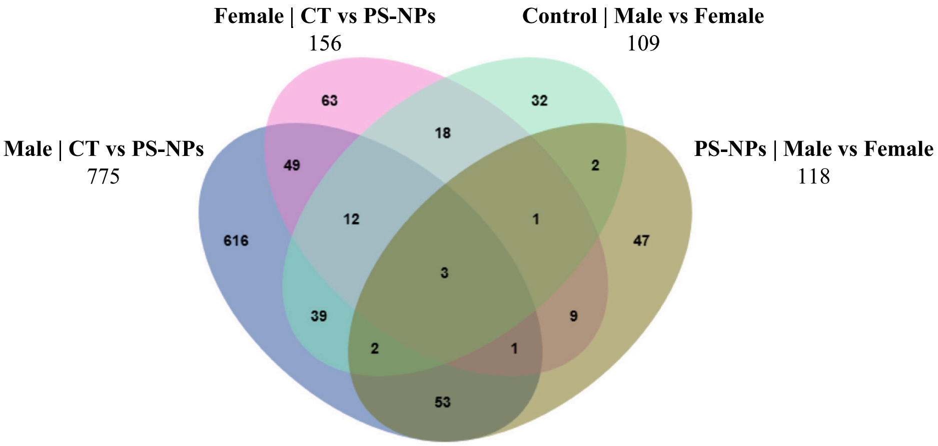

Fig. 1. Venn diagram showing the number of specific and common proteins between controls (CT) vs PS-NPs contaminated gonads in male and female *M. galloprovincialis*, and male vs female *M. galloprovincialis* for CT and PS-NPs contaminated gonads after 21-days of exposure.

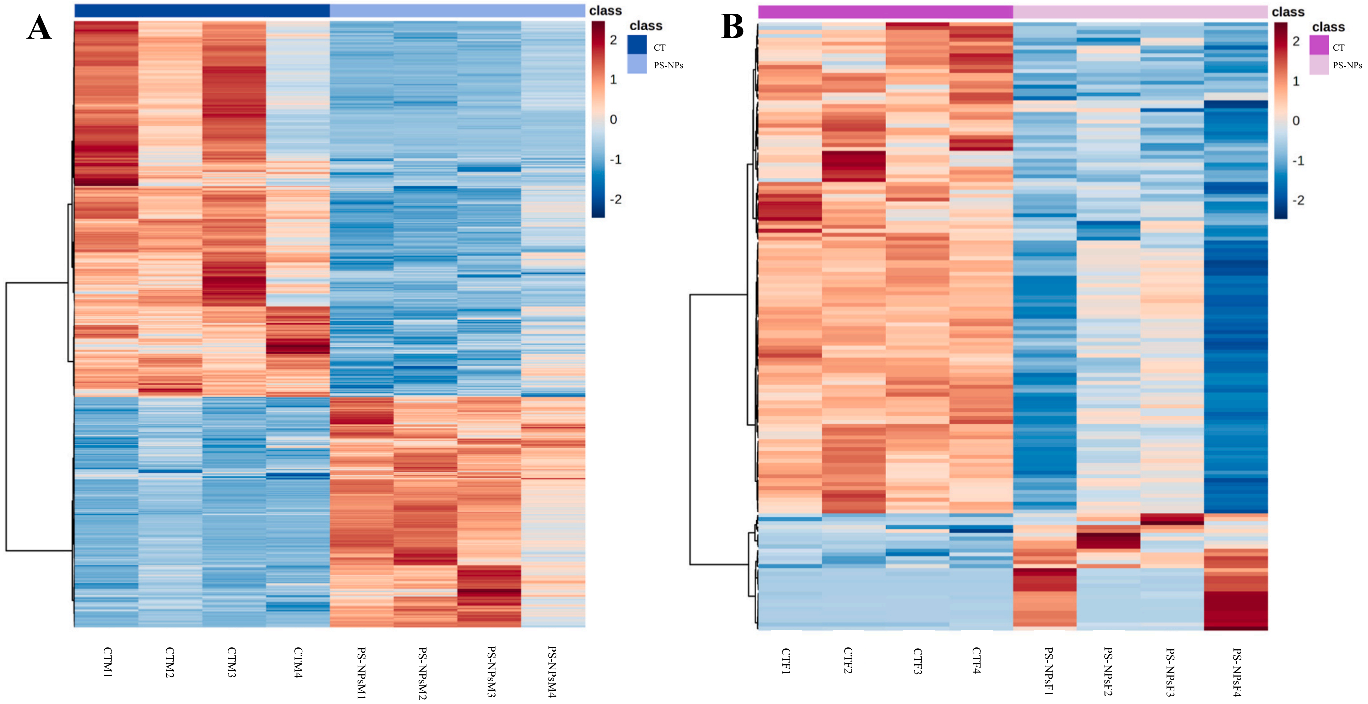

Fig. 2. HeatMap for the 775 proteins (A) in male gonads and 156 proteins (B) in female gonads between controls (CT) and PS-NPs exposed after 21-days with statistically significant differences (Wilcoxon rank-sum test) ($p < 0.05$).

and PS-NPs exposed male and female gonads ($p < 0.05$). In zebrafish exposed to PS-NPs (1.0 mg L^{-1} for 21 days) of similar size (50 nm), NPs influence reproductive toxicity that is gender-specific (Li et al., 2024). When zebrafish are exposed to PS-NPs (50 nm), NPs can pass the blood barrier and accumulate in the gonads (Li et al., 2024). Zebrafish submitted to acute exposure of the same type of PS – NPs but with a smaller size (45 nm; 5 mg L^{-1}, 96 h), NPs were able to penetrate the testicular barrier and be internalised in germ cell lines (Pujol et al., 2025). The exposure to high-density polyethylene (HPDE) microplastics also induced changes in the proteome of the haemolymph of *Mytilus edulis* (Green et al., 2019).

3.3. Impact of PS-NP exposure on the proteome of unexposed and exposed mussel male gonads

Gene Ontology (GO) enrichment analysis shows differences in protein expression related to putative biological processes (BP), cellular components (CC) and molecular functions (MF) between unexposed and PS-NPs exposure mussel male and female gonads (Fig. 4). It revealed that in control mussel male gonads compared to those exposed to PS-NPs, NPs affected proteins related to biological processes (9 proteins were upregulated and 8 proteins downregulated) ($p < 0.05$; Fig. 4). In comparison, 14 proteins of the cellular components were upregulated, and 16 were downregulated ($p < 0.05$; Fig. 4). Regarding proteins related to molecular functions, 16 proteins were upregulated, and 20 proteins were downregulated ($p < 0.05$; Fig. 4).

The inhibition of these differentially expressed proteins in male

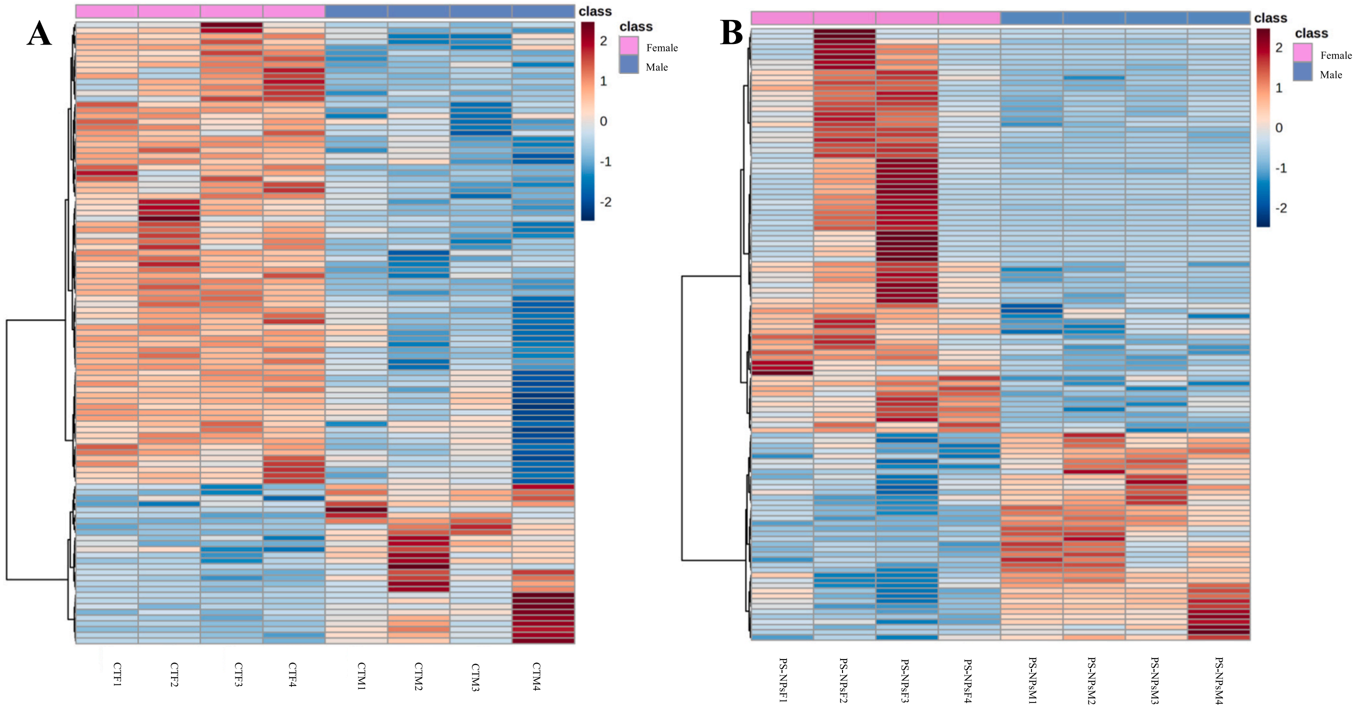

Fig. 3. HeatMap for the 109 proteins (A) between control male and female gonads and 118 proteins (B) between PS-NPs exposed male and female gonads after 21-days with statistically significant differences (Wilcoxon rank-sum test) ($p < 0.05$).

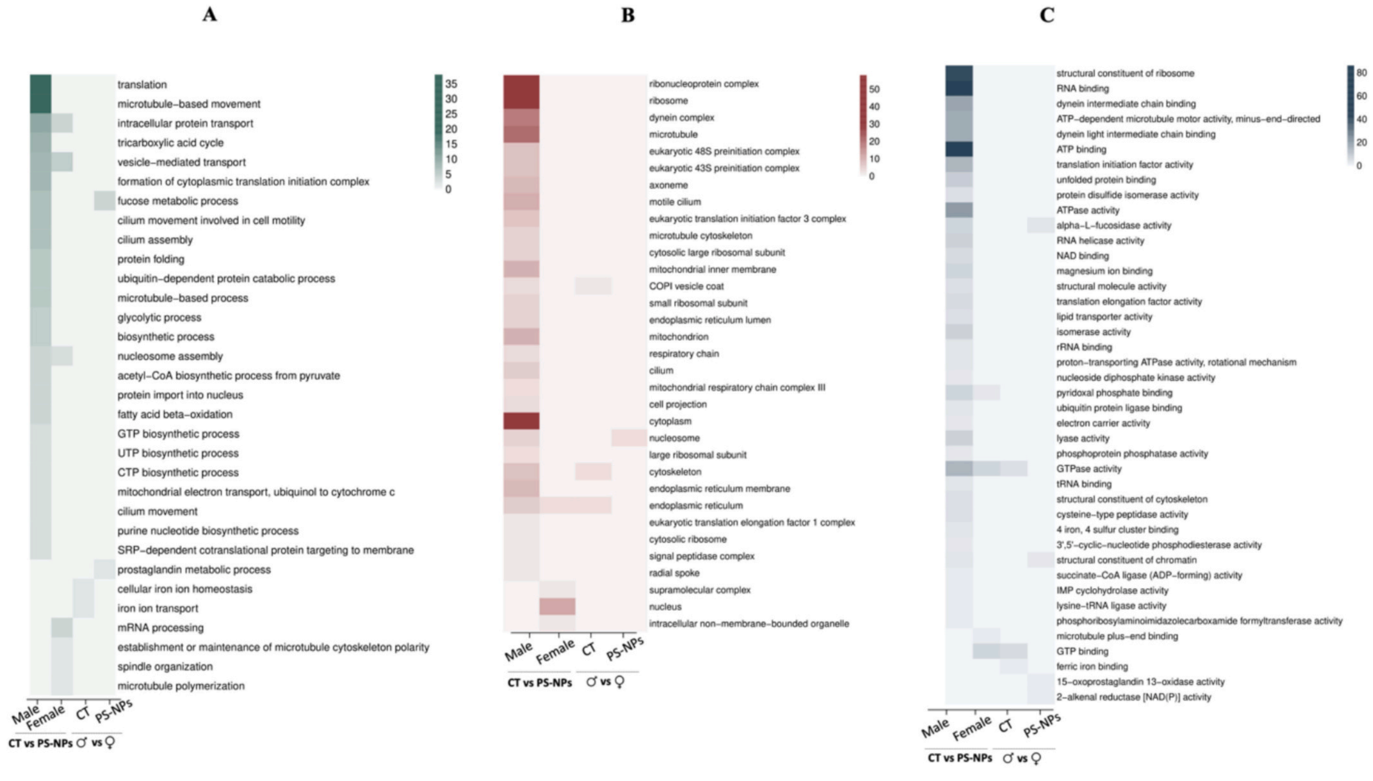

Fig. 4. Functional identification in *M. galloprovincialis* gonads identified by David GO enrichment analysis of (A) biological processes, (B) cellular components and (C) molecular function of differential proteins expressed between controls and PS-NPs exposed, and between male and females.

gonads because of the exposure to PS-NPs compared to control male gonads leads to changes in the following pathways: translation and ribosome biogenesis, protein folding and quality control, intracellular transport and vesicle trafficking, energy metabolism, RNA processing and tRNA charging, protein degradation, cytoskeletal dynamics and stress adaptation mechanisms. The reduction of the translation and maturation of LSU-rRNA affects the formation of the ribosome-mRNA complex, reducing protein synthesis, ribosomal assembly, and energy production. Other stress conditions, such as unfolded protein response, changes in cytoskeleton dynamics, oxidative damage and apoptosis, can also occur. Similarly in male gonads exposed to the same time, type and size NPs concentration oxidative damage was also detected after 21 days of exposure (Gonçalves et al., 2025). The inhibition of these proteins can severely compromise the ability of male mussel gonads to develop functional sperm, potentially impairing reproductive function. However, this needs to be confirmed. In the Pacific oyster *C. gigas* exposed to PS microplastics (6 μm; 0.023 mg L^{-1}), Sussarellu et al. (2016) showed that the exposure to this type of microplastics induced a decrease in the velocity of the sperm, compromising the ability to fertilise the oyster oocytes.

On the other hand, these interrelated processes represent the cell's integrated response to stress, particularly oxidative stress (Gonçalves et al., 2025). In male mussel gonads exposed to PS-NPs (50 nm; 10 μgL^{-1}; 21 days) a significant increase in superoxide dismutase (SOD) and glutathione-*S*-transferases (GST) activity and a decrease in lipid peroxidation (LPO) was registered (Gonçalves et al., 2025), Therefore, the cellular slowdown or inhibition of specific functions (like translation, ribosome biogenesis, and energy metabolism) can activate a protective antioxidant response, with increased SOD and GST activity and a reduction in LPO as part of the defense mechanism (Gonçalves et al., 2025).

However, the upregulation of proteins in male gonads induced changes in the following pathways: energy production microtubule-based processes, chromatin remodeling and DNA packaging, electron transport and oxidative stress response, nucleotide biosynthesis and motility and cell projection enhancing the need for energy and structural demand for spermatogenesis and sperm functionality. Moreover, the upregulation of the tricarboxylic acid cycle (TCA cycle), glycolysis, and mitochondrial electron transport chain and the induction of pyruvate dehydrogenase and acetyl-CoA biosynthetic processes highlights the need for high energy demand required for spermatogenesis and sperm motility. Previous data indicated that the exposure of PS-NPs reduced the number of spermatozoa dramatically, and spermatozoa motility was hindered (16.5 μg/L of 50 nm for 24 h) (Gonçalves et al., 2025), Moreover, PS-NPs can halt fertilisation by adhering to spermatozoa and forming agglomerates (González-Fernández et al., 2018).

When energy production is upregulated, cells may also need to upregulate their antioxidant response to counterbalance the oxidative stress induced by increased mitochondrial activity, as observed in Gonçalves et al. (2025) where oxidative stress was detected after male mussels were exposed to PS-NPs (10 μg/L of 50 nm for 21 days). Additionally, microtubules are essential for intracellular transport, cell division, and motility. Microtubule dynamics may be impacted by cell oxidative stress, which can interfere with motility and appropriate intracellular transport. However, as cells rearrange their cytoskeleton to support proper function under challenging circumstances, enhanced microtubule-based processes (such as polymerisation or stabilisation) may also be a stress reaction (Gurel et al., 2014). A stronger antioxidative response, including the increase of SOD and GST activity as observed in male gonads (Gonçalves et al., 2025), may result from stimulation of microtubule dynamics in response to stress or energy needs (Dalle-Donne et al., 2001; Apraiz et al., 2006).

Nevertheless, energy generation, chromatin remodeling, microtubule-based processes, and other cellular functions are upregulated in response to stress and increased metabolic demand, which may increase ROS production. Cells respond to oxidative stress by increasing the activity of GST to detoxify lipid peroxidation products and other reactive intermediates and SOD to neutralise superoxide radicals formed (Gonçalves et al., 2025). By maintaining membrane integrity and lowering LPO, this cellular adaptation ensures that vital functions like motility, DNA repair, and nucleotide biosynthesis run smoothly. Therefore, under stress, these systems coordinated activation helps maintain homeostasis, prevent cellular damage, and manage the oxidative burden (Rangaswamy et al., 2024). In zebrafish (*Danio rerio)* males exposed in water to an acute concentration of PS-NPs (45 nm; 5 mg L^{-1} for 96 h), NPs were able to penetrate the internal barrier of the spermatozoids and be internalised in germ cells, inducing the upregulation of several genes related to meiotic chromosomal assembly double-strand break and chromatic formation affecting sperm mobility (Pujol et al., 2025). Similarly, exposure for 28 days to PS-NPs (25, 50 and 100 nm; 5 mg mL^{-1}) indicates that PS-NPs decrease sperm concentration and viability and increase sperm abnormalities (Ma et al., 2024). Moreover, PS-NPs disrupt spermatogenesis in the testicular of male mice by inhibiting the expression of Pmfbp1/Ggn/Fsip2 and enabling Hsd3b5 protein expression to reduce dihydrotestosterone levels and affect sperm flagellar assembly by decreasing the expression of *Dnah8/Tekt5/Rsph6a* and the claudin family by destroying the tight junctions (Ma et al., 2024). PS-NPs also induce the expression of cathepsins (B/F/H), indicating that exposure to PS-NPs induces testicular cell apoptosis (Ma et al., 2024). However, in the present case, possible apoptosis needs to be confirmed.

On the other hand, sex reversal can occur due to changes in biotic and abiotic factors as well as due to anthropogenic effects. In the Black Sea, the sex of female *M. galloprovincialis* were reversed to males due to hypoxia, starvation and the presence of contaminants such as detergents, diesel oil and metals, or in the presence of sex hormones such as testosterone (Chelyadina et al., 2018, 2022, 2024). However, the duration of the exposure (30 days) was higher than in the present case. Although the changes of these environmental factors were not observed in the present case, sex determination in the present experiment was only made when mussels were collected at the beginning and at the end of exposure period to avoid further mussel stress or even mortality. However, it would be desirable to use the RT-qPCR sex identification assay developed by Evensen et al. (2024) to assess if sex reversal occurs during the experiment to confirm that all these protein changes in males were not the result of sex reversal of females.

3.4. Impact of PS-NP exposure in non-exposed and exposed mussel female gonads

Opposite to the effect of NPs in mussel male gonads, the exposure of mussel female gonads to PS-NPs compared to controls induced fewer protein changes at BP, CC and MF levels (Fig. 4). This might be related to the variability observed in the gonads of mussel females exposed to PS-NPs. The exposure to PS-NPs induced changes in seven proteins involved in BP, six of which were downregulated, and one was upregulated, while with CC, four proteins were up-regulated. At the same time, only one was down-regulated, and four proteins related to MF were down-regulated ($p < 0.05$). Pujol et al. (2025) also found that in zebrafish (*Danio rerio*) exposed for 96 h to a toxic concentration of the same type of NP (45 nm; 5 mg L^{-1}), oocytes were not as severely affected by PS-NPs exposure as the male gonads. This indicates that PS-NPs have a different impact on oocytes.

The exposure of mussel female gonads to PS-NPs inhibited the following proteins: vesicle-mediated transport, mRNA processing, establishment or maintenance of microtubule cytoskeleton polarity, intracellular protein transport, spindle organisation and microtubule polymerisation. Vesicle-mediated transport is crucial for the movement of proteins and lipids within the cell, especially between organelles like the endoplasmic reticulum (ER) and Golgi apparatus, and its inhibition could disrupt oocyte development by altering hormone signaling, nutrient storage, and protein secretion. The inhibition of mRNA processing affects gonad development and oocyte maturation by preventing protein translation required for cellular function. The establishment or maintenance of microtubule cytoskeleton polarity is essential for the maintenance of cell shape, intracellular transport, and cell division. The inhibition of this protein disrupts cell division and positioning of organelles during oogenesis. The downregulation of intracellular protein transport disrupts energy production, meiotic progression, and hormone biosynthesis, and the inhibition of spindle organisation can lead to aneuploidy in oocytes, reduce fertility or cause developmental abnormalities in offsprings. Moreover, inhibiting microtubule polymerisation by exposure to PS-NPs could impair meiotic progression, disrupt vesicle transport, and lead to oocyte or gonadal cell dysfunction. On the other hand, nucleosome assembly was induced to enhance the formation or restoration of nucleosomes, which can have several implications in different pathways, namely chromatin remodeling, gene transcription, and DNA damage repair. So, exposure to PS-NPs severely affects the meiotic cell cycle, cytoskeleton regulation, vesicle transport, mRNA processing, and hormonal signaling pathways.

The inhibition of these proteins affects the cytoskeleton organisation and dynamics, disrupting spindle formation and intracellular transport that are critical for oocyte maturation. The intracellular transport, vesicle-mediated transport pathway, spindle organisation, and chromosome segregation induce impaired spindle assembly that could disrupt meiosis. The inhibition of the energy metabolism and ATP-dependent processes pathway affects the ATPase and proton transport, which are critical for energy supply during oocyte development. The disruption of the GTPase-mediated signaling pathway affects the GTP-binding proteins that regulate vesicle trafficking and cytoskeleton assembly. GTPase activity activates the MAPk/ERK signaling pathway, which is involved in cell growth and differentiation and regulates actin polymerisation. Moreover, microtubule plus-end binding is crucial in cellular processes like mitosis, transport and cell shape maintenance. The impaired aminopeptidase activity could hinder protein turnover necessary for cellular remodeling, inducing changes in the aminopeptidase activity and protein degradation pathway. In Gonçalves et al. (2025), female gonads exposed to PS-NPs (50 nm; 10 μgL^{-1}; 21 days), the exponential increase in SOD, catalase (CAT), and GST activities did not prevent oxidative damage. Cellular stress is caused mainly by disrupting protein homeostasis, organelle function, and DNA integrity caused by suppressing vesicle-mediated transport, mRNA processing, microtubule dynamics, protein transport, and spindle organisation. Reactive oxygen species (ROS) produced by these disturbances lead to oxidative stress. Antioxidant defense mechanisms, such as SOD, CAT, and GST in mussel female gonads are upregulated by the cell to counteract ROS production and detoxify reactive compounds (Gonçalves et al., 2025).

Nevertheless, despite these efforts, LPO levels rise, indicating continuous oxidative damage, especially to cell membranes susceptible to ROS damage. The image shows a stressed cell strengthening its antioxidant defences to compensate for various dysfunctions. However, female gonads still suffer severe oxidative damage (Gonçalves et al., 2025).

The exposure of PS-NPs in mussel female gonads induced an upregulation in the nucleosome assembly, which is involved in the deposition of histones onto DNA to form nucleosomes, the basic unit of chromatin that was also induced, highlighting the need for histone synthesis to package replicated DNA during oocyte maturation. The upregulation of nucleosome assembly and related proteins suggests enhanced chromatin remodeling, transcriptional regulation, and genome stability processes. These changes are pivotal for oocyte maturation, meiosis, and preparation for fertilisation. The increase in SOD, CAT, GST activity and LPO levels observed in female gonads exposed to PS-NPs for 21 days (50 nm; 10 μgL^{-1}) are indicators of oxidative stress caused by the exposure (Gonçalves et al., 2025). The cell upregulates nucleosome assembly and associated proteins involved in DNA packing and chromatin remodeling in response to oxidative damage. This implies that to maintain healthy oocyte maturation and genome integrity, the cell is trying to shield its genome from the harmful effects of ROS and lipid peroxidation. While the upregulation of histone production and nucleosome assembly emphasises the cell's attempt to stabilise and repair the genome under this stressful exposure, the rise in antioxidant enzyme activity is part of the cell's attempt to combat oxidative damage (Pérez-Cremades et al., 2023).

3.5. Differences in protein expression between mussels' male and female gonads exposed to PS-NPs

When comparing differential protein expression between mussel male and female gonads, three GO terms were expressed in males associated with the molecular function of the proteins namely: GTPase activity, GTP binding and ferric iron binding ($p < 0.05$; Fig. 4). GTPase activity controls processes that underline gametogenesis and maturation. At the same time, GTP binding hydrolysis into GDP regulates various molecular functions in the gonads, while ferric iron binding is related to cellular respiration, DNA synthesis and enzyme function. These proteins are indispensable for the metabolic and enzymatic processes needed for successful reproduction in mussels.

When male and female gonads were exposed to PS-NPs, six proteins were up-regulated in male mussels, namely, fucose metabolic process, alpha-L-fucosidase activity, lipid transporter activity, hydrolase activity, structural constituent of cytoskeleton, and cysteine-type peptidase activity ($p < 0.05$; Fig. 4). This indicates that exposure to PS-NPs increases the fucosylation of proteins and lipids, enhancing cell communication and the immune system. On the other hand, alpha-L-fucosidase activity catalyses the hydrolysis of alpha-L-fucoside bonds, breaking down fucosylated glycoconjugates and causing lysosomal stress. In addition, the upregulation of lipids transport activity and hydrolase activity increases energy production and membrane synthesis. On the other hand, the structural constituent of cytoskeleton proteins maintains and regulates the structure and dynamics of the cytoskeleton (e.g., actin, tubulin) and its upregulation promotes immune responses (Dalle-Donne et al., 2001). Regarding cysteine-type peptidase activity, caspases and cathepsins use cysteine residues to cleave peptide bonds and induce apoptosis (Pujol et al., 2025).

On the other hand, PS-NP exposure induces the downregulation of the nitrogen compound metabolic process, nucleosome, structural constituent of chromatin and protein heterodimerisation activity ($p < 0.05$; Fig. 4). The downregulation of the nitrogen compound metabolic process involves the breakdown and recycling of nitrogen-containing compounds, like amino acids and nucleotides, impairing the amino acid metabolism, reducing growth and nucleotide synthesis and weakening cellular repair mechanisms by impacting DNA replication and RNA transcription, slowing cell proliferation and repair. The nucleosome and structural constituent of chromatin are the basic structural units comprising transcription and DNA wrapped around histone proteins. These two proteins are essential to organise and regulate DNA accessibility for replication and repair into a compact and functional structure of the cells. In male *D. rerio* gonads exposed to an acute concentration of PS-PNs (45 nm, 5 mg L^{-1}) for 96 h, abnormal sperm and clustering chromatin compact was observed that result in viable spermatozoids but with reduced motility that could lead to functional impairment that may be the result of oxidative stress and DNA damage (Pujol et al., 2025). Similarly in mussel males these changes can also be related to changes in the oxidative status once an increase of SOD and GST was also observed in mussel male gonads exposed to the same type and concentration of PS-NPs (Gonçalves et al., 2025). Gene expression involved in reproduction and chromatin remodeling was also observed in *D. rerio* testis as well as in the immune system, indicating that PS-NPs induce an increase in the immune response to cope with the phagocytosis of this type of NPs (Pujol et al., 2025). The downregulation of these proteins in male gonads due to PS-NP exposure can lead to DNA damage, mutations, and chromosomal abnormalities. In addition, the downregulation of protein heterodimerisation activity can affect cell growth and immune response and induce apoptosis. In male *D. rerio,* exposure to acute toxic concentrations of NPs (45 nm) induces caspase-3 levels, indicating that apoptosis occurred in prophase I (Pujol et al., 2025). These results indicate similar responses to the effects of PS-NPs at the genome and proteome level in zebrafish and *M. galloprovincialis* male gonads.

In summary, the exposure of PS-NPs in male gonads induces significant changes in the differential expression of these proteins, due to changes in the gonads oxidative status, leading to an increase in energy production and immune responses, alterations in lipid metabolism and carbohydrate pathways, DNA damage, as well as apoptosis. The elevated levels of these proteins in male mussel gonads reflect the physiological and reproductive demands of continuous sperm production, chromatin compaction, gamete motility, and metabolic regulation, responding to the effects induced by exposure to PS-NPs, which is more pronounced in males.

4. Conclusion

A quantitative proteomic analysis was conducted to assess the effects of PS-NPs in *M. galloprovincialis* male and female gonads. The results suggest that the accumulation of PS-NPs affects protein expression in male gonads more than in females. It modulates protein synthesis, energy production, intracellular transport, and cellular homeostasis, potentially leading to infertility. Regarding the effects of PS-NPs in female gonads, proteomic analysis revealed that exposure to PS-NPs disrupts proteins that are related to translation, RNA processing and signaling, ribosome biogenesis, cell cycle, and stress response, protein folding, lipid metabolism, and calcium signaling pathways impairing oogenesis, meiotic progression, intracellular trafficking, and energy metabolism affecting the reproductive success and cellular homeostasis. Therefore, with the increase of NPs expected to occur, there is an urgent need to reduce the presence of NPs in the ocean because this emerging contaminant may be an additional source that can induce biodiversity loss.

CRediT authorship contribution statement

Joanna M. Gonçalves: Investigation, Formal analysis, Data curation. **Vera M. Mendes:** Validation, Methodology, Formal analysis. **Bruno Manadas:** Validation, Methodology, Investigation, Formal analysis. **Maria João Bebianno:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Funding acquisition, 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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2025.180181.

Data availability

Data will be made available on request.

References

Anjo, S.I., Santa, C., Manadas, B., 2015. Short GeLC-SWATH: a fast and reliable quantitative approach for proteomic screenings. Proteomics 15 (4), 757–762, 508. https://doi.org/10.1002/pmic.201400221.

Apraiz, I., Mi, J., Cristobal, S., 2006. Identification of proteomic signatures of exposure to marine pollutants in mussels (*Mytilus edulis*). Mol. Cell. Proteomics 5 (7). https://doi.org/10.1074/mcp.M500333-MCP200, 1274–511 1285.

Auguste, M., Balbi, T., Miglioli, A., Alberti, S., Prandi, S., Narizzano, R., Salis, A., Damonte, G., Canesi, L., 2021. Comparison of different commercial nanopolystyrenes: behavior in exposure media, effects on immune function and early larval development in the model bivalve *mytilus galloprovincialis*. Nanomaterials 11 (12). https://doi.org/10.3390/nano11123291.

Bardou, P., et al., 2014. jvenn: an interactive Venn diagram viewer. BMC Bioinformatics 15 (1), 518, p. 293.

Bebianno, M.J., Mendes, V.M., O'Donovan, S., Carteny, C.C., Keiter, S., Manadas, B., 2022. Effects of microplastics alone and with adsorbed benzo(a)pyrene on the gills proteome of *Scrobicularia plana*. Sci. Total Environ. 842. https://doi.org/10.1016/j.scitotenv.2022.156895.

Capolupo, M., Valbonesi, P., Fabbri, E., 2021. A comparative assessment of the chronic effects of micro-and nano-plastics on the physiology of the mediterranean mussel *mytilus galloprovincialis*. Nanomaterials 11 (3), 1–17. https://doi.org/10.3390/nano11030649.

Chelyadina, N., Pospelova, N., Popov, M., Smyrnova, L., Kharchuk, I., Ryabushko, V., 2018. Sex inversion in cultivated mussels Mytilus galloprovincialis lam. (Crimea, Black Sea) under influence of external environmental factors. Ecologica Montenegrina 19, 26–31. https://doi.org/10.37828/em.2018.19.3.

Chelyadina, N.S., Popov, M.A., Pospelova, N.V., Smyrnova, L.L., 2022. Effects of heavy metals on sex inversion of the mussel *Mytilus galloprovincialis* Lam., 1819 in coastal zone of the Black Sea. Mar. Pollut. Bull. 185, 114323. https://doi.org/10.1016/J.MARPOLBUL.2022.114323.

Chelyadina, N.S., Popov, M.A., Pospelova, N.v, 2024. The effect of sex hormones on sex inversion in the mussel Mytilus galloprovincialis. Mar. Environ. Res. 201, 106710. https://doi.org/10.1016/J.MARENVRES.2024.106710.

Dalle-Donne, I., Rossi, R., Milzani, A., di Simplicio, P., Colombo, R., 2001. The actin cytoskeleton response to oxidants: from small heat shock protein phosphorylation to changes in the redox state of actin itself. Free Radic. Biol. Med. 31 (12), 1624–1632. https://doi.org/10.1016/S0891-5849(01)00749-3.

Evensen, K.G., Rusin, E., Robinson, W.E., Price, C.L., Kelly, S.L., Lamb, D.C., Goldstone, J.v, Poynton, H.C., 2024. Vertebrate endocrine disruptors induce sex-reversal in blue mussels. Sci. Rep. 14 (1), 23890. https://doi.org/10.1038/s41598-024-74212-y.

Gagné, F., 2019. Detection of polystyrene nanoplastics in biological tissues with a fluorescent molecular rotor probe. Journal of Xenobiotics 9, 7–9. https://doi.org/10.4081/xeno.2019.8147.

Gallo, A., Esposito, M.C., Tosti, E., Boni, R., 2021. Sperm motility, oxidative status, and mitochondrial activity: exploring correlation in different species. Antioxidants 10 (7). https://doi.org/10.3390/antiox10071131.

Gallo, A., Esposito, M.C., Boni, R., Tosti, E., 2022. Oocyte quality assessment in marine invertebrates: a novel approach by fluorescence spectroscopy. Biol. Res. 55 (1). https://doi.org/10.1186/s40659-022-00403-4.

Gonçalves, J.M., Sousa, V.S., Teixeira, M.R., Bebianno, M.J., 2022. Chronic toxicity of polystyrene nanoparticles in the marine mussel *Mytilus galloprovincialis*. Chemosphere 287. https://doi.org/10.1016/j.chemosphere.2021.132356.

Gonçalves, J.M., Benedetti, M., d'Errico, G., Regoli, F., Bebianno, M.J., 2025. Gender effects of nanoplastics and emerging contaminants mixtures in *Mytilus galloprovincialis*. Aquat. Toxicol. 279, 107219. https://doi.org/10.1016/j.aquatox.2024.107219.

González-Fernández, C., Tallec, K., le Goïc, N., Lambert, C., Soudant, P., Huvet, A., Suquet, M., Berchel, M., Paul-Pont, I., 2018. Cellular responses of Pacific oyster (*Crassostrea gigas*) gametes exposed in vitro to polystyrene nanoparticles. Chemosphere 208, 764–772. https://doi.org/10.1016/j.chemosphere.2018.06.039 549.

Green, D.S., Colgan, T.J., Thompson, R.C., Carolan, J.C., 2019. Exposure to microplastics reduces attachment strength and alters the haemolymph proteome of blue mussels (*Mytilus edulis*). Environ. Pollut. 246, 423–434. https://doi.org/10.1016/j.envpol.2018.12.017 553.

Gurel, P.S., Hatch, A.L., Higgs, H.N., 2014. Connecting the cytoskeleton to the endoplasmic reticulum and Golgi. In: Current Biology, 24(14). Cell Press. https://doi.org/10.1016/j.cub.2014.05.033.

Huang da, W., Sherman, B.T., Lempicki, R.A., 2009. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat. Protoc. 4 (1), 44–57.

Kautsky, N., 1982. Growth and size structure in a Baltic *Mytilus edulis* population. Mar. Biol. 68, 117–133. https://doi.org/10.1007/BF00397599.

Kögel, T., Bjorøy, Ø., Toto, B., Bienfait, A.M., Sanden, M., 2020. Micro- and nanoplastic toxicity on aquatic life: determining factors. In: Science of the Total Environment, vol. 709. Elsevier B.V. https://doi.org/10.1016/j.scitotenv.2019.136050.

Lewis, C., Ford, A.T., 2012. Infertility in male aquatic invertebrates: a review. Aquat. Toxicol. 120–121, 79–89. https://doi.org/10.1016/j.aquatox.2012.05.002.

Li, Z., Xian, H., Ye, R., Zhong, Y., Liang, B., Huang, Y., Dai, M., Guo, J., Tang, S., Ren, X., Bai, R., Feng, Y., Deng, Y., Yang, X., Chen, D., Yang, Z., Huang, Z., 2024. Gender-specific effects of polystyrene nanoplastic exposure on triclosan-induced reproductive toxicity in zebrafish (*Danio rerio*). Sci. Total Environ. 932. https://doi.org/10.1016/j.scitotenv.2024.172876.

López-Pedrouso, M., Varela, Z., Franco, D., Fernández, J.A., Aboal, J.R., 2020. Can proteomics contribute to biomonitoring of aquatic pollution? A critical review. In: Environmental Pollution, vol. 267. Elsevier Ltd. https://doi.org/10.1016/j.envpol.2020.115473

Ma, S., Wang, L., Li, S., Zhao, S., Li, F., Li, X., 2024. Transcriptome and proteome analyses reveal the mechanisms involved in polystyrene nanoplastics disrupt spermatogenesis in mice. Environ. Pollut. 342. https://doi.org/10.1016/j.envpol.2023.123086.

Martínez-Pita, I., Sánchez-Lazo, C., Ruíz-Jarabo, I., Herrera, M., Mancera, J.M., 2012. Biochemical composition, lipid classes, fatty acids and sexual hormones in the mussel *Mytilus galloprovincialis* from cultivated populations in south Spain. Aquaculture 358–359, 274–283. https://doi.org/10.1016/j.aquaculture.2012.06.003.

Mikhailov, A.T., Mendez, J., 1995. Sexual differentiation of reproductive tissue in bivalve molluscs: identification of male associated polypeptide in the mantle of *Mytilus galloprovincialis* Lmk. Int. J. Dev. Biol. 39 (3), 545–548. https://www.researchgate.net/publication/15622565.

Pérez-Cremades, D., Bueno-Betí, C., García-Giménez, J.L., Ibañez-Cabellos, J.S., Pallardó, F.V., Hermenegildo, C., Novella, S., 2023. Extracellular histones trigger oxidative stress- dependent induction of the NF-kB/CAM pathway via TLR4 in endothelial cells. J. Physiol. Biochem. 79 (2), 251–260. https://doi.org/10.1007/s13105-022-00935-z.

Plastics Europe, 2023. Plastics- the fast facts 2023. https://plasticseurope.org/knowledge-hub/plastics-the-fast-facts-2023/.

Pujol, G., Marín-Gual, L., González-Rodelas, L., Álvarez-González, L., Chauvigné, F., Cerdà, J., Teles, M., Roher, N., Ruiz-Herrera, A., 2025. Short-term polystyrene nanoplastic exposure alters zebrafish male and female germline and reproductive outcomes, unveiling pollutant- impacted molecular pathways. J. Hazard. Mater. 481. https://doi.org/10.1016/j.jhazmat.2024.136529.

Qi, P., Qiu, L., Feng, D., Gu, Z., Guo, B., Yan, X., 2023. Distinguish the toxic differentiations between acute exposure of micro- and nano-plastics on bivalves: an integrated study based on transcriptomic sequencing. Aquat. Toxicol. 254. https://doi.org/10.1016/j.aquatox.2022.106367.

Rangaswamy, B., An, J., Kwak, I.S., 2024. Different recovery patterns of the surviving bivalve *Mytilus galloprovincialis* based on transcriptome profiling exposed to spherical or fibrous polyethylene microplastics. Heliyon 10 (10). https://doi.org/10.1016/j.heliyon.2024.e30858.

Shang, Y., Chang, X., Khan, F.U., Dupont, S., Hu, M., Wang, Y., 2025. Sex-specific trade-off under marine heat wave and food limitation: insights from bioenergetic and intestinal microbiome in mussels. Aquaculture 594, 741440. https://doi.org/10.1016/j.aquaculture.2024.741440.

Sherman, B.T., et al., 2022. DAVID: a web server for functional enrichment analysis and functional annotation of gene lists (2021 update). Nucleic Acids Res. 50, W216–W221.

Shi, C., Liu, Z., Yu, B., Zhang, Y., Yang, H., Han, Y., Wang, B., Liu, Z., Zhang, H., 2024. Emergence of nanoplastics in the aquatic environment and possible impacts on aquatic organisms. Sci. Total Environ. 906, 167404. https://doi.org/10.1016/J.SCITOTENV.2023.167404.

Sussarellu, R., Suquet, M., Thomas, Y., Lambert, C., Fabioux, C., Pernet, M.E.J., Goïc, N. le, Quillien, V., Mingant, C., Epelboin, Y., Corporeau, C., Guyomarch, J., Robbens, J., Paul-Pont, I., Soudant, P., Huvet, A., 2016. Oyster reproduction is affected by exposure to polystyrene microplastics. Proc. Natl. Acad. Sci. USA 113 (9), 2430–2435. https://doi.org/10.1073/pnas.1519019113.

Tallec, K., Huvet, A., di Poi, C., González-Fernández, C., Lambert, C., Petton, B., le Goïc, N., Berchel, M., Soudant, P., Paul-Pont, I., 2018. Nanoplastics impaired oyster free living stages, gametes and embryos. Environ. Pollut. 242, 1226–1235. https://doi.org/10.1016/j.envpol.2018.08.020.

Tallec, K., Blard, O., González-Fernández, C., Brotons, G., Berchel, M., Soudant, P., Huvet, A., Paul-Pont, I., 2020. Surface functionalization determines behavior of nanoplastic solutions in model aquatic environments. Chemosphere 225, 639–646. https://doi.org/10.1016/j.chemosphere.2019.03.077.

Tang, D., et al., 2023. SRplot: a free online platform for data visualization and graphing. PLoS One 18 (11), e0294236.

Tong Chen, Y.-X.L., 2022. Luqi Huang, ImageGP: an easy-to-use data visualization web server for scientific researchers. iMeta 1 (1).

Wegner, A., Besseling, E., Foekema, E.M., Kamermans, P., Koelmans, A.A., 2012. Effects of nanopolystyrene on the feeding behavior of the blue mussel (*Mytilus edulis* L.). Environ. Toxicol. Chem. 31 (11), 2490–2497. https://doi.org/10.1002/etc.1984.

Zhang, W., Wu, F., 2023. Effects of adverse fertility-related factors on mitochondrial DNA in the oocyte: a comprehensive review. In: Reproductive Biology and Endocrinology, vol. 21, 633 Issue 1. BioMed Central Ltd. https://doi.org/10.1186/s12958-023-01078-6.

Zhong, M., Wu, H., Li, F., Shan, X., Ji, C., 2020. Proteomic analysis revealed gender-specific responses of mussels (*Mytilus galloprovincialis*) to trichloropropyl phosphate (TCPP) exposure. Environ. Pollut. 267. https://doi.org/10.1016/j.envpol.2020.115537.