



Nanoplastic and metal-oxides transcriptomic responses and oxygen consumption analysis in the Antarctic clam *Laternula elliptica*

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ABSTRACT

Human activities are impacting even the most remote regions of our planet, including Antarctica. The Fildes Bay coastal area (King George Island) is among those considered more at risk to anthropogenic stressors, with increasing concern on consequences to marine life. Here we investigated the potential hazard posed by contaminants of emerging concern (CECs) as nanoplastics and metal-oxide nanomaterials either as single or combined exposure in the Antarctic clam *Laternula elliptica*. The digestive gland was used to assess the transcriptomic response and oxygen consumption upon in vivo exposure to polystyrene (PS-COOH-NP) and titanium-dioxide nanoparticles (TiO₂-NP) at environmental realistic concentrations (5–50 µg · L⁻¹). A total of 2852 differentially expressed genes were found in clams exposed to either single contaminant or in combination versus the control group. The transcript functions affected included autophagy, cell cycle, proteases co-chaperones and their transcription factors, antioxidants, membrane fatty acids, cell pH balance enzymes, mitochondria stress and ATP production in PS-COOH-NP (5 µg · L⁻¹) and TiO₂-NP (5 and 50 µg · L⁻¹) groups as well as in co-exposure at 50 µg · L⁻¹. Exposure to TiO₂-NP (5 and 50 µg · L⁻¹) and co-exposure at 50 µg · L⁻¹ affected transmembrane transport enzymes and histones. Co-exposure at 5 µg · L⁻¹ shows ribosomal proteins, protease and apoptosis transcripts affected and PS-COOH-NP (at 50 µg · L⁻¹ only) protease as transcripts with known functions. Oxygen consumption decreased significantly upon combined exposure to nano-pollutants at the two concentrations, suggesting a synergistic effect impairing clam metabolism. Our results highlighted several molecular pathways affected by treatments and metabolism decreased in co-exposures, demonstrating the hazardous behavior and toxicity of these nanoparticles.

Introduction

Human activities are impacting even the most remote regions of our planet including Antarctica. It is commonly recognized that anthropogenic pollution in Antarctica can originate from either global or local sources (da Silva et al., 2023). In King George Island (South Shetland

Islands), marine coastal areas such as Fildes Bay are among those considered more at risk with increasing concern of consequences on marine life, as human activities such as logistics and the expansion of station facilities are increasing (Convey, 2024). Nowadays, there is an increasing evidence of plastic pollution in Antarctica, with evidence of microplastics (below 5 mm) of various shape found in sea ice (Kelly

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et al., 2020), surface waters and sediments (Antaceli et al., 2024; Aves et al., 2022; Cunningham et al., 2022; Isobe et al., 2017; Lacerda et al., 2019; Leistenschneider et al., 2024, 2021; Monrás-Riera et al., 2023; Munari et al., 2017; Rowlands et al., 2023; Suaria et al., 2020) and in marine wildlife (Bergami et al., 2023; Bessa et al., 2019; Bottari et al., 2022; Corti et al., 2023; Geng et al., 2023; González-Aravena et al., 2024; Gonzalez-Pineda et al., 2024; Leuenberger et al., 2024; Lv et al., 2024; Mancuso et al., 2023; Sfriso et al., 2020; Wilkie Johnston et al., 2023; Zhu et al., 2023a, 2023b). This issue is further intensified by certain planktonic species, such as the Antarctic krill that are capable of fragmenting microplastic into nanoplastic (Dawson et al., 2018), which may increase bioavailability for filter-feeder organisms depending on particle properties. Additionally, wastewater discharges from research stations, fishing activities and tourism vessels may also introduce these pollutants into Antarctic waters (Rota et al., 2022). The combination of natural transformation processes by marine organisms and direct human inputs creates a complex pollution scenario that poses significant challenges for conservation efforts and ecosystem management in the Antarctic region.

Regarding engineered metal oxide nanoparticles (NPs), many personal care products used in Antarctic scientific stations and ending up into sewages contain them such as sunscreen, including titanium-dioxide NPs (TiO₂-NP) (Musial et al., 2020; Weir et al., 2012). However, only a few studies have been conducted in Antarctica on marine benthic species in relation to such exposure. For instance, TiO₂-NP has been detected in other regions, such as the Mediterranean Sea, where its concentration increases during the summer because of its use as an inorganic UV filter in sunscreen products (Labille et al., 2020). Similarly to nanoplastics, engineered metal oxide NPs deserve a high attention for Antarctic marine organisms to determine the effects in key species at sublethal concentrations. Although there is increasing evidence and probabilities of the presence of these contaminants of emerging concern (CECs), there is currently a gap about their effects on Antarctic marine biota. This gap mainly exists for environmental realistic and sublethal concentrations and particularly in key physiological parameters such as oxygen (O₂) consumption as a proxy of metabolism and global gene expression as an early molecular response to the pollutant using a metabolic important organ.

Currently few studies have addressed efforts to determine the toxicological effects of NPs integrating macro-physiological approach, such as O₂ consumption, and molecular physiology such as transcriptomic analyses, even less assessing mixture of two types of particles. Both nanoplastic and metal oxide NPs exposure are now raising concerns about their toxic and hazardous outcomes in marine species. Although these CECs have not yet been regulated, there is evidence of their physiological and molecular toxicity in aquatic organisms (Gardon et al., 2024; Imran et al., 2024; Lu et al., 2023; Queiroz et al., 2024; Rondon et al., 2024; Zhu et al., 2024). In aquatic metazoans worldwide, engineered NPs have been found to cause negative physiological and molecular effects following single exposures. Mainly, they have been linked to alterations in O₂ consumption in fish, suggesting metabolism impairment such as O₂ consumption increase (Chackal et al., 2022) and also provoking decrease of this physiological parameter in fish and bivalve species (Vidya et al., 2016; Zha et al., 2022). At the molecular level, transcriptomics studies on the digestive glands of mollusks have revealed significant changes. For instance, individuals of the Mediterranean mussel *Mytilus galloprovincialis* exposed to TiO₂-NP have shown an enrichment of upregulated genes associated with cytoskeletal biological processes (Banni et al., 2016). In addition, the combination of TiO₂-NP with 2,3,7,8-tetrachlorodibenzo-p-dioxins (TCDD) produced an enrichment of differentially expressed genes associated with response to chemical stimulus. Similarly, short-term experimental exposure of *M. galloprovincialis* to PVP/PEI coated Ag-NPs led to significant regulation of genes associated with cytoskeleton, lipid transport and metabolism during spring while carbohydrate transport and metabolism were specifically altered during autumn (Duroudier et al., 2019). Despite

these findings, few studies have explored the synergistic effects of these two types of nano-pollutants in marine metazoans (Rondon et al., 2024b), and even less on Antarctic filter feeders that play an important role in the benthic ecosystem.

In terms of current knowledge on effects on Antarctic species this is still limited, and there are no reports of studies examining their effects on digestive gland. Considering that the digestive gland is the ultimate destination for these nanopollutants, and as mentioned by Canesi and Corsi (2016), as it serves as the primary site of NP accumulation in filter-feeder bivalves, it is anticipated that the effects on this organ would be even more pronounced. Also, this is a key metabolic organ for the whole organism, and the results could be a support to respirometry data. Among the available research, one study examined the effects of surface-charged polystyrene nanoparticles (PS-NPs) on the immune cells (coelomocytes) of the Antarctic sea urchin *Sterechnus neumayeri*. In this study, fluorescently labelled PS-COOH-NP were found to modulate the expression of genes related to antioxidant responses and cell protection against stress and apoptosis (Bergami et al., 2019). In another work on the effects of TiO₂-NP on the fairy shrimp *Branchinecta gainii* it was found that the gene Hsp70a was significantly upregulated at 200 µg · ml⁻¹ TiO₂-NP. Similarly, cytochrome P450 expression increased at both 100 and 200 µg · ml⁻¹ TiO₂-NP. In contrast, the expression levels of cathepsin L and antioxidant-related genes such as superoxide dismutase and glutathione peroxidase, were significantly reduced with the highest concentrations of TiO₂-NP (100 and 200 µg · ml⁻¹) (González-Aravena et al., 2022).

Nanoplastics has been found in Antarctic sea-ice at the average concentration of 52.3 µg · L⁻¹ (Materić et al., 2022). Therefore in our study, tested concentrations on the Antarctic soft-shelled clam *Laternula elliptica* were close as 5 and 50 µg · L⁻¹ in line with previous studies (Al-sid-cheikh et al., 2018; Sun et al., 2016). O₂ consumption analysis as a proxy of metabolism status and complemented with genome-wide gene expression approach to determine the possibility of congruent results has not yet been explored in aquatic metazoan, and it is important to investigate it in a benthic filter-feeder species. Taking this information into account, we hypothesize that single and mixed exposure of plastic and engineered metal oxide NPs could have an effect in O₂ consumption and gene expression of relevant molecular pathways, in a metabolic key organ as the digestive gland of the Antarctic clam *L. elliptica*. We also expected to find different levels of effects at the two concentrations studied. The aim of the present study was to determine if metabolic impairment occurs following exposure to nanoplastics and metal-oxide NPs and if transcriptomic data could bring support suggesting this possible impairment. Furthermore, it could reveal novel results investigating whether transcriptomic alterations in the digestive gland could be associated with O₂ consumption. Several gene expression changes were observed following nanoparticle exposure, with synergistic treatments also affecting O₂ consumption.

Methodology

A total of 120 adult individuals of the Antarctic soft-shelled clam *L. elliptica* collected by SCUBA diving in January 2020 at Fildes Bay, King George Island (South Shetland Islands), with an average shell length of 71.66±11.75 mm, mean ± standard deviation, SD were used for the study. Preparation of NP suspensions and experimental exposure are fully described below and for a further reference in our previous study (Rondon et al., 2024b).

Carboxyl-modified polystyrene NPs (PS-COOH-NP) had a nominal size of 62 nm and were purchased from Bangs Laboratories Inc. (catalog code: PC02N, suspension at 10.1%). TiO₂-NP were kindly provided by Degussa Evonik as powder composed of 82–18% anatase - rutile crystal structure (Aeroxide ® P25) having a nominal size of 25 nm. The stock suspension of PS-COOH-NP was used to prepare an intermediate suspension in MilliQ water at the concentration of 10 mg · mL⁻¹ in order to prepare two working solutions of 250 and 25 µg · mL⁻¹ diluting and

sonicating with the bath sonicator DENTSPLY Ultra-sonik 57 H for 2 min and then vortexing prior to use according to (Bergami et al., 2020). These two working suspensions at 250 and 25 $\mu\text{g} \cdot \text{mL}^{-1}$ were used in the experimental exposure by adding 1 mL of each suspension to 5 L tanks of natural sea water (NSW) to achieve final concentrations of 50 and 5 $\mu\text{g} \cdot \text{L}^{-1}$, respectively. Concerning the TiO_2 -NP solutions, first a stock suspension (10 $\text{mg} \cdot \text{mL}^{-1}$) was prepared in MilliQ water, vortexed and sonicated for 60 min, always keeping the suspension in ice, in order to avoid any damage of nanoparticles (changing water each ~ 10 min) (Della Torre et al., 2015). Similar to nanoplastics, two intermediate suspensions were prepared at 250 and 25 $\mu\text{g} \cdot \text{mL}^{-1}$, and 1 mL of each was added to 5 L tanks containing NSW to reach concentrations of 50 and 5 $\mu\text{g} \cdot \text{L}^{-1}$, respectively. The NSW used for the experiments had a temperature of $0 \pm 1^\circ\text{C}$, pH of 8.42 and salinity of 32.8, and was taken from Fildes Bay where the specimens were also collected. Both tested suspensions of PS-COOH and TiO_2 were replaced every 24 h for a semi-static in vivo exposure.

The Dynamic Light Scattering (DLS) and electrophoretic mobility were used for secondary characterization of PS-COOH-NP (at 50 $\mu\text{g} \cdot \text{mL}^{-1}$, following Bergami et al., 2019, 2020) and TiO_2 -NP (at 5 $\mu\text{g} \cdot \text{mL}^{-1}$, following Della Torre et al., 2015). Such high concentrations were used only to run DLS analyses, in line with previous studies, due to limitation of the instrument to characterize particles below 1 $\mu\text{g} \cdot \text{mL}^{-1}$. In order to allow the removal of large suspended natural colloids that would interfere with the analysis, the NSW was first filtered at 0.22 μm . Three independent measurements were obtained at 0°C (Z-average) and 4°C (ζ -potential) for nanoparticle size-related parameters (e.g., Z-average, expressed in nm) and surface charge (ζ -potential, expressed in mV) evaluations.

For the experimental design firstly, 2 days of acclimation of the specimens were achieved in well oxygenated NSW with constant temperature, pH and salinity, two independent experiments (96 h) at each concentration used were carried out, in order to avoid different stress noise of results. Individuals ($n = 5$) were placed in 5 L tanks (three replicate tanks for condition), with the following test solutions: NSW only (controls), PS-COOH-NP at 5 and 50 $\mu\text{g} \cdot \text{L}^{-1}$, TiO_2 -NP at 5 and 50 $\mu\text{g} \cdot \text{L}^{-1}$ and combined exposure (PS-COOH-NP and TiO_2 -NP) at 5 and 50 $\mu\text{g} \cdot \text{L}^{-1}$. The 5 L tanks (dimension $32 \times 16 \times 42$ cm) with specimens were maintained with a light/obscurity interval photoperiod of 18/6 h and were permanently well oxygenated. After 96 h, clams were dissected for tissue extractions (digestive glands), frozen with liquid nitrogen, put in 1 mL of RNAlater and kept at -80°C .

Thirty-nine clam individuals were used for O_2 consumption analyses (5 individuals per condition; excluding the control at 5 $\mu\text{g} \cdot \text{L}^{-1}$ condition having 4 individuals). The clams were placed in 1 L glass respirometry chambers with 18 cm high and 9 cm of circumference, hermetic closed, for up to 5 h all individuals at the same time and O_2 concentrations were measured, as $\text{O}_2\%$ saturation at the beginning and the end with an O_2 -sensitive sensor type pst3 (model Fibox4, Presens). A blank chamber without organisms was used as a control. Then, % O_2 was transformed to mg O_2 considering temperature recorded and salinity and atmospheric pressure of Fildes Bay according to Benson and Krause table, and afterwards O_2 consumption were calculated for time and wet weight ($\text{mg O}_2 \cdot \text{h}^{-1} \cdot \text{g}^{-1}$), with the following formula: $\text{VO}_2 = ((C_i - C_f) \cdot V) / (W \cdot T)$ (Data in supplementary material 1). Normality and homoscedasticity assumption of data were checked for size of clams with Shapiro and Bartlett test respectively, then two Kruskal-Wallis analysis was performed to evaluate significant differences between treatments and concentrations with RStudio (version 2026.01.1–403) using the package rstatix. Due that no significant differences of size between treatments and concentrations were revealed, an ANOVA-Two-Way was performed with the same version of Rstudio and the packages rstatix version 0.7.3 (Kassambara, 2025) and a post-hoc analysis of Tukey with emmeans packages version 2.0.1 (Lenth and Piaskowski, 2025). The boxplot figure was created using packages ggplot2 (Wickham, 2016) and viridis 0.4.2 (Garnier et al., 2023) (r codes and results provided in

supplementary materials 2).

RNA isolations were performed using 4 digestive glands tissues for each treatment. In brief, the E.Z.N.A.® Total RNA Kit I (Omega Bio-Tek) was used following the protocol instructions, using between 15–30 mg of tissue. The Tecan infinite m200 pro spectrophotometer was used to determine the RNA concentration and quality. Afterward, the RNA of the 32 samples were used for library preparation and sequencing, with TrueSeq Stranded mRNA Kit (Illumina) and Illumina NovaSeq 6000 sequencing respectively in DNALink Company (Los Angeles, United States).

Following the sequencing, thirty-two paired-end libraries were produced from Illumina NovaSeq 6000 sequencing and were uploaded to NCBI libraries bank as Bioproject PRJNA1287504. The raw sequences were analyzed under Galaxy Platform (Abueg et al., 2024) ABIMS from Station Biologique de Roscoff (<https://galaxy.sb-roscoff.fr/>) and Institut Française de Bio-informatique (<https://usegalaxy.fr/>), until finding the differentially expressed transcripts between controls and treatments. Quality-check of the raw sequences were performed using a Phred score > 30 as criteria with FastQC read quality reports galaxy version 0.74+galaxy1. The 10 first nucleotides did not pass this criterion for all libraries, and for some of them, several nucleotides in average for some reads do not correspond with the expected quality. For this reason, trimmomatic software version 0.39+galaxy2 (Bolger et al., 2014) was used in order to cut the first 10 nucleotides and a sliding window trimming an average of 5 nt of reads with < 30 Phred quality score. Then, the reference transcriptome was assembled using all libraries, after quality filters, with Trinity (Grabherr et al., 2011), and an ortholog regrouping was done to the assembled transcriptome to reduce functional redundancy using CD-Hit (Fu et al., 2012) (with 80% of similarity for clustering). The following step was to annotate the final transcriptome with the Trinotate workflow. BUSCO software was used to determine the Completeness and redundancy degree of the final transcriptome (Simão et al., 2015). Beginning the expression differential analysis, the count of reads and alignment using the assembled reference transcriptome and filtered libraries as input was performed with Salmon (Patro et al., 2017). The normalized counts data in TPM units were used to produce expression matrices of transcript expression considering each experimental condition. In order to detect the differentially expressed genes (DEG) at the isoform level, the software DESeq2 (Love et al., 2014) was used, taking into account the DEG with a $\log_2$ (fold change) > 1.5 (fold-change > 3) and false discovery rate < 0.05 . Venn diagrams were built using the id of transcripts in the Galaxy package Venn diagram [JVenn] Galaxy Version 2021.05.12. The list of DEG by treatment were used to detect enriched pathways, against the *Homo sapiens* database, and construct protein-protein interaction network using the online software STRING (<https://string-db.org/>). A heatmap of key DEG based on $\log_2$ fold-change values was generated in R v4.4.0. Visualization and hierarchical clustering (Euclidean distance, complete linkage) were carried out using pheatmap v1.0.12 (Kolde, 2019). Diverging color gradients centered at zero were implemented with RColorBrewer v1.1–3 (Neuwirth, 2022).

Results and discussion

Nanoparticles characterization

PS-COOH-NP displays a high agglomeration in NSW with average size of 752.7 ± 137 nm and a negative surface charge of -25.2 ± 1.8 mV. Such results are aligned with our previous findings in which these negative charges PS NPs undergo high agglomeration in high ionic media as NSW although less prone to this in Antarctic NSW probably due to its peculiar NOM content compared to more eutrophic ones as the one from Mediterranean (Bergami et al., 2019). Similarly TiO_2 -NPs showed high agglomeration in NSW up to 872.9 ± 73.8 nm and a low negatively surface charge of -11.7 ± 0.7 mV in agreement with our previous findings using the same batch and Antarctic NSW (González-Aravena

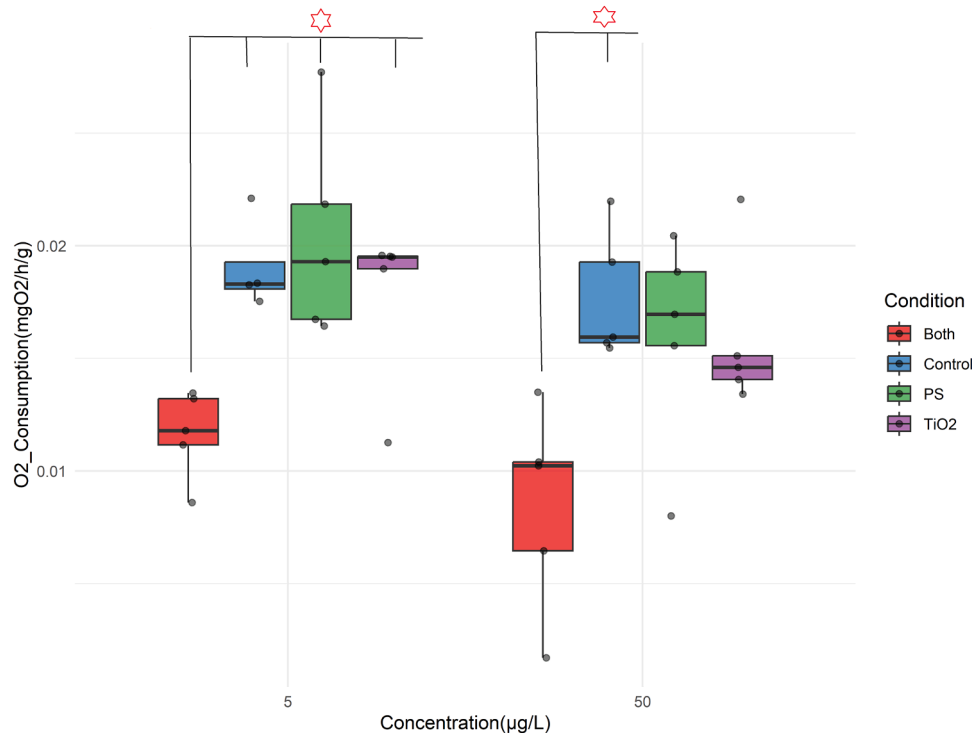


Fig. 1. Boxplots of Oxygen consumption in mg of O₂ per minute and per grams of wet weight of clams, for each condition at concentrations, 5 (A) and 50 (B) µg/L. The bars represent the maximum and minimum values, the centered line the median and the red stars indicate significant differences with p-value < 0.005 in the pairwise comparison. Only differences between same concentration experiment are indicated.

et al. 2022).

Co-exposures of TiO₂-NP and PS-COOH-NP leads to a decrease in O₂ consumption at both concentrations tested

The assessment of O₂ consumption in *L. elliptica* exposed to PS-COOH-NP and TiO₂-NPs provides a functional window into the respiratory physiology of this key species in Antarctic benthic ecosystems. This parameter, which is sensitive to metabolic alterations, allows the detection of early responses to environmental stress induced by emerging pollutants. However, to understand the molecular basis of these responses and their potential adaptive implications, it is essential to integrate these data with transcriptomic analysis. As it is known, the digestive gland in bivalves is not only specialized in digestive functions, but in xenobiotic detoxification (Faggio et al., 2018) and metabolism (Balbi et al., 2017) including reserve storage during seasonal changes (Ahn et al., 2003).

To explore potential correlations between gene transcription and metabolic changes in clams, we assessed the O₂ consumption in 5 individuals per treatment except for control at 5 µg · L⁻¹ experiment with 4 individuals (n = 39). A significant decrease in O₂ consumption was observed in the co-exposures to both NPs at 5 µg · L⁻¹ and the control, PS-COOH-NP and TiO₂-NP at the same concentration (p-value adjusted hsd < 0,05) (Fig. 1; supplementary materials 1–2). Also, the co-exposure at 50 µg · L⁻¹ showed significant differences with the control (p-value adjusted hsd < 0,05) suggesting that the combination induces effective hypometabolism at both the lower (5 µg · L⁻¹) and the higher (50 µg · L⁻¹) concentration tested. Such findings correlate with the observed down-regulation of protein synthesis transcripts, with a mean fold-change of –1.69 at 5 µg · L⁻¹ where the O₂ consumption fold-change was 1.64 and up-regulation of energy catabolism transcripts with mean fold-change of 4.24 at 50 µg · L⁻¹ where the fold-change of O₂ consumption was 1.87, suggesting an attempt to compensate for energy deficit by increasing ATP production while reducing glycogen and lipid synthesis. These

Table 1
Quantity of sequences (reads) obtained by condition after sequencing, quality control filter, mapped from *Laternula elliptica* digestive gland transcriptome and percentage of aligned reads in transcriptome considering raw reads.

Condition	Sequenced reads	Quality Control	Mapped Reads	% alignment
Control 5 µg · L ⁻¹	109,797,397	101,543,285	86,526,031	85.21%
PSNP 5 µg · L ⁻¹	117,305,354	109,294,379	88,667,601	81.12%
TiO ₂ -NP 5 µg · L ⁻¹	113,158,477	104,357,923	87,828,022	84.16%
Both NP 5 µg · L ⁻¹	112,960,616	105,302,563	85,670,642	81.35%
Control 50 µg · L ⁻¹	114,245,649	103,797,076	87,470,420	84.27%
PSNP 50 µg · L ⁻¹	113,675,623	103,714,485	88,903,505	85.71%
TiO ₂ -NP 50 µg · L ⁻¹	118,457,156	109,303,693	92,009,654	84.17%
Both NP 50 µg · L ⁻¹	114,576,182	107,320,778	91,873,086	85.60%
Total	914,176,454	844,634,182	708,948,960	-
Mean	114,272,057	105,579,273	88,618,620	83.95%

results highlight how mixtures of NPs in the environment may impact the whole metabolism of Antarctic filter-feeder bivalves. Other studies, performed on marine mussels (*M. edulis* and *M. galloprovincialis*) have shown that exposure to microplastic leachates, leads to a decrease in respiration rate (Uguen and Cozzolino, 2025), while decreased O₂ consumption upon exposure to engineered metal oxide NPs in the blood cockle *Tegillarca granosa* (Zha et al., 2022). Our results, showing a decrease in O₂ consumption after NP exposure, are consistent with these reports but differ in the sense that only the co-exposure treatments lead to such a statistically significant decrease although a tendency in decrease is also observed after single exposure to TiO₂-NP (50 µL L⁻¹). In

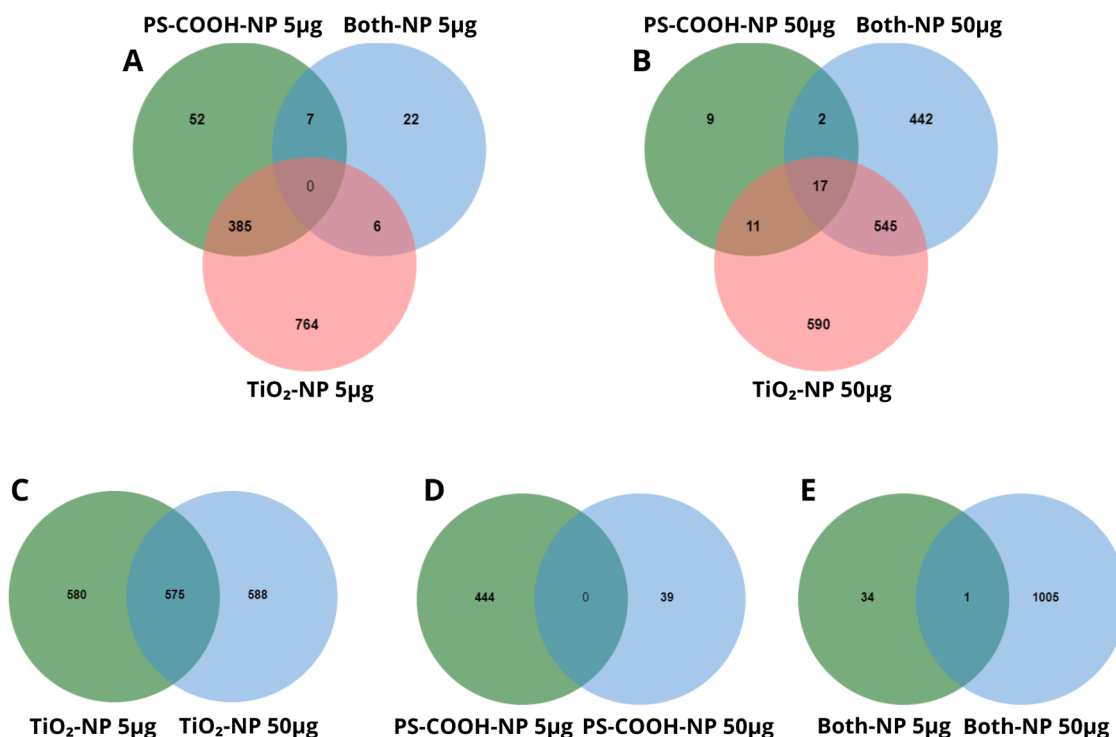


Fig. 2. Venn diagrams illustrating the number of shared differentially expressed genes between nanoparticles exposure treatments to A) Shared DEGs among treatments at the 5 µg/L exposure level. B) Shared DEGs among treatments at the 50 µg/L exposure level. C) Shared DEGs between the two concentrations of TiO₂-NP exposure. D) Shared DEGs between the two concentrations of PS-COOH-NP exposure. Shared DEGs between the two concentrations of co-exposures to both NPs pollutants.

contrast, opposite trends have been observed in zebrafish exposed to lower and higher doses of nanoplastic, where an increase in metabolic activity was reported (Chackal et al., 2022). These results raise the attention of the metabolic effects of nano and micro-pollutants, with possible consequences at population and community levels.

Single and co-exposure to TiO₂-NP and PS-COOH-NP leads to profound changes in the transcriptome of the digestive glands

The global paired-end RNA sequencing yielded 914,176,454 reads for the 32 digestive gland samples (with an average for each library of 28,568,014 paired-end reads), of which 844,634,182 reads remained after the quality control filter (92.4%) and 708,948,960 reads mapped into the digestive gland clustered transcriptome (83.9%) (details in Table 1). The transcriptome assembled and clustered with CD-Hit had 223,856 transcripts and 154,502,073 nt assembled, with a GC average of 38.5%, N50 of 1013 nt and contig length average of 690.19 nt. The Benchmarking Universal Single-Copy Orthologs (BUSCO) analysis against the Mollusca database showed 89.4% completeness (79.4% with single copy and 10.0% with duplicity), 2.4% with fragmentation and only 8.2% missing, revealing a high degree of coverage of transcriptome of *Laternula elliptica* digestive gland for a mollusk species.

Overall, 2852 genes were differentially expressed, across all experimental comparisons between controls and treatment groups. At the 5 µg · L⁻¹ exposures, we identified 1155, 444 and 35 differentially expressed genes (DEGs) in response to TiO₂-NP, PS-COOH-NP and their co-exposure respectively. At the 50 µg · L⁻¹, we found 1163, 39 and 1006 DEGs in response to TiO₂-NP, PS-COOH-NP and their co-exposure respectively (see Supplementary Material 3 for data of DEG with blast results). Therefore, these results indicate that the effect on the digestive glands were more pronounced than those observed in the gills under similar conditions in our previous investigations (Rondon et al., 2024b). Another notable difference between the digestive gland and gill tissues was the absence of a clear dose dependent global transcriptomic

response between low and high NP concentrations. However, engineered metal oxide NPs exposure resulted in a higher number of DEGs compared to nanoplastic, along with a higher degree of shared DEGs between the two concentrations in TiO₂-NP exposure. At the 5 µg · L⁻¹, there was generally low overlap in DEGs between treatments, except for comparison between PS-COOH-NP and TiO₂-NP which shared 385 DEGs (Fig. 2A). In contrast, only 6 and 7 DEGs were shared between TiO₂-NP and the co-exposure treatment, and between PS-COOH-NP and co-exposure to both NPs respectively. Surprisingly, no DEGs were shared between all three treatments at 5 µg · L⁻¹ concentration. Regarding the comparison between TiO₂-NP and co-exposure to both nanoparticles at 50 µg · L⁻¹ concentration, 545 DEGs were shared. Only 2 DEGs were shared between PS-COOH-NP and the co-exposure and 11 DEGs were shared between the two types of NPs, PS-COOH-NP and TiO₂-NP (Fig. 2B). Interestingly, between all three treatments at 50 µg · L⁻¹ concentration 17 gene functions were shared. When comparing the transcriptomic responses across concentrations with the same NPs, 575 DEGs were shared between the 5 and 50 µg · L⁻¹ exposure to TiO₂-NP (Fig. 2C). In contrast, no shared DEGs were found between the two concentrations of PS-COOH-NP (Fig. 2D) and only one DEGs was shared between the co-exposure's treatments (Fig. 2E).

Functional enrichment analysis and protein-protein interaction pathway reveal specific response to each treatment

After detecting enriched biological processes, we performed a protein-protein interaction (PPI) network analysis to investigate the interacting genes involved in these pathways, and to better understand the main effects of nanopollutants on the *L. elliptica* digestive gland. Following exposure to 5 µg · L⁻¹ of PS-COOH-NP we observed a clear clustering of interacting genes associated with five enriched biological process GO terms (Fig. 3). These were "Organic compound Metabolic Process" (I), "Catabolic Process" (II), "Cellular Localization" (III), "Regulation of Cell Cycle" (IV) and "Proteolysis" (V). At higher

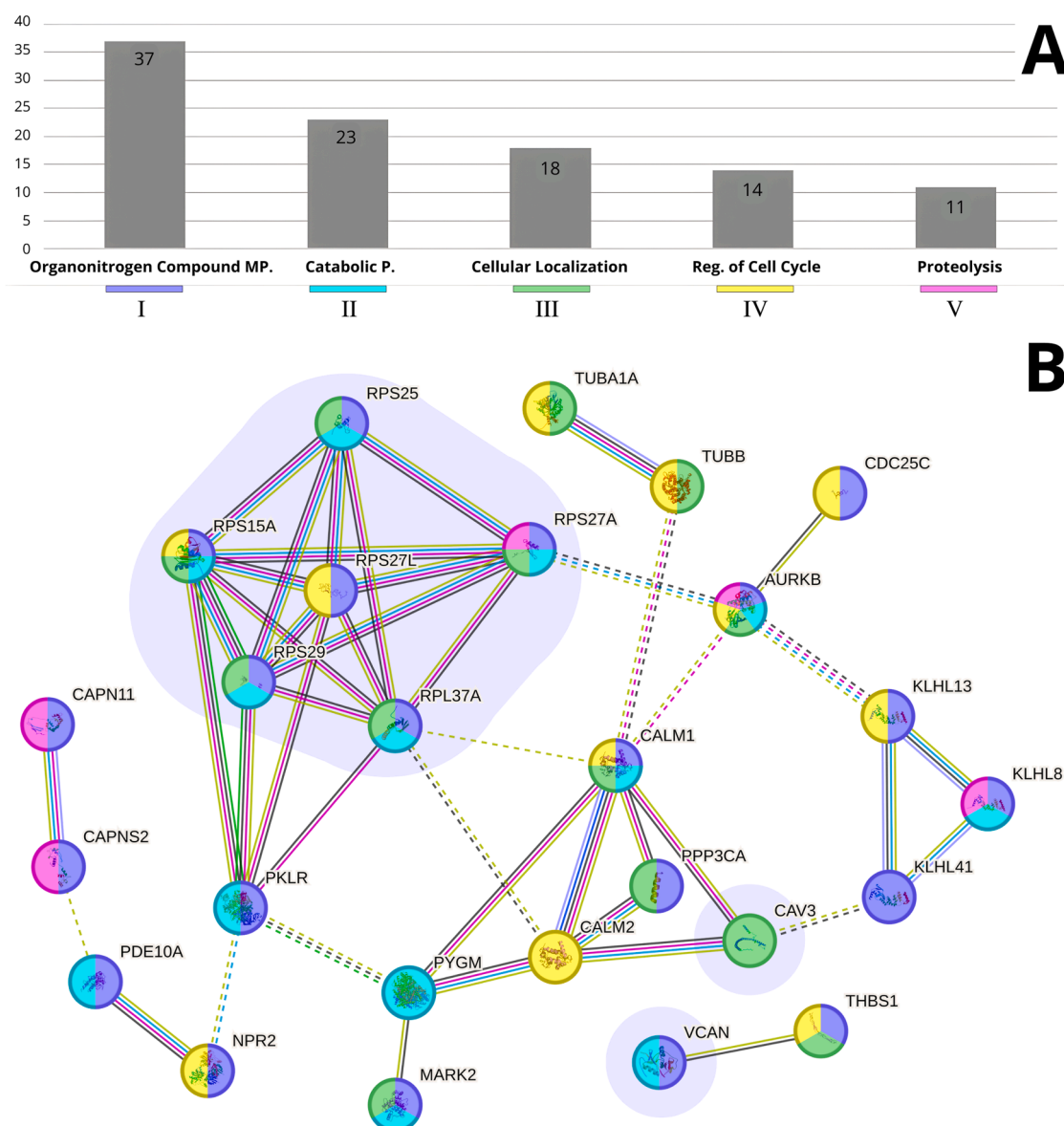


Fig. 3. Enrichment analysis, where Y-axis corresponds to the number of differentially expressed genes (DEG) by GO term (A) with Biological Process GO terms from the most abundant to the less abundant in response to the exposure to PS-COOH-NP at 5 $\mu\text{g/L}$, with colors relating to the protein-protein network analysis of DEG (B). Each circle is a transcript with color from enrichment Biological Process GO term, and lines represented each time this circle is connected. Complete lines represent experimentally proven interactions, and dashed lines represent predictive interactions.

concentration ($50 \mu\text{g} \cdot \text{L}^{-1}$) of PS-COOH-NP exposure, among all DEGs, only one had a known function, a Neurotrypsin gene, which is involved in protein degradation. Similar effects on protein degradation pathways have been reported, in the hepatopancreas of the red swamp crayfish (*Procambarus clarkii*) exposed to the two-concentration studied (Capanni et al., 2021).

Exposures at both 5 and $50 \mu\text{g} \cdot \text{L}^{-1}$ of TiO_2 -NP resulted in the differential expression of several genes associated with biological processes which were shared between the two treatments. The enriched biological processes were “Cellular Composition Organization” (I), “Response to Stimulus” (II), “Positive Regulation to Biological Process” (III), “Localization” (IV), “Cell Cycle” (V) and “Response to Inorganic Substances” (VI) for $5 \mu\text{g} \cdot \text{L}^{-1}$ (Fig. 4). Under this condition, no clear clustering was observed, and the associated GO terms appeared scattered throughout the network (Fig. 4). Similarly, in the $50 \mu\text{g} \cdot \text{L}^{-1}$ TiO_2 -NP exposure transcript functions also showed a dispersed pattern, with no distinct clustering (Fig. 5). The Biological Processes GO terms overrepresented were: “Transport” (I), “Catabolic Process” (II), “Organelle Organization”

(III), “Response to Stress” (IV), “Vesicle-Mediated Transport” (V), “Secretion” (VI) and “Cell Cycle” (VII) (Fig. 5).

The enrichment analysis of the co-exposure to both nano-pollutants at $5 \mu\text{g} \cdot \text{L}^{-1}$ showed several GO terms (Fig. 6), all of which were related to ribosomal proteins, implicated mainly in gene expression. In contrast, the enrichment network analyses for the co-exposure to both nano-pollutants at $50 \mu\text{g} \cdot \text{L}^{-1}$ (Fig. 7) showed a broader variety of DEGs that were well-clustered. The Enriched GO terms were: “Organelle Organization” (I), “Cell Cycle” (II), “Metabolic Based Process” (III), “Post Mitotic Cell Projection Organelles” (IV), “Regulation of Transferase Acts” (V) and “Chromosome Organization” (VI).

An overall stress response seems to be a conserved pattern following exposure to each treatment

Interestingly, some GO biological processes appeared enriched across most treatments, involving genes such as cell division regulators (for the cell cycle), tubulins (for cellular localization and organelle

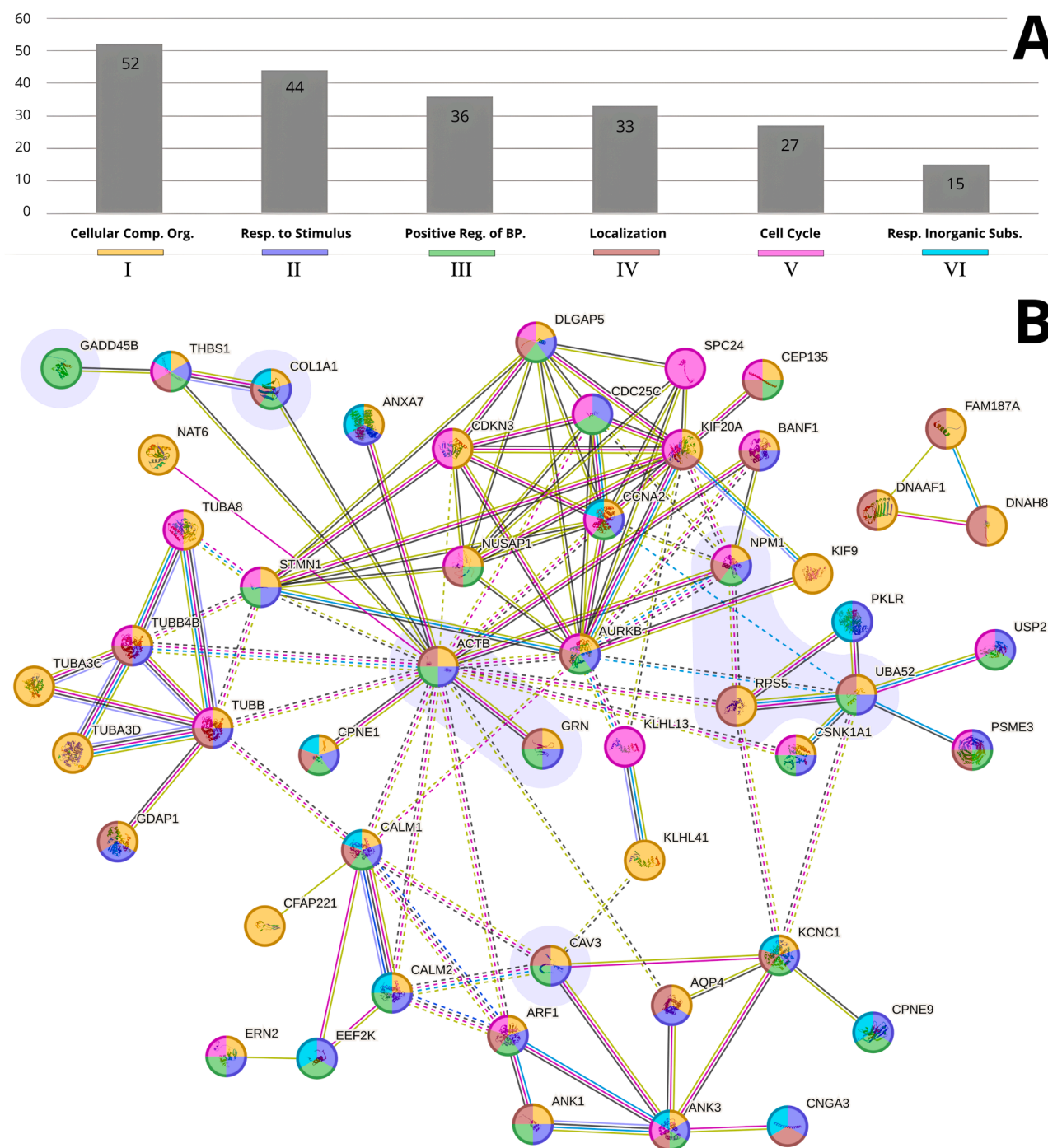


Fig. 4. Enrichment analysis, where Y-axis corresponds to the number of differentially expressed genes (DEG) by GO term (A) with Biological Process GO terms from the most abundant to the less abundant in response to the exposure to TiO_2 -NP at $5 \mu\text{g/L}$, with colors relating to the protein-protein network analysis of DEG (B). Each circle is a transcript with color from enrichment Biological Process GO term, and lines represented each time this circle is connected. Complete lines represent experimentally proven interactions, and dashed lines represent predictive interactions.

organization) suggesting that NPs exposure - both individual and combined- may disrupt internal cellular structure and cell division regulation in digestive cells. To our knowledge, the observation of altered expression in genes related to cell division represents a novel finding in marine invertebrate digestive cells exposed to NPs. Additionally, the differential expression of several cytoskeleton related transcripts aligns with previous findings in the digestive gland of the bivalves *M. galloprovincialis* exposed to TiO_2 -NP (Banni et al., 2016). Other enriched GO terms, such as those related to metabolism, were shared across two or three treatments. These include transcripts involved in

glycolysis, glycogenolysis and lipase activity. Besides, some treatment specific enrichments were also notable, such as responses to inorganic substances in TiO_2 -NP exposure at $5 \mu\text{g} \cdot \text{L}^{-1}$, response to stress in TiO_2 -NP exposure at $50 \mu\text{g} \cdot \text{L}^{-1}$ and chromosome organization in the co-exposure to both NPs at $50 \mu\text{g} \cdot \text{L}^{-1}$. While the responses to stress and inorganic substances were consistent with previous studies (Banni et al., 2016), the observed dysregulation of histones related genes brings new insights into the potential toxicity of combined nanoplastic and engineered metal oxide NP exposure in a filter-feeding species.

Some marine invertebrates lack the classical heat stress response,

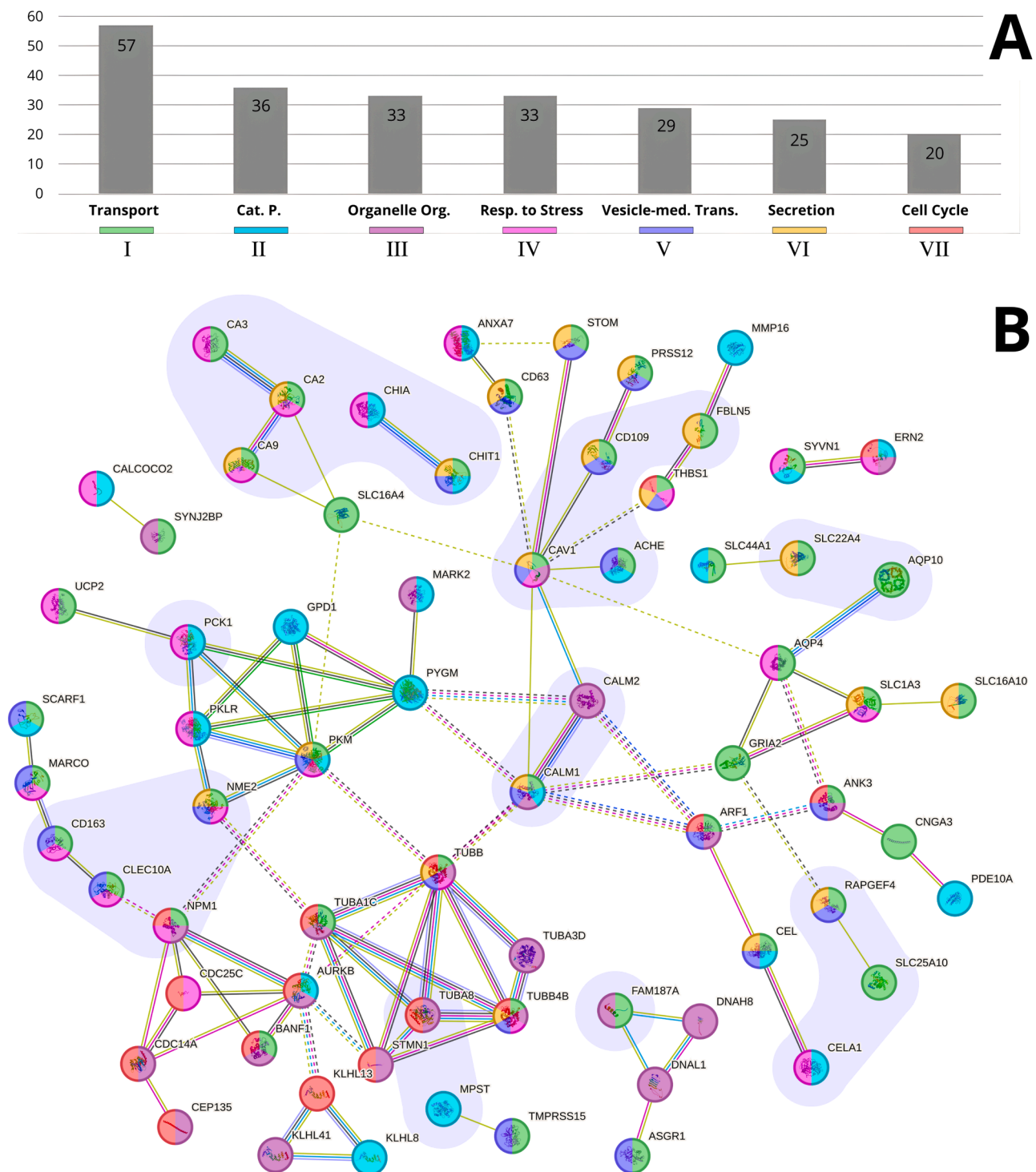


Fig. 5. Enrichment analysis, where Y-axis corresponds to the number of differentially expressed genes (DEG) by GO term (A) with Biological Process GO terms from the most abundant to the less abundant in the treatment $\text{TiO}_2\text{-NP}$ at $50 \mu\text{g/L}$, with colors relating to the protein-protein network analysis of DEG (B). Each circle is a transcript with color from enrichment Biological Process GO term, and lines represented each time this circle are connecting. Complete lines represent experimentally proven interactions and dash lines represent predictive interactions.

which is characterized by the expression of heat shock proteins such as Hsp (Clark and Peck 2009). In *L. elliptica*, these changes are induced by heat stress, hypoxia, pH changes, injury, and starvation (Clark et al. 2008; Park et al. 2007; Clark et al. 2013; Cummings et al. 2011; Husmann et al. 2014.). The current study reveals that *L. elliptica* constitutively expresses several Hsps, including Hsp90 and Hsp70. However, the challenge with NPs does not affect their expression, as is the case in other invertebrates (Bergami et al., 2019). By other hand, several

chaperones' transcripts showed significant differential expression. Notably, "DnaJ homolog subfamily C member 4" was up-regulated in response to both concentration of TiO_2 NP and in the co-exposure treatment at $50 \mu\text{g} \cdot \text{L}^{-1}$. Also "DnaJ homolog subfamily B member 13", was also up-regulated in the co-exposure treatment at $50 \mu\text{g} \cdot \text{L}^{-1}$. The DNAJ/Hsp40 proteins play a crucial role in determining Hsp70 activity by stabilizing their interaction with substrate proteins. It is plausible that a substantial pool of cognate Hsp70s may contribute to the

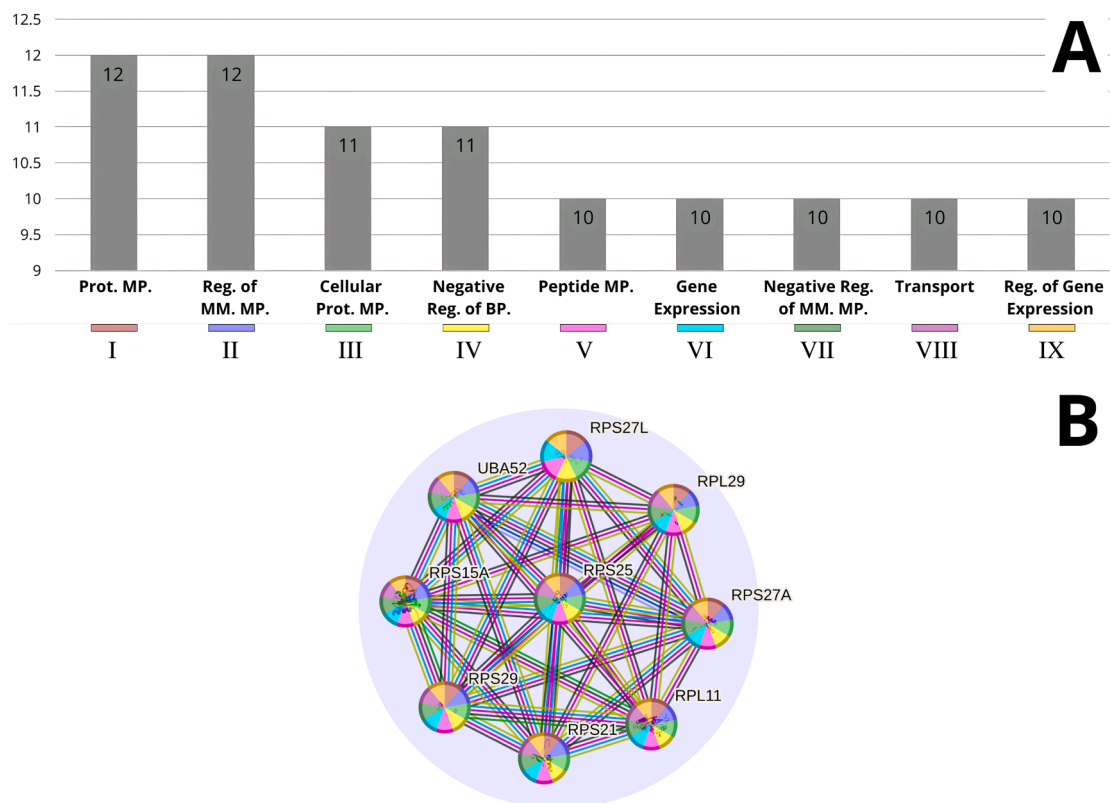


Fig. 6. Enrichment analysis, where Y-axis corresponds to the number of differentially expressed genes (DEG) by GO term (A) with Biological Process GO terms from the most abundant to the less abundant in the treatment Co-exposure to both nanoparticles at 5 $\mu\text{g/L}$, with colors relating to the protein-protein network analysis of DEG (B). Each circle is a transcript with color from enrichment Biological Process GO term, and lines represented each time this circle is connected. Complete lines represent experimentally proven interactions and dash lines represent predictive interactions.

stabilization of these Hsp70s. Additionally, in all three of these treatments, the transcription factor “Heat shock factor protein 2” was also up-regulated. These results suggest that TiO_2 -NP may induce a molecular stress response involving co-chaperones and their transcription factor, suggesting a cellular need to repair misfolded proteins, potentially due to endoplasmic reticulum stress (ERS). This hypothesis is further supported by the up-regulation “Thrombospondin-1” (THBS1), a gene implicated in ERS response (Lynch et al., 2012). Similar up-regulations of chaperones have been observed in the digestive gland of the gastropod *Helix aspersa* exposed to silver nanoparticles (Turki et al., 2022). Despite these protective responses, all treatments in our study also showed an up-regulation of proteases and ubiquitin related enzymes, suggesting that misfolding proteins may be beyond repair and are instead targeted for degradation. Similar response has been observed in the green mussel *Perna viridis* gills where acute exposure to zinc oxide NPs led to up-regulation of chaperones, ERS-related transcripts and peptidase enzymes (Vysakh et al., 2025). In that case, mitochondrial stress and mitophagy and autophagy were also observed (Vysakh et al., 2025). In our current study on *L. elliptica* digestive gland, mitochondrial stress was similarly indicated by the upregulation of “Mitochondria-eating protein” following exposure to PS-COOH-NP and TiO_2 -NP at 5 $\mu\text{g} \cdot \text{L}^{-1}$ and “Mitochondrial uncoupling protein 2” on these two treatments and TiO_2 -NP at 50 $\mu\text{g} \cdot \text{L}^{-1}$. This suggested a cellular attempt to resolve mitochondrial damage. Furthermore, transcripts involved in autophagy, were also up-regulated, most notably the “Tumor protein p53-inducible nuclear protein 1” in both TiO_2 -NP treatments, in PS-COOH-NP at 5 $\mu\text{g} \cdot \text{L}^{-1}$ and in the co-exposures at 50 $\mu\text{g} \cdot \text{L}^{-1}$. This suggests that many of the treatments triggered cellular dysfunction requiring autophagic response. Similar cellular stress responses have been observed in the digestive gland of the blue mussel *M. edulis* exposed to Fullerene C60 (Sforzini et al., 2020), highlighting a common pattern

of defense and damage control in marine invertebrates following NP exposure.

Antioxidant-related genes are frequently found among those affected by nanomaterial exposures (Canesi and Corsi, 2016). This was the case in the digestive gland of *L. elliptica* exposed to TiO_2 -NP and combined NP treatments showed significant alterations in antioxidant transcripts. Notably, the transcript “Thioredoxin domain-containing protein 6” was up-regulated in both concentrations of TiO_2 -NP. Additionally, two other antioxidant transcripts “Glutaredoxin-C3” and “Oxidative stress-responsive serine-rich protein 1” were up-regulated in the TiO_2 -NP at 5 $\mu\text{g} \cdot \text{L}^{-1}$ and the co-exposure to both NPs at 50 $\mu\text{g} \cdot \text{L}^{-1}$. Antioxidant transcripts have been up-regulated in the digestive gland of other bivalves responding to NP exposure, such is the case of a *Mytilus* species exposed to a low concentration of TiO_2 -NP over prolonged period (Barmo et al., 2013). Conversely, down-regulation of antioxidant transcripts were also observed, particularly in the TiO_2 -NP and co-exposure treatments at 50 $\mu\text{g} \cdot \text{L}^{-1}$. Down-regulated transcripts included “Glutaredoxin-1”, “Glutathione peroxidase” and “DBH-like monooxygenase protein”. Previous research has shown similar down-regulation patterns in *Mytilus* digestive gland, following acute exposure (24 h) to various NP suspensions (NCB, C60, TiO_2 -NP, SiO_2 -NP), indicating potential dysregulation of key antioxidant defenses in bivalve digestive tissues (Canesi et al., 2010). In addition to antioxidant genes, we also recorded down-regulations in pH balance transcripts, including various Carbonic anhydrases: “Carbonic anhydrase 3” was down-regulated in PS-COOH-NP and TiO_2 -NP at 5 $\mu\text{g} \cdot \text{L}^{-1}$ and TiO_2 at 50 $\mu\text{g} \cdot \text{L}^{-1}$, “Carbonic anhydrase 2” and “Carbonic anhydrase 9” in TiO_2 at 50 $\mu\text{g} \cdot \text{L}^{-1}$ and “Carbonic anhydrase 14” in the co-exposure to both nanoparticles at 50 $\mu\text{g} \cdot \text{L}^{-1}$. Maintaining pH homeostasis is critical for the proper function of digestive cells and this downregulation may compromise cellular efficiency. Notably, previous research on *L. elliptica*

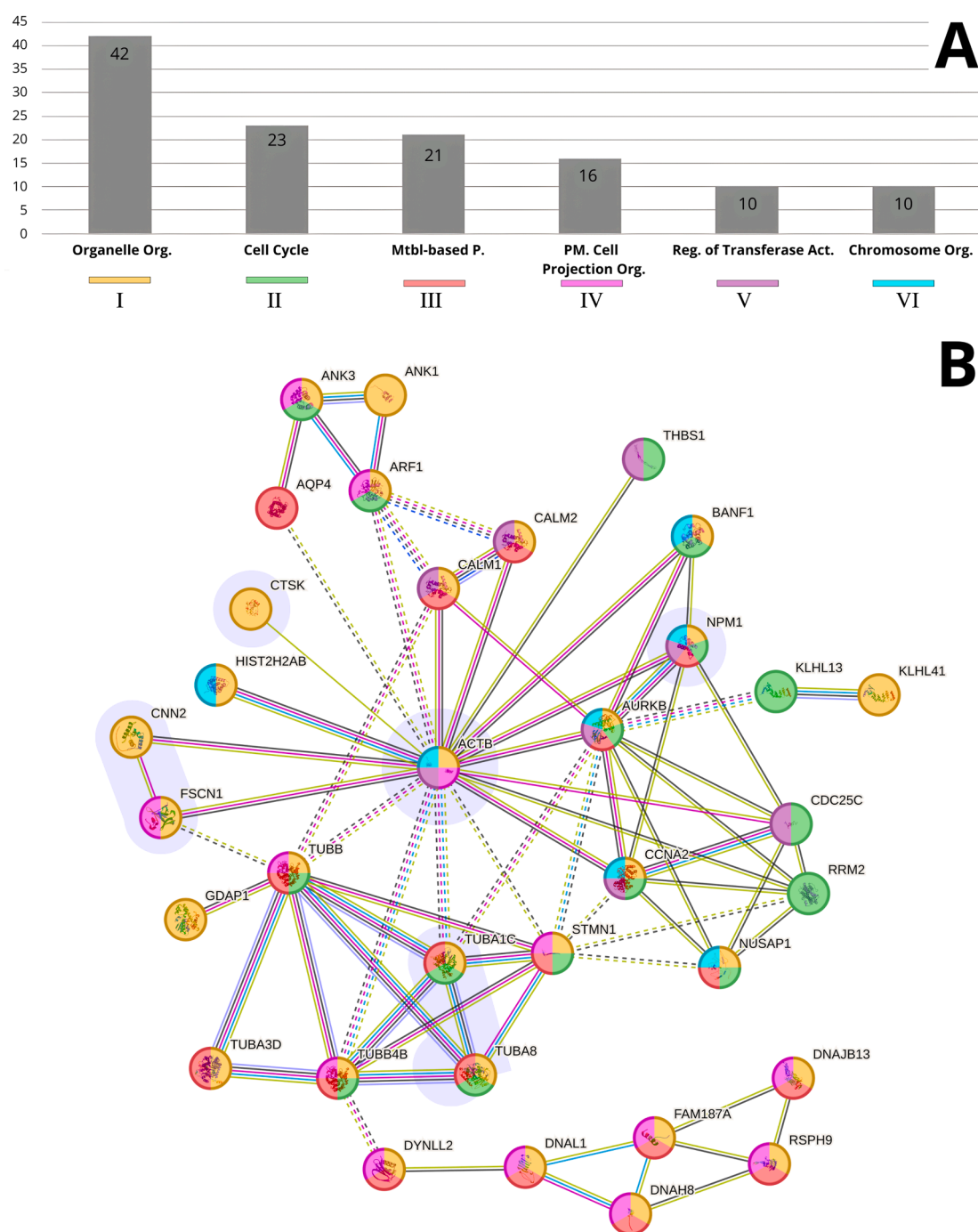


Fig. 7. Enrichment analysis, where Y-axis corresponds to the number of differentially expressed genes (DEG) by GO term (A) with Biological Process GO terms from the most abundant to the less abundant in the treatment Co-exposure to both nanoparticles at 50 $\mu\text{g/L}$, with colors relating to the protein-protein network analysis of DEG (B). Each circle is a transcript with color from enrichment Biological Process GO term, and lines represented each time this circle is connected. Complete lines represent experimentally proven interactions and dash lines represent predictive interactions.

also identified alterations in Carbonic anhydrase transcript level in gills tissues following combined NPs exposures at 50 $\mu\text{g} \cdot \text{L}^{-1}$ (Rondon et al., 2024b), suggesting the sensitivity of this species to these types of pollutants.

All these stress signals could lead to an energy imbalance, characterized by the up-regulation of catabolic pathways and the down-regulation of anabolic ones. In this context, we observed an up-regulation of transcripts related to glycolysis, such as “Pyruvate kinase PKLR” and glycogenolysis, such as “Glycogen phosphorylase, muscle form” in four out of six treatments (PS-COOH-NP at 5 $\mu\text{g} \cdot \text{L}^{-1}$, TiO_2 -NP at

both concentrations and the co-exposure to both NPs at 50 $\mu\text{g} \cdot \text{L}^{-1}$). Additionally, the gene encoding for “Probable pyruvate dehydrogenase E1 component subunit alpha, mitochondrial” involved in the production of Acetyl-CoA prior to the Krebs Cycle was up-regulated only in the co-exposure to both NP at 50 $\mu\text{g} \cdot \text{L}^{-1}$. Similarly, the gene encoding for “Isocitrate dehydrogenase [NAD] subunit beta, mitochondrial” implicated in ATP production via the Krebs cycle, was upregulated exclusively in TiO_2 -NP at 5 $\mu\text{g} \cdot \text{L}^{-1}$. Furthermore, the gene encoding for, the “Bile salt-activated lipase”, an enzyme involved in lipase energy production through lipid metabolism, was up-regulated only in TiO_2 -NP at

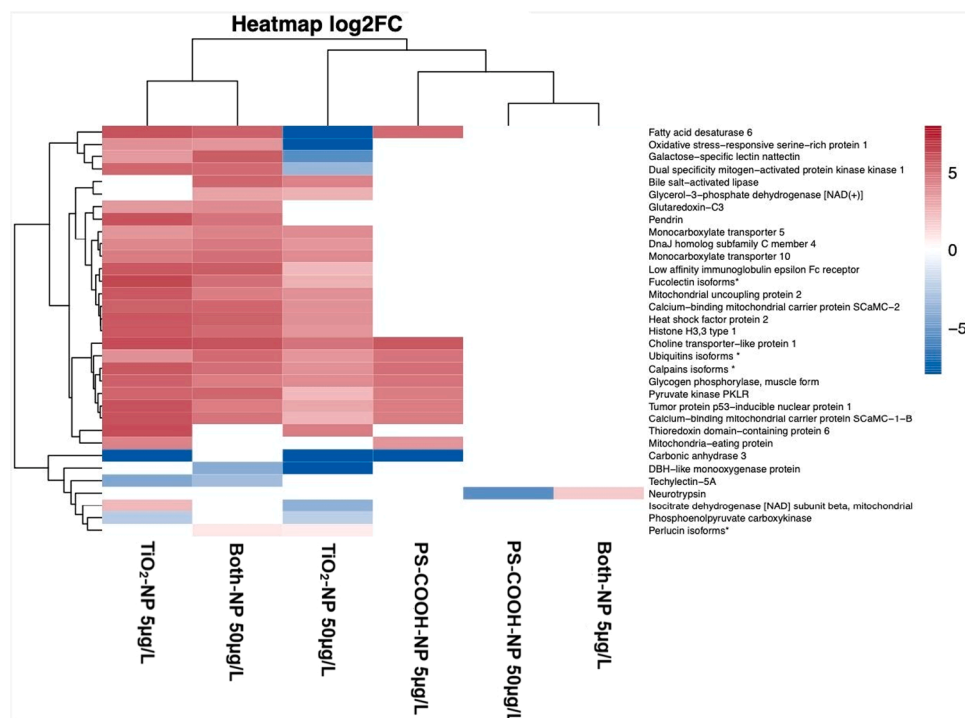


Fig. 8. Heatmap of relevant transcripts, showing some shared functions, being down and up-regulated by the different treatments. Blue color intensity represents the degree of down-regulation and red color intensity represents up-regulation degree. The * indicates when several isoforms were revealed, and an average was used for heatmap design. The samples and transcripts are ordered according with the Euclidean distance.

$50 \mu\text{g} \cdot \text{L}^{-1}$. Similar results were reported in the digestive gland of *M. galloprovincialis* after exposure to PVP/PEI coated silver NPs during the autumn conditions (Duroudier et al., 2019). Conversely, the gene encoding for “Phosphoenolpyruvate carboxykinase”, a key enzyme implicated in gluconeogenesis, was down-regulated in PS-COOH-NP at $5 \mu\text{g} \cdot \text{L}^{-1}$ and the gene encoding for “Glycerol-3-phosphate dehydrogenase [NAD(+)]”, involved in lipid biosynthesis via carbohydrates, was down-regulated only under the co-exposure to both NPs at $50 \mu\text{g} \cdot \text{L}^{-1}$. The downregulation of these anabolic transcripts supports the hypothesis of an increased energy demand under these treatments. Regarding lipid metabolism, similar results were observed in the digestive gland of *M. galloprovincialis* exposed to both biodegradable and anthropogenic nanoplastic (Limonta et al., 2024).

Along with the induction of catabolic transcripts, several membrane transporters were also up-regulated, each implicated in various cellular functions. Genes encoding for “Monocarboxylate transporters 5 and 10”, involved in the transport of small carbohydrates and aromatic amino acids respectively, were up-regulated following exposure to TiO_2 -NP at both concentrations and the co-exposure to both NPs at $50 \mu\text{g} \cdot \text{L}^{-1}$ suggests an increased demand for these nutrients after NP exposures. Additionally, two of these transporters, “Calcium-binding mitochondrial carrier protein SCA2” and the “SCA2” isoforms which facilitates the mitochondrial transporters of ATP-Mg2 +/Pi in a calcium dependent manner (del Arco et al., 2016) and “Choline transporter-like protein 1” involved in Choline transport for phospholipid synthesis (Hedtke and Bakovic, 2019; Taylor et al., 2021), were up-regulated following exposure to PS-COOH-NP at $5 \mu\text{g} \cdot \text{L}^{-1}$, TiO_2 -NP at both concentrations and the co-exposure to both NPs at $50 \mu\text{g} \cdot \text{L}^{-1}$. These could suggest that digestive gland cells may be attempting to cope with NP exposure by increasing ATP transport and supporting cytosolic and mitochondrial membrane's integrity.

Lectins in mollusks play a crucial role in their immune defense, acting as carbohydrate-binding proteins that recognize and neutralize pathogens and particles. These lectins in Antarctic clams, including Fucosyltransferase, Perlucin and Techlectin-5A, enhance immune responses by

facilitating hemocyte adhesion and phagocytosis (Jeyachandran et al., 2024). The classical response associated with immune effectors, such as antimicrobial peptides, is absent in *L. elliptica*. This is reinforced by the non-induction of the bactericidal/permeability-increasing protein, as occurs in snail *Helix aspersa* associated with the effect of silver NPs in the digestive gland (Turki et al., 2022). Further investigation is required to clarify the potential effects of NPs on the immune system and to assess whether these effects are advantageous or detrimental to the population over time.

In the latest treatment, expression of the genes encoding for “Deleted in malignant brain tumors 1 protein (DMBT1)” and “Tumor protein p53-inducible nuclear protein 1” involved in anti-inflammatory responses and tumor suppression, were down-regulated. Similar effects of NP exposure on hepatopancreas and digestive gland tissues have been reported in mussel *M. galloprovincialis* and snail *H. aspersa* (Limonta et al., 2024; Turki et al., 2022). In the co-exposure treatment at $5 \mu\text{g} \cdot \text{L}^{-1}$, a gene encoding for an apoptosis inhibitor was downregulated along with other genes encoding for several ribosomal proteins implicated in translation, suggesting a potential increase in apoptosis and a decrease in protein synthesis at this concentration. Comparable results regarding apoptosis-related transcripts alterations have been observed in the digestive gland of the freshwater clam *Corbicula fluminea* exposed to PS-NP (Li et al., 2021), while the downregulation of ribosomal proteins coding transcripts related to translation has also been reported in the marine rotifer *Brachionus koreanus* (Jeong et al., 2021). On the other hand, and contrary to expectation, transcripts involved in xenobiotic metabolism were predominantly down-regulated. For instance, the gene encoding for “Glutathione-S-Transferase Mu 3” was down-regulated by PS-COOH-NP $5 \mu\text{g} \cdot \text{L}^{-1}$ and the “Carboxylesterase 5A” was down-regulated following TiO_2 -NP exposure at $50 \mu\text{g} \cdot \text{L}^{-1}$. Similarly, the genes encoding for “Cytochrome P450 2A13” and “Cytochrome P450 17A1” were down-regulated after the co-exposure to both NPs at $50 \mu\text{g} \cdot \text{L}^{-1}$. Only the gene encoding for the “Cytochrome P450 4A4” was up-regulated in response to TiO_2 -NP treatment at $50 \mu\text{g} \cdot \text{L}^{-1}$. These outcomes could indicate that digestive gland cells of *L. elliptica* are less

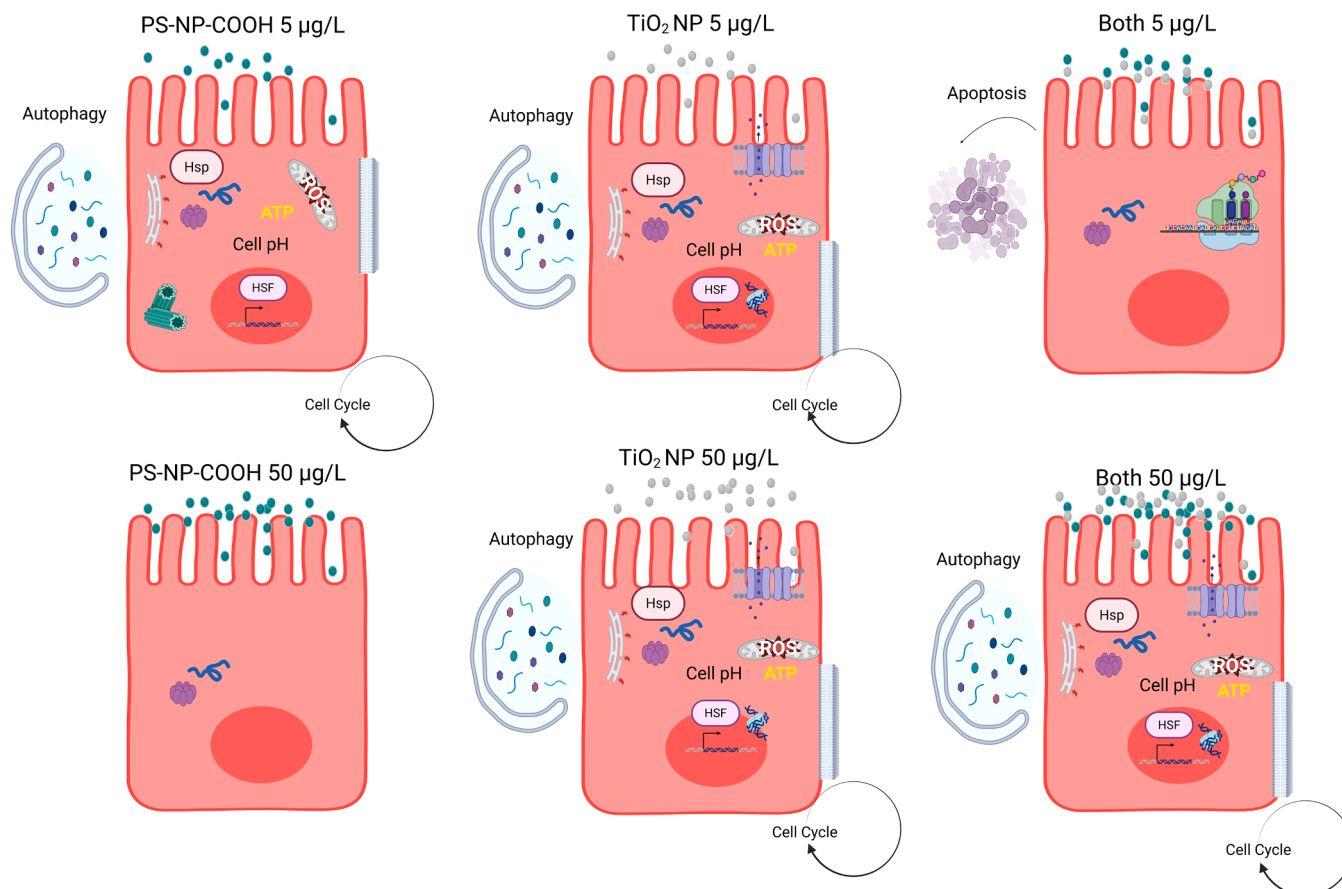


Fig. 9. Graphical representation of resumed cellular consequences according to molecular functions affected after exposures to each nanoparticle at each centration. This figure was made with Biorender online software. The main functions affected were Autophagy, represented by auto-degraded cell beside the functional cell; Cell cycle represented by a circle arrow in the down-right corner of the cells; protease represented by the violet circle with cylinders near to un-folding proteins represented by the blue line and the stressed Endoplasmic Reticulum represented by light-blue reticulum with red lines in the exterior part; the co-chaperones proteins represented by the letters “HSP” near of unfolding proteins; the cell pH balance enzymes; Antioxidant enzymes by an augmentation of Reactive Oxygen Species (ROS), affecting the mitochondria behind of ROS letters and in some cause the energy production (ATP); Membrane fatty acids represented as lipid membrane in the exterior of the cell; HSP transcription factors represented as HSF in the nucleus and histones represented by a circle with DNA in the nucleus; cytoskeleton represented by tubules green; apoptosis represented by a programmed cell death beside of the cell; and finally ribosomes proteins represented by two irregular forms with three space for mRNA codons nucleotides.

capable of metabolizing xenobiotics under NP exposure compared to normal conditions.

Some genes display the same patterns of expression following exposure to either TiO₂-NP or PS-COOH-NP or co-exposure at both concentrations

The global transcriptomic analysis revealed a common response across most treatments for the majority of the transcript functions (Fig. 8), with a few notable exceptions, particularly in the co-exposure at both NPs at $5 \mu\text{g} \cdot \text{L}^{-1}$, where several ribosomal protein coding transcripts were down-regulated accompanied by decreased transcription of an apoptosis control gene, and an increase in gene encoding for protease. Interestingly, all treatments exhibited up-regulation of proteases, potentially indicating that digestive gland cells experienced protein unfolding following NP exposures, and on most treatments an induction was observed for co-chaperones transcripts and a transcript factor. As discussed, it could be an early response to cope with environmental stress for a cold-water mollusk. In the case of PS-COOH-NP at $50 \mu\text{g} \cdot \text{L}^{-1}$ the digestive cells of *L. elliptica* appeared to completely lose control with only one protease up-regulated while the remaining transcripts corresponded to proteins of unknown functions, representing a very distinct response to the highest concentration tested. Initially, in treatment with PS-COOH-NP at $5 \mu\text{g} \cdot \text{L}^{-1}$, with TiO₂-NP at both concentrations and

following co-exposure to $50 \mu\text{g} \cdot \text{L}^{-1}$, very similar transcriptomic responses were recorded. These included effects on genes related to the cell cycle, autophagy, transmembrane transport proteins and synthesis of membrane fatty acids, a global cell pH balance as well as antioxidant effect and ERS. For PS-COOH-NP at $5 \mu\text{g} \cdot \text{L}^{-1}$, cytoskeleton transcripts were also affected. Together with TiO₂-NP at $5 \mu\text{g} \cdot \text{L}^{-1}$, mitochondrial stress was evident. Finally, vesicular protein transcripts were affected by TiO₂-NP at both concentrations and by the co-exposure of NPs at $50 \mu\text{g} \cdot \text{L}^{-1}$. This has revealed a global picture of molecular functions and cellular mechanisms of which respond and are affected for the set of nanopollutants assessed.

Conclusion

Physiological and molecular assessments, alongside respirometry, demonstrated that co-exposure to PS-COOH NPs and TiO₂-NP decreases metabolism. This indicates that a synergistic effect between these nanopollutants induces hypometabolism (increase in O₂ consumption) at the concentrations tested. As such, RNAseq analysis also provides confirmation for several treatments in the main metabolic organ only in co-exposures. The fold-change values of relevant metabolic transcripts with significant differences were correlated with fold-change of O₂ consumption with p-value significantly providing a dimension of how

the two analyses reflect the synergy effect of nanopollutants. In the other hand, several relevant transcript functions critical for digestive cell activity were affected by NP exposures (Fig. 9), revealing that organelle stress, particularly in mitochondria and more notably in Endoplasmic reticulum (ER), as well as a decrease in protein synthesis and an increase in protease activity. Additionally, disruption in transmembrane transport proteins, membrane permeability, vacuolar proteins and energy balance were observed in most treatments, suggesting impaired nutrient uptake in essential cells. Consequently, these effects could contribute to the observed reduction in metabolic activity particularly in the co-exposures at both tested concentrations. This link between O₂ consumption and gene expression is a novel result and an important knowledge in nano-toxicology, of which could be improved including proteomics and metabolomic approaches. As mentioned before, the digestive gland is the organ where the NP accumulation in filter-feeder bivalves is observed and plays metabolisms functions, which likely explains the more pronounced effects observed in this organ compared to others, such as gills which are the initial point of contact. This integrative approach not only strengthens the interpretation of the sublethal effects of pollutants but also provides evidence of the resilience or vulnerability of *L. elliptica* in the face of anthropogenic pressures in the context of global change.

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CRedit authorship contribution statement

Rodolfo Rondon: Writing – original draft, Visualization, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Felipe León:** Software, Methodology, Investigation, Formal analysis, Data curation. **Marcelo González-Aravena:** Writing – review & editing, Validation, Methodology, Investigation, Conceptualization. **Elisa Bergami:** Writing – review & editing, Validation, Resources, Methodology, Conceptualization. **Cristián Chaparro:** Writing – review & editing, Validation, Software, Methodology, Data curation. **César A. Cárdenas:** Writing – review & editing, Methodology, Conceptualization. **Caroline Pérez-toledo:** Methodology, Formal analysis. **Ignacio Garrido:** Resources, Investigation. **Alejandro Font:** Writing – review & editing, Software, Formal analysis. **Garance Perrois:** Investigation, Formal analysis. **Kurt Paschke:** Validation, Resources, Investigation, Formal analysis, Data curation. **Erwan Corrê:** Validation, Software, Resources, Data curation. **Iliaria Corsi:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Conceptualization. **Céline Cosseau:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Formal analysis, Conceptualization.

Declaration of competing interest

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Supplementary materials

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Data availability

Data will available at NCBI web page after publication

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