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FOCUS ISSUE ARTICLE

Insights into the molecular response of *Dioithona rigida* to selenium nanoparticles: *de novo* transcriptome assembly and differential gene expression analysis

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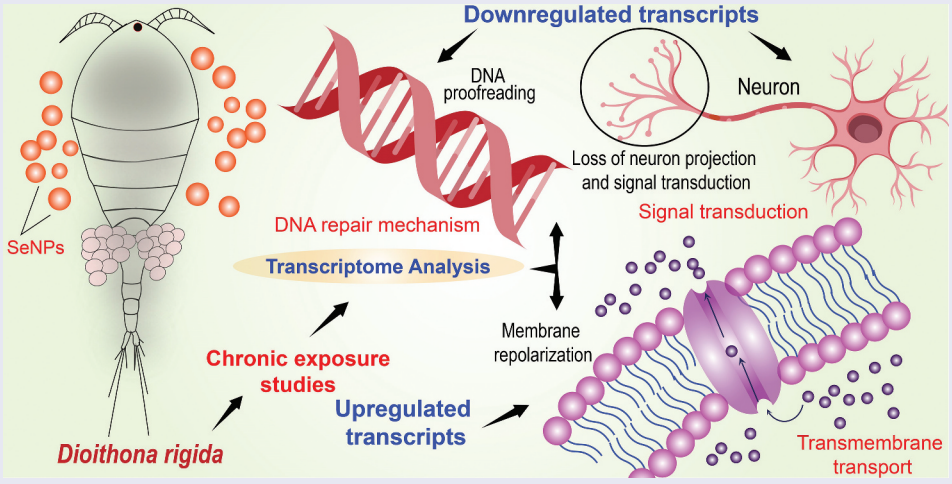
ABSTRACT

The impact of contaminants on Copepod sp. and its molecular response is least explored, despite their abundance and dominance among invertebrates in aquatic environments. In the present investigation, *Dioithona rigida*, a cyclopoid zooplankton, was treated with selenium nanoparticles (SeNPs) to determine the associated biochemical changes, and the chronic exposure effects were recorded using transcriptomic analysis. It was found that, SeNPs were acutely toxic with a lethal dose 50% of 140.9 mg/L. The *de novo* assembled transcriptome of the copepod comprised 81,814 transcripts, which underwent subsequent annotations to biological processes (23,378), cellular components (21,414), and molecular functions (31,015). Comparison of the expressed transcripts against the treated sample showed that a total of 186 transcript genes were differentially expressed among the *D. rigida* treatments (control and SeNPs). The significant downregulated genes are coding for DNA repair, DNA-templated DNA replication, DNA integration, oxidoreductase activity and transmembrane transport. Similarly, significant upregulations were observed in protein phosphatase binding and regulation of membrane repolarization. Understanding the impact of SeNPs on copepods is crucial not only for aquatic ecosystem health but also for human health, as these organisms play a key role in marine food webs, ultimately affecting the fish consumed by humans. By elucidating the molecular responses and potential toxicological effects of SeNPs, this study provides key insights for risk assessments and regulatory policies, ensuring the safety of seafood and protecting human health from the unintended consequences of nanoparticle pollution.



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IMPACT STATEMENT

The toxicity analysis in *Dioithona rigida* is the first of its kind as a copepod model for analysis on dietary fixation of metal toxicity at the trophic level. Since this copepod is a major zooplankton fed by fish and crustacean larvae in marine ecosystems, the toxicity analysis on this copepod will give us more insights of the trophic-level food transfer. As far as our knowledge, this is the first study that opted to construct the *de novo* transcriptomic pipeline for this copepod, treated with selenium nanoparticles. The effectiveness of this work may be further extrapolated to assess the effect of other metal nanoparticles in this model organism. Although the selenium toxicity in marine ecosystem is an established sector, through our combined approach of biochemical analysis and omics approach, the solid framework and comprehensive insight of the selenium toxicity in reproductive fitness and molecular changes has been studied. This study chose to seek a reliable alternative in the sense of new copepod model and omics approach to analyse the relevant metal nanoparticle toxicity in the marine ecosystem.

1. Introduction

The importance of invertebrates in determining the impact of environmental pollutants on the marine biosystem is being evidenced by the growing body of research. Tremendous efforts have been made in present years to find effective and environmentally realistic, invertebrates toxicity testing model organisms [1]. One of the major criteria in providing ecotoxicological and eco-friendly genomic studies to select a good model organism is that it could substantiate the toxicity of marine contaminants with repeatable results and it elucidates the mechanism of toxic action of the pollutants at different stages of biological organisations, mainly at the cellular and genomic levels [2]. Copepods represent one of the most anatomically and functionally diverse groups of invertebrates, playing a crucial role in the dynamics of food webs, serving as indicators of pollution, key participants in various biogeochemical cycles, and acting as a carbon sink [3–5]. With their significant role in different aquatic ecosystems, copepods are considered as model organisms in ecotoxicological assessments [6]. In toxicological investigations, the commonly examined physiological endpoints include mortality, fecundity, egg viability, and larval development ratio are significant in this model organism [7]. To identify the environmental indicators and elucidate the molecular mechanisms triggered by chemical exposure, it is now possible by accessing the genome and transcriptome data for different copepods. Genotoxicity studies enable the characterisation of sensitive biomarkers and the elucidation of the mechanism of action by various nanomaterials [8]. As an important primary consumers, these copepods aid in spreading pollution to higher trophic levels by bioaccumulating toxins [9]. The small size and brief lifespan of copepods are frequently employed in chemical testing. They serve as potent ecotoxicology model species [10]. *Dioithona rigida*, a species of copepod, has been selected as an experimental model due to its unique characteristics. Its small size and short lifespan make it suitable for chemical testing in terms of cost efficiency and reliability. Additionally, *D. rigida* occupies an important ecological niche as a primary consumer in aquatic food webs, making it relevant for assessing the impact of pollutants on higher trophic levels and the potential bioaccumulation of toxins. This relevance extends to human health, as copepods are integral to marine food webs, affecting the fish and seafood consumed by humans.

Nanoparticles (NPs) pose a severe hazard to the environment due to their severe toxicity and durability [11,12]. The intrinsic properties like size, surface composition, crystalline structure, chemical constituents and hazardous properties like aggregation and dissolution to the environment by different NPs are responsible for the significant threat [13]. When these NPs are released to the environment many of the planktonic organisms like copepods are exposed for shorter time because of their lifestyle, the swimming capabilities and their behaviour of rapid transformation when compared to the benthic organisms [14,15]. Therefore, reproductive characteristics and molecular mechanisms including the egg production rate, hatching process, biomarkers, and genomic profiles are significant ways of assessing the effects of different pollutants disposed in the environment [16]. The increased production and utilization of nanomaterials, coupled with the absence of a dedicated waste management system, contribute to their discharge into the environment [17]. Due to the influence of physicochemical properties and various factors such as pH, surface charge, biomass concentration, and the chemical composition of the surroundings, NPs undergo environmental transformations, including aggregation, agglomeration, and dissolution, that play a crucial role in determining their toxicity [18]. The assessment of pollution also should include an ecotoxicological evaluation of the fate and stability of NPs [19]. The ecology is impacted by bioaccumulation in phytoplankton and zooplankton due to the greater ambient concentration of the contaminants [20,21]. Due to their ecological significance, short life cycle, widespread distribution, and sensitivity to low levels of contaminants, planktons are therefore regarded as one of the best reference organisms for toxicological studies [22]. These are evidenced from reports regarding the toxicity values for metals, pesticides and other contaminants on copepods and diatoms [23].

Selenium (Se) is a vital micronutrient for organisms, with a narrow concentration range where it can be either beneficial or toxic [24]. At lower concentrations (approximately 0.1–0.2 μg/g per day), Se aids in detoxification and scavenging reactive oxygen species (ROS) [25]. However, at higher concentrations (> 5 μg/g per day), it becomes toxic due to its pro-oxidant properties and its ability to replace sulfur in proteins [25]. Se contamination in aquatic ecosystems can result from industrial discharges associated with mining operations, thermoelectric power plants, petrochemical refineries, and other sources. Elemental Se is used as a catalyst in many chemical reactions and is essential for the manufacturing of solar cells and photocells. Nonetheless, the USEPA has enacted strict regulations to prevent its discharge into natural waters [26]. It has been found that the potential for biotransformation to impact higher trophic levels, including humans, is increased by the higher Se absorption and biotransformation in primary producers [27]. Thus, toxicity studies are much needed for sensitive marine organisms like copepods to understand their broader ecological impacts and implications for human health [28]. Therefore, the present study aims to

(i) analyse the acute toxicity of SeNPs on the zooplankton model, *D. rigida*.
(ii) generate a *de novo* assembled transcriptome for *D. rigida* exposed to SeNPs.
(iii) evaluate the chronic toxicity effects by examining the molecular and biochemical changes in transcriptomic expressions upon exposure to nanoparticles.

2. Materials and methods

In the present investigation, a lab-based analysis of the acute and chronic toxicity of SeNPs on the marine zooplankton *D. rigida* was conducted. The acute toxicity analysis reveals the immediate toxic effects of SeNPs on the marine ecosystem. Consequently, the chronic exposure analysis provides deeper insights into the long-term biochemical and molecular responses of the organism to SeNPs. Biochemical assays, such as measuring the lactose dehydrogenase (LDH) activity and ROS production, will help to assess the immediate impact of SeNPs on the organism. Meanwhile, comprehensive molecular-level analyses offer a detailed understanding of the negative effects of chronic exposure. These findings not only enhance our understanding of aquatic ecosystem health but also have significance on human health, as these organisms play a crucial role in marine food webs, ultimately affecting the quality of fish consumed by humans.

2.1. Synthesis and characterization of SeNPs

The top-down approach of nanoparticle synthesis was employed using sodium selenite (Na_2SeO_3) and L-cysteine, a natural amino acid, non-toxic and biocompatible, chosen as precursors and synthesized with slight modifications of parameters from the previous literature [29,30]. Briefly, a 50 mM L-cysteine stock solution was prepared by dissolving it in 0.1 M hydrochloric acid (HCl). Then, 2.5 mL of this L-cysteine solution was gradually mixed into 10 mL of 0.1 M Na_2SeO_3 solution in a 1:4 ratio, while stirring continuously for 15 min at ambient temperature. Consequently, the solution was centrifuged at 8000 rpm for 5 min, rinsed with 70% ethanol, left to air dry for 24 h, and then stored for later experimental use.

2.2. Copepod culture

The copepod *D. rigida* were cultivated in 20-L tanks containing filtered seawater (0.22-μm filter) with a salinity of 30 ppt and a pH of 8.0 ± 0.1. They were provided with an algal species, i.e. *Chaetoceros gracilis* during its exponential growth phase, at a final concentration of 1×10^5 cells/mL. Copepod cultures were maintained in a controlled environment at 20 ± 1°C and 8:16 light: dark and photoperiod using a cool white luminescent bulb (20 W, 1900 Lumen).

Approximately 2000 healthy adult *D. rigida* were carefully separated from the main culture tank using a 250-μm mesh net. These adults were acclimated for 3 days in 800-mL beakers at a density of 1 adult/mL and were fed with the algal culture prior to the incubation experiments.

2.3. Acute toxicity analysis

To determine the lethal toxicity (median lethal concentration (Lethal Concentration 50% (LC_{50})) of SeNPs, 96-h acute toxicity tests were performed on the adult *D. rigida* individuals. The tests were conducted according to the ISO 14669:1999 guidelines, with certain modifications [31]. For every concentration of SeNPs examined, three replicate groups consisting of 25 individuals each were placed in 150 mL glass beakers filled with 100 mL of the test solution. A preliminary range-finding test was conducted to determine the toxicity of SeNPs on *D. rigida*. The test involved exposing *D. rigida* to various concentrations of SeNPs, including 10, 20, 50, 100, 200, 500, and 1000 mg/L. Based on the results, it was determined that the LC_{50} of SeNPs for *D. rigida* fell within the range of 100–300 mg/L.

Following the range-finding test, a total of six SeNP concentrations were selected for further analysis: 50, 100, 150, 200, 250, and 300 mg/L. Photoperiod was

16:8 h dark:light cycle, with a salinity of 30 ppt, and temperature controlled at 24°C. Copepods were starved during the experiments. The mortality rates were assessed after 24, 48, 72, and 96 h of exposure. A copepod was deemed deceased if no swimming or movement of its appendages was observed for a duration of 10–15 s [32]. Dose response fit analysis was facilitated to determine the LC_{50} value of the organism. The LC_{50} was determined as the concentration of SeNPs that resulted in 50% mortality of the organisms, and it was compared to the control tanks from the experiment.

2.4. Assessment of biochemical changes

For the estimation of biochemical changes, LDH and ROS produced during the NPs treatment were measured. Briefly, 25 adults were treated with 50, 100, 150, 200, 250 and 300 mg/L of SeNPs concentrations. Individuals were retrieved from NPs treatment after 24, 48, 72 and 96 h to analyse the biochemical changes. The collected organisms were washed with filtered seawater and milli Q water to remove any unbounded NPs. Subsequently the organism was homogenized uniformly using a mortar and pestle in an ice cool condition. After homogenizing the copepod, the pellet was obtained by centrifuging the mixture at 13,000 rpm for 10 min at 4°C. The resulting supernatant was collected for additional biochemical assays [33].

2.4.1. Analysis of ROS generation

The reproductive impairment and biochemical changes observed in marine species are predominantly due to the oxidative stress. Therefore, ROS test was applied in the current investigation to determine the oxidative stress. Using the dichloro-dihydro -fluorescein diacetate (DCFH-DA) test, with a slight alteration, the total amount of ROS generated was determined [34,35]. In brief, the collected supernatant, obtained after centrifugation of homogenized individuals, was combined with DCFH-DA at a final concentration of 100 µM. The resulting mixture was then incubated for 30 min at room temperature (27°C). The fluorescence intensity was measured using a Synergy™ H1 Hybrid Multi-Mode Reader, Biotek, U.S.A., with excitation and emission wavelengths set at 485 nm and 530 nm, respectively.

2.4.2. Membrane permeability analysis

As a measure of the membrane permeability, LDH released due to the membrane damage was employed. The damage to the cell was measured by monitoring the LDH released outside of the organism, which was achieved by employing the conventional methodology with a minor modification [36]. Approximately 100 µL of the supernatant obtained from the homogenized sample was combined with 100 µL of 30 mM sodium pyruvate, followed by the addition of 2.8 mL of 0.2 M Tris-HCl. To initiate the reaction, around 100 µL of 6.6 mM nicotinamide adenine dinucleotide (NADH) was added. The decrease in absorbance was measured by performing 10 readings at 340 nm using a Synergy™ H1 Hybrid Multi-Mode Reader, Biotek, U.S.A., in the UV-visible range. This procedure allowed for the quantification of changes in absorbance associated with the reaction [37].

2.5. Chronic exposure studies and RNA extraction

A total of 250 adult copepods of *D. rigida* were selected and separated from the stock culture. They were then transferred to a 1 L beaker filled with filtered seawater. Throughout the duration of the experiment, the copepods were provided with a food source consisting of *C. gracilis* at a concentration of 1×10^5 cells/mL and was used in the exponential phase of growth. This feeding regimen was maintained consistently during the entire experiment period. To this experiment group, SeNPs (125 mg/L) was introduced at a concentration lower than the LC_{50} value. Similarly, a control group was incubated in filtered seawater and algal culture without toxicants.

The copepods were kept under gentle agitation at a temperature of 20 ± 1°C and subjected to a light–dark photoperiod of 8 h of light and 16 h of darkness. This maintenance condition was maintained for a period of 7 days. Each day, the adult copepods were carefully collected using a 250 µm mesh net filter and transferred to new beakers containing fresh medium and algae. After the 7-day exposure period, approximately 75–80 adult copepods from each duplicate beaker were collected and transferred to 1.5-mL tubes. Until extraction, to preserve the RNA, 1 mL of Trizol® reagent (Invitrogen, Sandiego, CA, U.S.A.) was added to each tube. The samples were then flash-frozen in liquid nitrogen and stored at −80°C until RNA extraction. To extract the RNA from *D. rigida*, the standardized Trizol method as described in the manufacturer's protocol was followed. The quantity and quality check of extracted RNA was carried out using the Qubit RNA HS Assay Kit (Invitrogen, Sandiego, CA, U.S.A.) and RNA Screen Tapes, Tapestation (Agilent, Santa Clara, CA, U.S.A.), respectively.

2.5.1. Transcriptome sequencing, de novo assembly and annotation

The library was prepared using the 'TruSeq RNA Library Prep Kit' from Illumina® (CA, U.S.A.) with Illumina standardized protocol. The final enriched library was validated and quantified on Qubit using dsDNA HS kit and on real-time PCR (Kansas Administrative Procedure Act (KAPA) library

quantification kit). The library quality was validated on TapeStation using the DNA1000 screen tape. The quality of the raw data was assessed using FastQC and MultiQC software. Various parameters were examined, including the distribution of base call quality, the percentage of bases above the quality thresholds (Quality score 20 (Q20) and Quality score 30 (Q30)), the percentage of guanine-cytosine content (GC content), and the presence of sequencing adapter contamination. These analyses were performed to evaluate the overall quality of the data and to identify any potential issues or biases that could impact subsequent analyses.

The *de novo* transcriptome was assembled using Trinity v2.8.5 with the default parameters [38]. Contigs with a length shorter than 200 bp were excluded from subsequent analysis. The assembled transcripts were utilized for predicting the protein coding sequences with the aid of TransDecoder v5.5.0. To annotate the protein sequences, the Basic Local Alignment Search Tool (BLASTp) module of Diamond v2.0.15 was employed to align them against the National Center for Biotechnology Information non-redundant (NCBI nr) database, Universal Protein Resource (Uniprot Swissprot), Protein Families Database (Pfam), Kyoto Encyclopedia of Genes and Genomes (KEGG) orthologous database (KO), and UniProt Reference Cluster (Uniref100) protein database. This process allowed for the identification and characterization of the proteins based on similarities with known sequences in these databases [39]. Transcripts that exhibited a mapping to the database with an E-value lower than 1e-04 were selected for further analysis. Pfam hits for the transcribed proteins were generated using hmmscan v3.3.2, employing the pfam database. The annotations of the transcripts were further refined using TransdecoderPredict. To obtain EuKaryotic Orthologous Groups (KOG) annotations, the protein sequences were submitted to KAAS (KEGG Automatic Annotation Server). These processes allowed for the identification of functional domains and the assignment of putative functions to the transcribed proteins based on their similarity to known protein families and KOG categories [40]. The Gene Ontology (GO) annotations were obtained by submitting the Uniref100 ids to the Uniprot ID mapping.

2.5.2. Differential expression analysis

To identify the differentially expressed genes (DEGs) in *D. rigida*, the raw reads obtained from each sample underwent quality control and were mapped to the assembled transcriptome using Bowtie2 v2.4.5. The read counts per transcript were then extracted using featureCounts v2.0.3 [41]. DEGs were identified in the treated groups of *D. rigida* using the DESeq2 v1.34.0 package [42]. Transcripts with lesser than 5 reads in any of the samples were excluded from further analysis. The read counts were normalized using DESeq2's variance stabilized normalization method, and differential expression analysis was conducted. Genes were considered differentially expressed with a threshold of 1% as the false discovery rate, and when changes in term of log2FC were more or equal to 2. This approach allowed for the identification of genes that exhibited significant changes in expression between the treated groups of *D. rigida*.

2.5.3. Functional insights using ToppFun database

To better understand the functional implications of the DEGs in *D. rigida*, the results of the differential expression analysis were compared with the ToppFun database (https://toppgene.cchmc.org/enrichment.jsp) [43]. The ToppFun database provides a comprehensive range of functional annotations, including GO terms, biological processes, molecular functions, and pathways. By conducting this comparison, the study aimed to identify enriched functional categories and pathways associated with molecular response of *D. rigida* to chronic exposure to SeNPs.

The DEGs were characterized and annotated by comparing them with the ToppFun database, offering insights into their putative functions and involvement in various biological processes. This integration of transcriptomic data with the ToppFun database enabled the identification of key molecular pathways, biological processes, and cellular functions that may contribute to *D. rigida* response to SeNPs. Exploring these functional annotations aimed to shed light on the underlying mechanisms and pathways through which SeNPs impact the biological functions of copepod.

2.6. Data analysis and statistics

Experiments were carried out in replicates, and the data are expressed as mean ± standard error.

3. Results and discussion

3.1. Selenium nanoparticle characterization

The physico-chemical characteristics of SeNPs used in the present study were thoroughly investigated in a previous study conducted by our group [44]. UV-Vis spectroscopic analysis showed a maximum absorption at 258 nm, indicating the reduction of Na_2SeO_3 to form SeNPs. High-resolution transmission electron microscopic (HRTEM) imaging revealed that the SeNPs were spherical and polydisperse in nature, consistent with previous literature. Dynamic light scattering measurements and zeta potential analysis confirmed the stability of the colloidal solution, with a zeta potential of −15.2 mV and a size range of 50–250 nm. Raman scattering spectra

identified characteristic peaks at 140, 236, and 254 cm^{-1}, representing the bonding state of the SeNPs. X-ray photoelectron spectroscopy (XPS) analysis detected the presence of elements Se, O, C, and S on the surface of the SeNPs, with Se 3d spectra showing characteristic spin orbitals. The O1s spectrum indicated the involvement of the carbonyl group in the synthesis of SeNPs, while the C1s spectra revealed the presence of different carbon bonding environments. In the current study, acute toxicity analysis of the NPs was conducted on the copepods (LC_{50}) and biochemical changes due to the exposure (LDH assay and ROS production).

3.2. Acute toxicity analysis

During the experiment, 25 adult *D. rigida* individuals were exposed to SeNPs at six different concentrations across four-time intervals (24, 48, 72, and 96 h). The mortality rate of the exposed adults was determined and compared to a control group, which exhibited 100% viability (Figure 1). In the samples exposed to SeNPs, the mortality rate increased with longer exposure times at all the six test concentrations. Notably, at 96 h and a concentration of 300 mg/L (Figure 1 (a)), the viability dropped to a minimum, with fewer than four viable organisms remaining. This observation indicates concentration and time-dependent toxicity of SeNPs on *D. rigida*. Furthermore, an acute toxicity analysis was conducted, leading to the calculation of the LC_{50}. The resulting LC_{50} value was determined to be 140.9 mg/L as shown in Figure 1b. This value represents the concentration of SeNPs at which 50% mortality of *D. rigida* is expected.

Studies have demonstrated that aquatic organisms are impacted in terms of their growth, development, and reproductive capabilities when they consume diets that contain significant amounts of non-dietary substances including several toxic contaminant accumulation causing reproductive impairment and reduced fecundity [45]. Literatures suggest that the reduced survival rates observed in individuals exposed to NPs may be attributed to the possibility of NPs entrapment and consequent blockage within the gastrointestinal tract of the affected organisms [46].

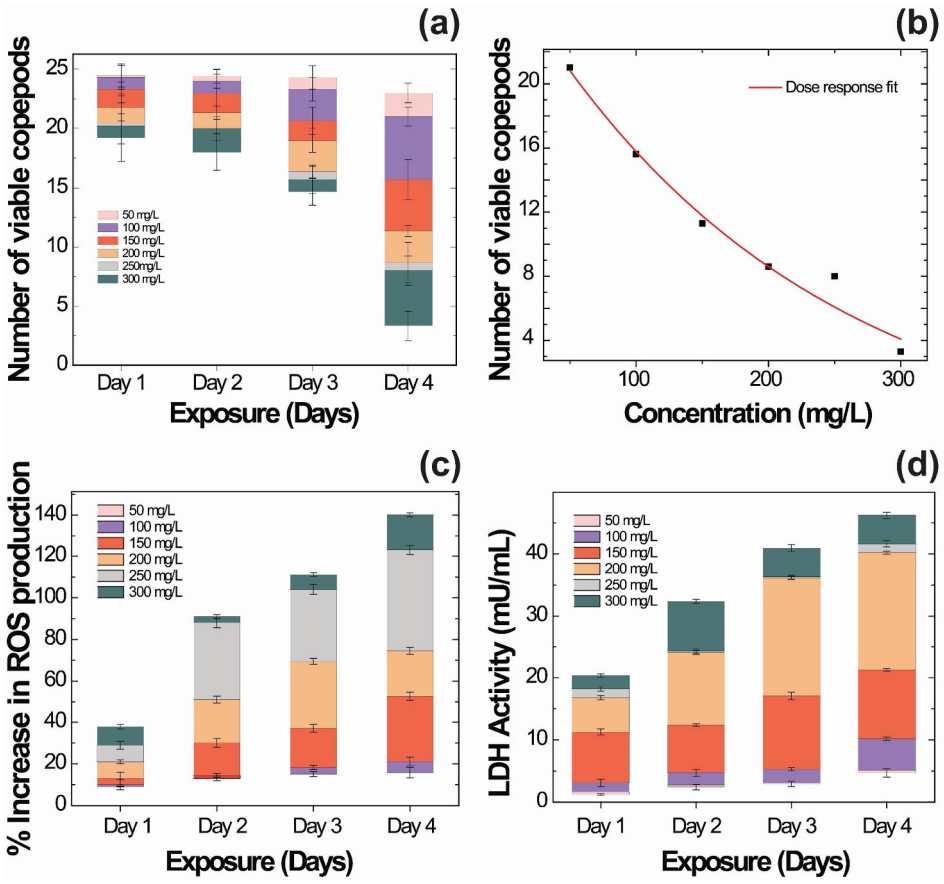

Figure 1. (a) The mortality of adults was calculated and found to be decreasing with respect to control (100% viability) in interacted samples. (b) Dose–response curve shows LC_{50} from the acute toxicity analysis is 140.9 mg/L. (c) In comparison to the corresponding controls, ROS generation increased for all three exposure concentration cases and all time periods for *D. rigida* treated with SeNPs. (d) When compared with control, a significant amount of LDH release was observed upon exposure to SeNPs after 96 h.

3.3. Biochemical changes

3.3.1. Estimation of ROS generation

The generation of ROS was thought to be the most refined illustration of how NPs might be toxic. Oxidative stress assessment is a crucial biochemical technique to investigate the oxidative stress caused by the antimicrobial activities of NPs [47,48]. Here, the DCFH-DA method was employed to quantify the amount of ROS produced. This method works as follows: intracellular esterase breaks down DCFH-DA into 2,7-dichlorofluorescein (DCF), a non-fluorescent molecule capable of permeating the cell membrane and diffusing through the cell wall. In the presence of ROS, DCF undergoes rapid oxidation, resulting in the formation of fluorescent DCF. The intensity of DCF fluorescence directly corresponds to the quantity of intracellular ROS produced [49]. Intracellular ROS generation was observed using a previously reported calculation with slight modifications [50]. In comparison to the corresponding controls, ROS generation increased for all six exposure concentration cases (50, 100, 150, 200, 250 and 300 mg/L) and all time periods (24, 48, 72, and 96 h) for *D. rigida* treated with SeNPs (Figure 1(c)). After 96-h treatment with 300 mg/L of SeNPs, a significant increase in ROS production was observed, reaching levels of 140.2 ± 1.8%. This elevated ROS generation has the potential to cause cellular damage, particularly in terms of membrane integrity. One way to detect this damage is by quantifying the release of LDH, which serves as an indicator of membrane disruption at the cellular level. High concentrations of SeNPs can impair the antioxidant defense mechanisms, leading to an imbalance between ROS production and antioxidant capacity, thereby contributing to the rapid accumulation of ROS within the system [51]. A similar occurrence has been observed in *Tigriopus japonicus* when exposed to zinc, wherein the activities of oxidative enzymes remained active, and presence of ROS was found within the organism. However, these antioxidant responses were ineffective as the resulting oxidative stress caused cellular and DNA damage that was irreparable, ultimately resulting in apoptosis and high mortality rates among the copepods after being exposed for 96 h [52].

3.3.2. Membrane permeability analysis

Membrane permeability assay was done to understand the cytotoxic activity of SeNPs toward the *D. rigida* at the concentration of 50, 100, 150, 200, 250, 300 mg/L for four different time points. After 24 h of exposure, *D. rigida* exposed to 250 and 300 mg/L of SeNPs exhibited LDH release levels of 18.03 ± 0.34 and 20.033 ± 0.23 mU/mL, respectively (Figure 1(d)). Subsequently, after 96 h, the LDH release further increased to 40.3 ± 0.14 and 45.3 ± 0.340 mU/mL, respectively. Notably, when compared to the control group, a significant LDH release of 45.3 ± 0.340 mU/mL was observed in samples exposed to 300 mg/L of SeNPs after 96 h. This considerable increase in LDH release indicates a change in membrane permeability and the cytotoxic effects of SeNPs on *D. rigida*. The elevated LDH release confirms the potential disruption of cellular membranes and the induction of cytotoxicity caused by SeNPs. The fact that zooplankton exposed to NPs had higher LDH levels than the control group is well-known from previous research. This increase in permeability is mainly due to the NPs being absorbed by the organism [53].

3.4. Transcriptome sequencing and de novo assembly

The Illumina sequencing of the control and treated samples yielded 32,173,320 and 43,061,462 high-quality raw reads. The control sample has a GC content of 41.15% and a Q30 score of 91.13%. On the other hand, the treated sample has a higher GC content of 53.39% and a higher Q30 score of 93.84%.

The results generated in *de novo* assembly of *D. rigida* utilizing all high-quality reads obtained from each sample were taken for consideration. This generated an assembled transcriptome size of 453,586,853, which were further assembled to total trinity transcripts 929,350 in which annotated protein coding transcripts were 81,814 (71.52%).

3.4.1. Functional annotation of combined assembly

The protein sequences were subjected to annotation using the BLASTp module against various

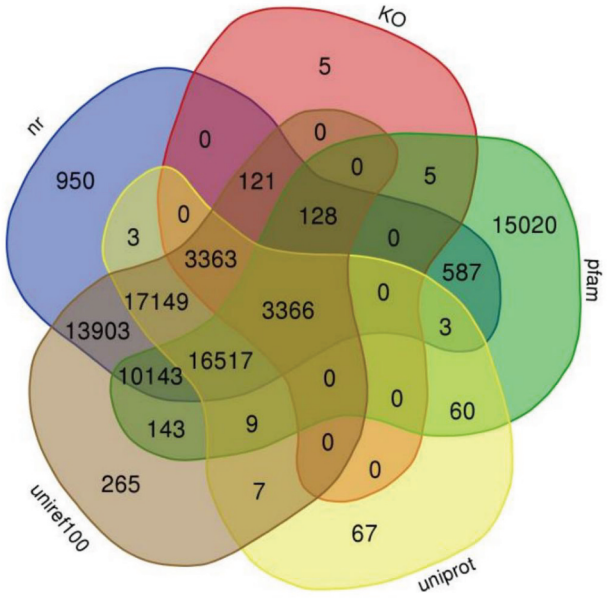

Figure 2. Venn diagram showing the annotated transcripts on different databases such as pfam, uniprot, uniref100, NCBI nr, and KO.

databases, including the NCBI nr, Uniprot, and Uniref100 protein databases. The results of the annotation are presented in Figure 2. For a better annotation efficiency, only contigs greater than 300 bp were considered. A total of 81,814 transcripts were annotated against the different databases and the found annotations are summarized and represented in the Venn diagram. To assess the representation of cellular components, biological processes, and molecular functions among the assembled transcripts, the GO terms were collected and analysed. The GO distribution of transcripts showed that 23,378 annotate to biological process, 21,414 to cellular components and 31,015 to molecular functions.

The transcripts of *D. rigida* were annotated with KEGG Orthologous (KO) annotation, resulting in the assignment of 35 functional classes (Figure 3). These functional classes encompass a wide range of biological processes, including cellular processes, environmental information processing, genetic information processing, metabolism, and organismal systems. Notably, a significant portion of the transcripts were found to be associated with environmental information processing, particularly in the context of signal transduction. This finding aligns with previous reports, further supporting the observation of similar transcript annotation patterns in *D. rigida* [54]. Cell growth and death, transport, and catabolism, translation, folding sorting and degradation, amino acid metabolism endocrine system, nervous system are the most represented annotations.

3.5. Differential expression analysis

Comparison of the expressed transcripts against the treated sample showed that a total number of 186 transcript genes were differentially expressed among the *D. rigida* treatments (Control and SeNPs). In the DEGs 132 transcripts were upregulated and 54 transcripts were downregulated upon chronic exposure. This amount of DEG observed was slightly higher than that which reported for *Eurytemora affinis* exposed to endocrine disrupting pesticides (3–8% DEG), as well for *T. japonicus* (0.1% DEG) and *Pseudodiaptomus annandalei* (0.01% DEG), when those species were exposed to low doses of mercury chloride. Additionally, *P. annandalei* primarily exhibited upregulated genes, but *T. japonicus* displayed an equal number of up- and downregulated genes. Therefore, it is likely that species- and toxicant-specific differences in gene expression levels between copepods and treatments exist [55,56].

The significant downregulated genes are coding for DNA repair, DNA templated DNA replication, DNA integration, DNA mediated transposition, oxidoreductase activity, cell redox homeostasis, transmembrane transport, methylation, ubiquinone biosynthetic process and carbohydrate biosynthetic process. Similarly, significant upregulations occured in protein phosphatase binding and regulation of membrane repolarization.

Significantly, the signal transduction functional category within the environmental information processing was the most represented KO functional category in the transcriptome of *D. rigida*. It is intriguing

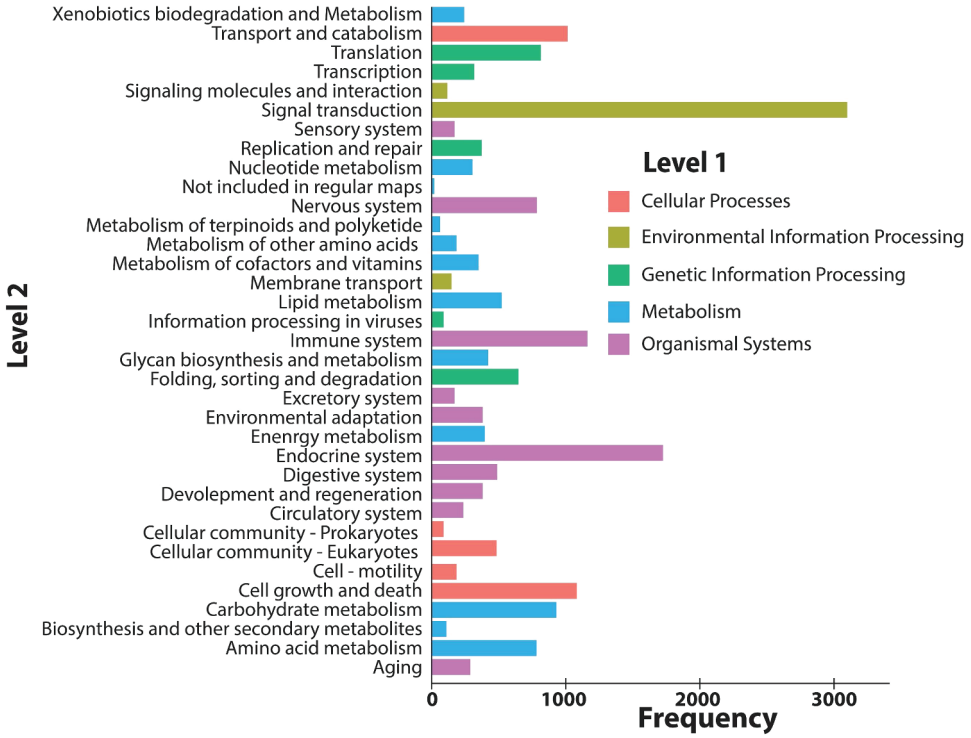

Figure 3. KO classification of *D. rigida* transcripts. The KO classification had been divided into five functional classifications such as cellular processes, environmental information processing, genetic information processing, metabolism, and organismal systems.

to observe that the human disease category, encompassing transcripts associated with cancer, neurodegenerative diseases, and infectious diseases, was also prominently present. However, further investigations are required to ascertain whether these findings are directly linked to the specific toxic effects of SeNPs on *D. rigida*. Additional studies will help to elucidate the underlying mechanisms and provide a clearer understanding of the relationship between SeNPs and the observed transcriptome annotations related to signal transduction and human diseases.

DEGs of *D. rigida* were further mapped against the ToppFun database to infer the cellular component, biological processes, molecular function, and pathway using the GO ID. From the upregulated genes, regulation of membrane repolarization and protein phosphatase binding were found to be significantly low compared to the genes in common (Figure 4). The proper functioning of the nervous system, which includes sensation, integration, and response, relies on the regulation of membrane repolarization during action potential in neurons. This crucial process is essential for the effective transmission of signals and

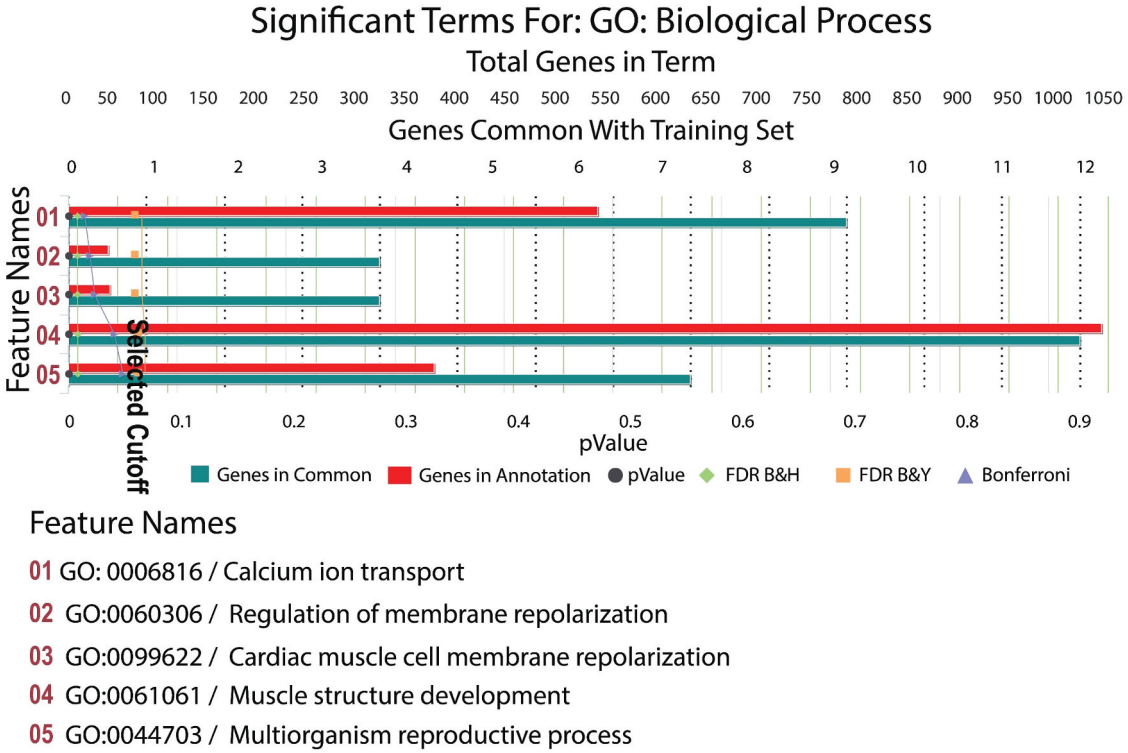

Figure 4. ToppFun database annotation with the upregulated gene ontology ID in the biological process category, which accounts for the regulation of membrane repolarization. This could disrupt cellular homeostasis and affect other cellular processes dependent on these ion gradients.

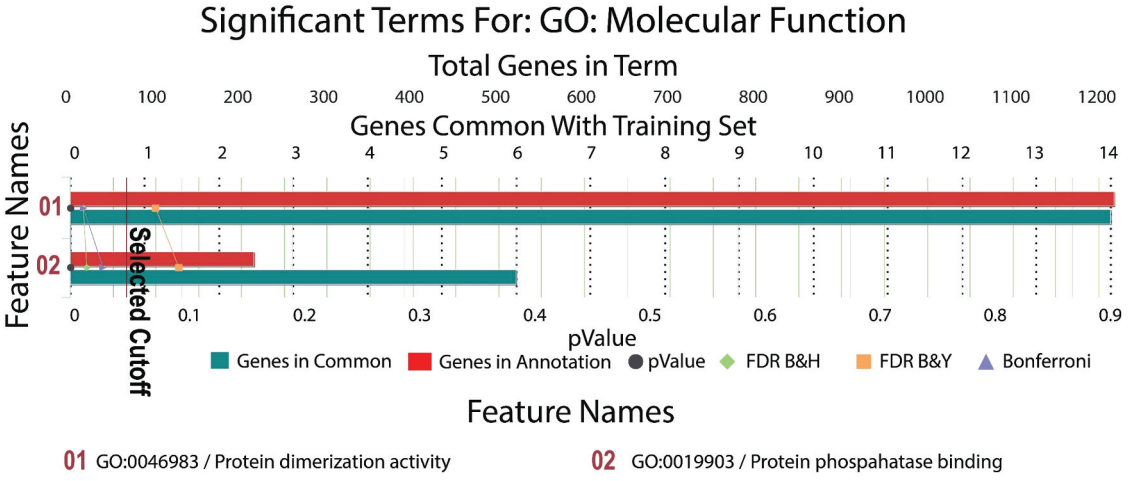

Figure 5. The upregulated genes are compared with the ToppFun database using the gene ontology ID, revealing significant dysfunction in protein phosphatase binding. This leads to a significant lack of action in metabolic pathways and translation.

the functioning of neurons that underlie these pathways [57].

The upregulation of protein phosphatase binding is associated with a wide range of cellular processes, including cell cycle regulation, cell morphology, development, signal transduction, translation, apoptosis, and stress response (Figure 5). This upregulation of protein phosphatase has several potential implications for the endoplasmic reticulum (ER) stress response. For instance, chronic ER stress can lead to programmed cell death through the activation of proapoptotic pathways involving molecules such as inositol-requiring enzyme (IRE1), tumor necrosis factor (TNF) receptor associated factor-2 (TRAF2), caspase-12, caspase-3, cyclophosphamide, doxorubicin hydrochloride (hydroxydaunorubicin), vincristine sulfate (Oncovin), and prednisone (CHOP), and members of the B-cell leukemia/lymphoma 2 (BCL-2) family. The proapoptotic transcription factor CHOP, induced by the Activating Transcription Factor 4 (ATF4) and Activating transcription factor 6 (ATF6), inhibits the transcription of anti-apoptoticBCL-2, thereby promoting cell death [58,59].

The downregulated genes were also mapped using the ToppFun database, and in the gene ontology analysis related to cellular components, it was observed that the integrin complex, specifically the protein complex involved in cell adhesion, had fewer annotated genes compared to the commonly observed genes (Figure 6). Cell adhesion complexes play a crucial role in facilitating cell attachment to each other or to the extracellular matrix [60,61]. The regulation of cell adhesion plays a vital role in maintaining tissue homeostasis and is involved in important processes such as cell migration and differentiation [62,63]. In this study, it was observed that the signal transduction by L1, a cell adhesion molecule predominantly found in the nervous system and concentrated on the axons, was significantly downregulated (Figure 7). L1, as a culture substrate, has been known to have a strong influence on promoting neurite outgrowth and eliciting specific growth cone behaviour. The environmental signalling and signal decoding from the other organism will be disrupted by the downregulation [64]. In addition, the downregulation of integrin family cell surface interactions and intermediate filaments (Figure 8) raises questions about the impact on cellular architecture and function. Integrins are important for cell adhesion and communication with the extracellular matrix, while intermediate filaments contribute to the formation of distinct elongated structures, typically 10 nm in diameter, within the cytoplasm of eukaryotic cells. The observed downregulation of these components raises concerns about potential alterations in cell structure and function [65,66].

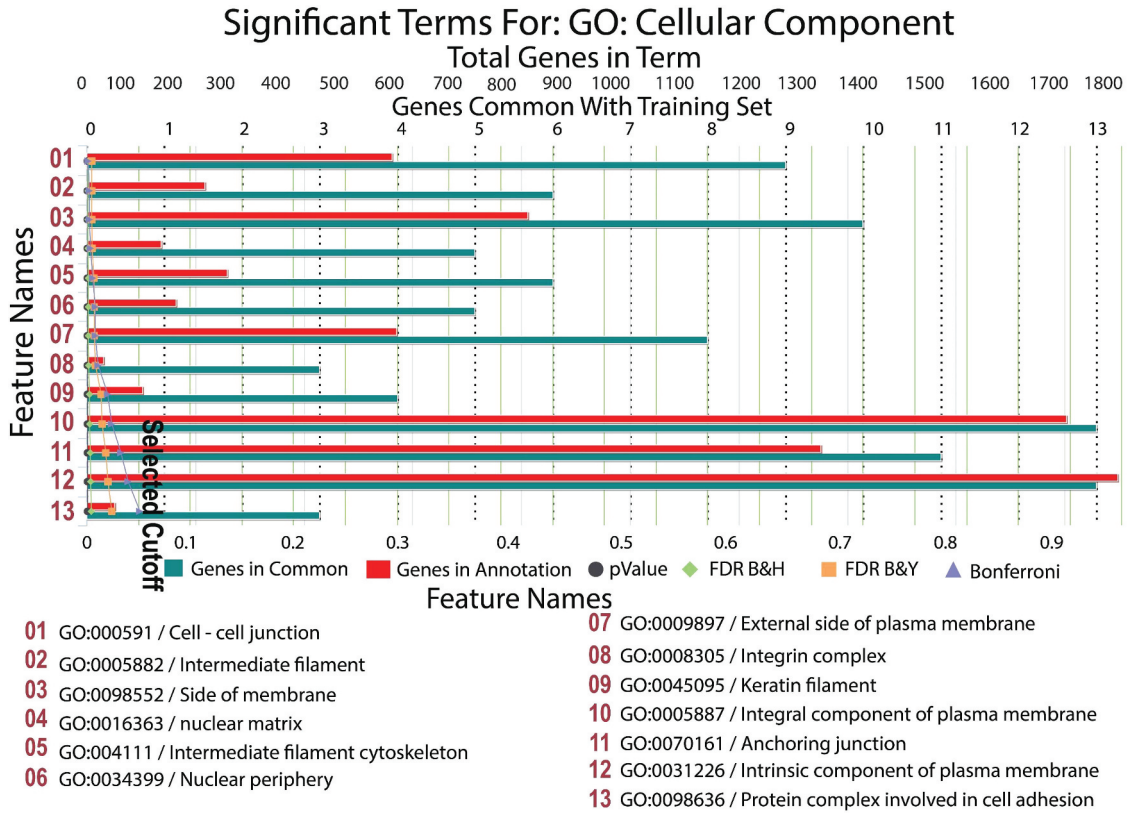

Figure 6. ToppFun database annotation with the downregulated gene ontology ID showed less genes in annotation than the genes in common in cellular component functional class. This includes Integrin complex and protein complex involved in cell adhesion.

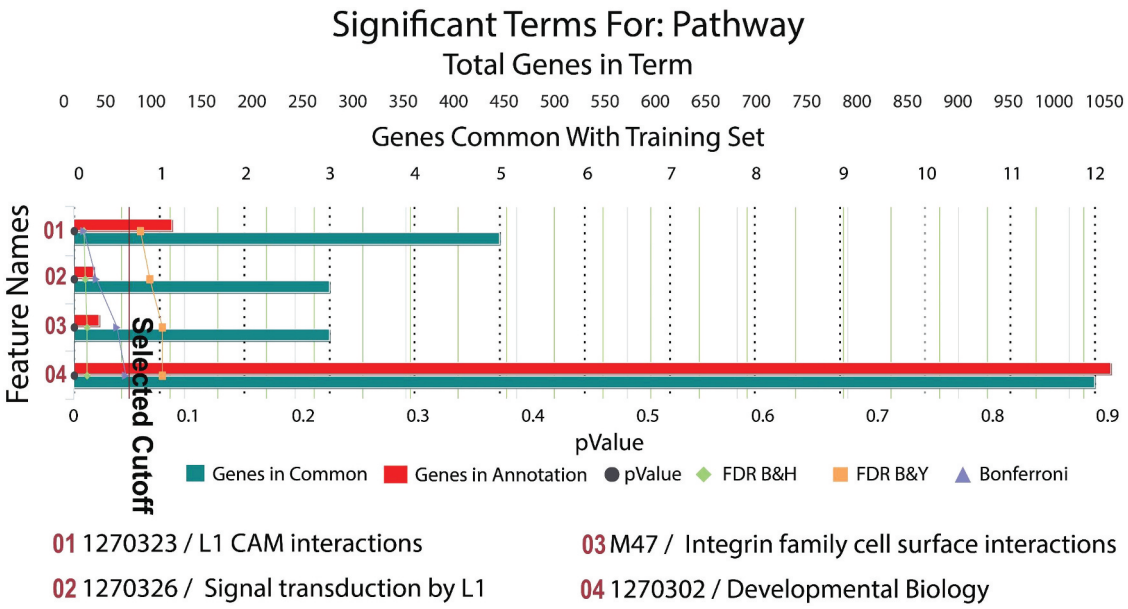

Figure 7. ToppFun database annotation with the downregulated gene ontology ID in the functional class of pathway shows genes in annotation less than the cutoff selection when compared to the ToppFun database. This includes signal transduction and integrin family cell surface interactions.

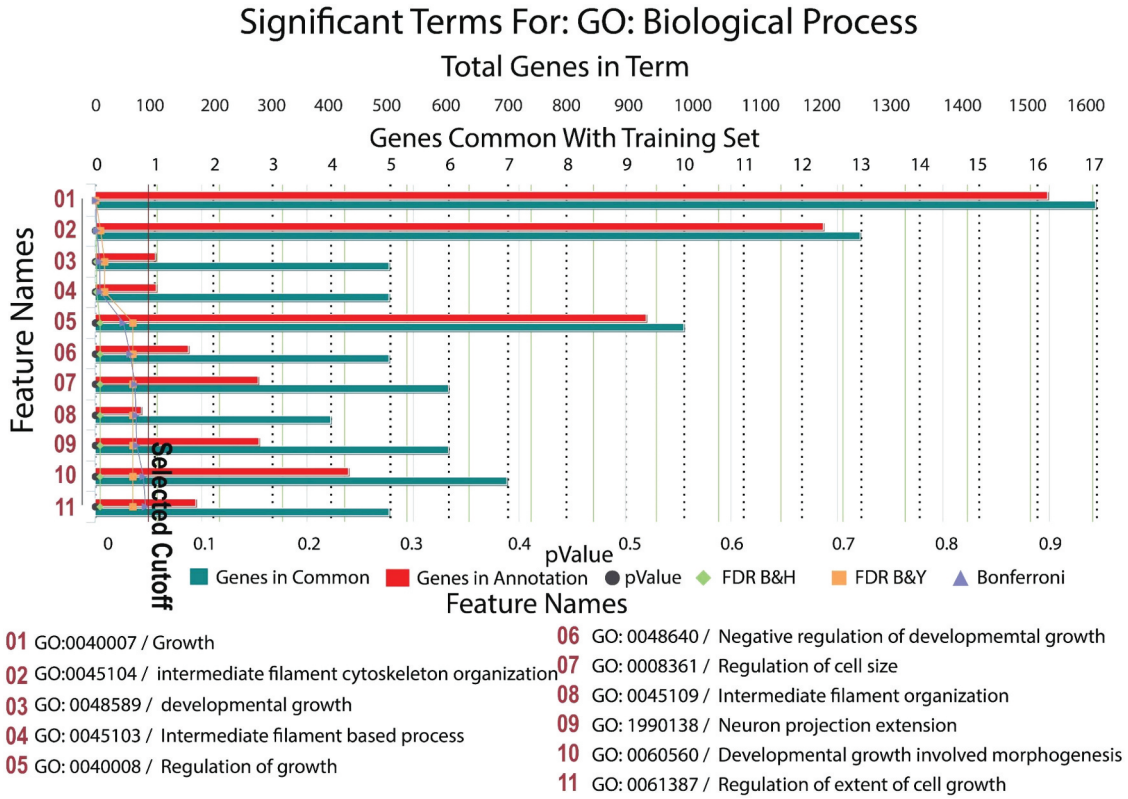

Figure 8. ToppFun database annotation with the downregulated gene ontology ID in the functional class biological processes. The intermediate filament organization and intermediate filament cytoskeleton organization had been found to be less than genes in common. This implies specific damage to biological processes.

The downregulated signalling cascade will be leading to the mutations in the next generations, and it may cause reproduction of the organism difficult [67]. It will be challenging to find the mate due to the inaccuracy of the signal transduction. This will have a mutative effect in the next generation, analysing the progeny of the *D. rigida* exposed to SeNPs will be giving more information on the same [68]. A thorough investigation on the larval morphology and physiological activities in the next generation should be done to conclude the mutation.

Toxicity associated with downregulation of DNA repair and DNA integration leads to genetic instability. During stressful conditions, especially those that can cause DNA damage, it is reasonable to assume that the cells would prioritize the protection of their DNA even more than under normal circumstances. DNA repair is an energy-intensive process that relies heavily on adenosine triphosphate (ATP) for its execution. Therefore, when faced with adverse conditions, cells may down-regulate DNA repair pathways to conserve energy. It is worth noting that this downregulation of DNA repair pathways during hypoxic conditions, which can be accompanied by a reduction in cell membrane transport, may seem counterintuitive as it could potentially hinder the cellular response [69].

One of the downregulated genes encoding oxidoreductase activity corresponds to the biochemical change analysis performed. The production of ROS in response to SeNPs exposure was dose dependent. This is because the oxidoreductase enzyme was reduced due to the exposure, leading to an increase in ROS levels, which ultimately resulted in cell death. Here, chronic administration of SeNPs negatively affected the DNA repair, DNA integration, protein phosphatase binding, oxidoreductase activity and transmembrane transport of *D. rigida* which results in the stress and apoptosis to the organism.

In line with the above results, there are a few reports on the molecular responses of lower trophic organisms. For example, nickel NPs showed toxicity towards the copepod species *Acartia tonsa* associated with xenobiotic exposure and resulted in the reduction of gene expression levels of several ribosomal proteins, with a consequent impairment of the ribosome synthesis pathways [70,71]. Some of the NPs induce negative effects on cell growth and the synthesis of new proteins and a recent study showed the downregulation of oogenesis, cell division and translation [72,73,74]. A filter-feeding marine invertebrate, the ascidian *Ciona intestinalis*, was recently studied to determine how nickel NPs affected its the sperm quality and capacity to fertilize. In their research, the authors showed that nickel NPs changed sperm morphology and mitochondrial membrane potential, which led to developmental abnormalities, oxidative stress, lipid peroxidation, and DNA fragmentation in *C. intestinalis* progeny.

3.5.1. Implications of gene expression changes to copepod physiology

The observed changes in gene expression in *D. rigida* upon exposure to SeNPs have significant implications for copepod physiology, ecosystem health, and potential human disease. The downregulation of genes involved in DNA repair, DNA integrity, oxidoreductase activity, and transmembrane transport, alongside the upregulation of genes associated with protein phosphatase binding and membrane repolarization, indicates a profound impact on the cellular and molecular functions of the organism [75].

In terms of copepod physiology, the downregulation of genes related to DNA repair and integrity suggests that *D. rigida* may experience increased genetic instability under chronic SeNPs exposure. This genetic instability can lead to mutations, impaired cell division, and compromised cellular function, which collectively affect the overall health and the survival of the organism [76]. The reduction in oxidoreductase activity leads to ROS levels, causing oxidative stress, cellular damage, and apoptosis. Elevated ROS levels disrupt normal cellular processes, and damage the proteins, lipids, and DNA, which further exacerbates the physiological stress on *D. rigida* [77]. Additionally, the downregulation of genes involved in cell adhesion, such as those coding for the integrin complex, and membrane transport compromises cellular communication and structural integrity. This disruption affects crucial processes including cellular migration, differentiation, and response to environmental signals, which are essential for maintaining tissue homeostasis and overall organismal function. Furthermore, the significant presence of signal transduction pathways among the downregulated genes indicates potential disruptions in nervous system function, which relies on accurate signal transmission and integration. This can affect the sensory and motor functions, impacting the ability of the organism to respond to environmental stimuli and find mates, further threatening the viability of the species [77].

These physiological changes in *D. rigid*a have broader implications for ecosystem health. As a primary consumer, the population of *D. rigida* is critical for the stability of marine food webs. Impaired copepod populations can lead to reduced availability of prey for higher trophic levels, affecting predator species and potentially leading to cascading effects throughout the ecosystem [70]. Moreover, copepods play a vital role in nutrient cycling by consuming phytoplankton and recycling nutrients. Reduced copepod populations and impaired physiological functions can disrupt these processes, leading to imbalances in nutrient availability and ecosystem productivity. The bioaccumulation of SeNPs in copepods can result in the transfer of toxic effects to higher trophic levels, including fish grade species. This poses risks not only to marine biodiversity but also to human health through the consumption of contaminated seafood [71].

The implications for human disease are also notable. The presence of transcripts associated with cancer, neurodegenerative diseases, and infectious diseases in the downregulated gene set raises concerns about the potential health impacts of nanoparticle exposure [72]. These molecular pathways, if disrupted, could mirror

the effects seen in human cells, highlighting the need for caution in the use of nanoparticles. Understanding the molecular responses of marine organisms to nanoparticle exposure can inform the development of biomarkers for monitoring environmental health and assessing the risks associated with nanoparticle pollution. This knowledge is crucial for regulatory policies aimed at minimizing environmental release and protecting both ecosystem and human health [73].

The changes in gene expression observed in *D. rigida* upon SeNPs exposure underscore the complex interplay between molecular responses and organismal health. These findings highlight the need for comprehensive studies to unravel the long-term ecological and health implications of nanoparticle pollution, ultimately guiding safer nanotechnology practices and environmental protection measures. By understanding the detailed molecular and physiological impacts of nanoparticle exposure on marine organisms, we can better predict and mitigate the cascading effects on ecosystems and human health, ensuring a more sustainable approach to nanotechnology development and application [74].

3.6. Practical applications and future research prospects

The current investigation into the toxicity risk posed by SeNPs in marine environments, focusing on the primary consumer organism *D. rigida*, has unveiled critical insights into the molecular responses to nanoparticle exposure. The study revealed the downregulation of transcripts essential for the vital functions of the organism, such as DNA repair, DNA integrity, oxidoreductase activity, and transmembrane transport, alongside a few significantly upregulated transcripts associated with severe translational damage. This result is corroborated by the biochemical experiments, which demonstrates an increase in ROS production and its impact on enzyme production, as well as correlations with cell membrane integrity. However, the long-term consequences of these genetic alterations in multigenerational model organism remain to be fully elucidated.

The implications of these findings are vast, ranging from the development of specific biomarkers for monitoring aquatic health and nanoparticle pollution to refining nanoparticle risk assessments in marine ecosystems. Such insights could inform regulatory policies to minimize environmental release and guide the design of less harmful nanoparticle formulations. Future research directions should focus on longitudinal studies to assess the generational effects, mechanisms of nanoparticle toxicity, and expand the scope to include a wider range of organisms. Additionally, exploring modifications to nanoparticle properties could mitigate the adverse effects while maintaining their beneficial uses. Through comprehensive understanding and strategic research, it is possible to harness the advantages of nanotechnology while safeguarding the marine ecosystems and their inhabitants against potential toxicological risks.

4. Conclusions

In the current investigation, the toxicity risk of SeNPs under marine conditions was assessed on the primary consumer class organism *D. rigida*. The study revealed the downregulation of several transcripts coding to the vital functions of the organism, particularly, DNA repair, DNA integrity, oxidoreductase activity and transmembrane transport. Significantly few upregulated transcripts coding to the protein phosphatase binding also cause severe effects which lead to damage of translation in the organism. Some of the DEGs are in line with the wet lab experiments, such as an increase in ROS production which leads to the correlation of reduction in oxidoreductase enzyme production and LDH assay correlated with the downregulation of cell integrin complex as well as cell membrane transport. Nevertheless, it is still unclear whether the changes observed in DNA repair mechanisms, protein phosphatase binding, and signal transduction will have an impact on the subsequent generation of the organism. Further investigation is needed to determine the potential consequences of these alterations and their implications on the overall fitness and reproductive success of the organism. A detailed study on generational exchange of the toxic effects should be thoroughly investigated to analyse the chronic toxicity effects of SeNPs.

These findings of this study have profound ecological implications. The disruption of vital molecular functions in *D. rigida* suggests potential adverse effects on marine food webs, as copepods are key secondary consumers and nutrient recyclers. The impaired health and reproductive success of these organisms could lead to reduced populations, thereby impacting predator species that rely on them for food. Additionally, the bioaccumulation of SeNPs in copepods might result in the transfer of toxic effects to higher trophic levels, including commercially important fish species consumed by humans. Understanding these impacts is crucial for developing strategies to mitigate nanoparticle pollution and protect marine biodiversity and ecosystem health.

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References

[1] Lim ZZJ, Li JEJ, Ng CT, et al. Gold nanoparticles in cancer therapy. Acta Pharmacol Sin. 2011;32(8):983–990. doi: 10.1038/aps.2011.82
[2] Raisuddin S, Kwok KWH, Leung KMY, et al. The copepod *Tigriopus*: a promising marine model organism for ecotoxicology and environmental genomics. Aquat Toxicol. 2007;83(3):161–173. doi: 10.1016/j.aquatox.2007.04.005
[3] Baek MJ, Son J, Park J, et al. Quantitative prediction of mixture toxicity of $AgNO_3$ and ZnO nanoparticles on *Daphnia magna*. Sci Technol Adv Mater. 2020;21(1):333–345. doi: 10.1080/14686996.2020.1766343
[4] Mayor DJ, Gentleman WC, Anderson TR. Ocean carbon sequestration: particle fragmentation by copepods as a significant unrecognised factor?: explicitly representing the role of copepods in biogeochemical models may fundamentally improve understanding of future ocean carbon storage. Bioessays. 2020;42(12):1–7. doi: 10.1002/bies.202000149
[5] Russo E, Lauritano C, d'Ippolito G, et al. RNA-Seq and differential gene expression analysis in *Temora stylifera* copepod females with contrasting non-feeding nauplii survival rates: an environmental transcriptomics study. BMC Genomic. 2020;21(1):693. doi: 10.1186/s12864-020-07112-w
[6] Kwok KWH, Souissi S, Dur G, et al. Copepods as references species in estuarine and marine waters. Aquatic ecotoxicology. 2015. 281–308. doi: 10.1016/B978-0-12-800949-9.00012-7
[7] Buttino I, Vitiello V, Macchia S, et al. Larval development ratio test with the calanoid copepod *Acartia tonsa* as a new bioassay to assess marine sediment quality. Ecotoxicol Environ Saf. 2018;149:1–9. doi: 10.1016/j.ecoenv.2017.10.062
[8] Wonga SWY, Zhoua G-J, Leung PTY, et al. Sunscreens containing zinc oxide nanoparticles can trigger oxidative stress and toxicity to the marine copepod *Tigriopus japonicus*. Mar Pollut Bull. 2020;154:11107. doi: 10.1016/j.marpolbul.2020.111078
[9] Tiphaine1 C, Emilie S, Mireille H-V, et al. Patterns of trace metal bioaccumulation and trophic transfer in a phytoplankton-zooplankton-small pelagic fish marine food web. Mar Pollut Bull. 2019;146:1013–1030. doi: 10.1016/j.marpolbul.2019.07.047
[10] Legranda E, Forget-Leraya J, Duflo A, et al. Transcriptome analysis of the copepod *Eurytemora affinis* upon exposure to endocrine disruptor pesticides: focus on reproduction and development. Aquat Toxicol. 2016;176:64–75. doi: 10.1016/j.aquatox.2016.04.010
[11] Rajput V, Minkina T, Sushkova S, et al. ZnO and CuO nanoparticles: a threat to soil organisms, plants, and human health. Environ Geochem Health. 2020;42(1):147–158. doi: 10.1007/s10653-019-00317-3
[12] Danabas D, Ates M, Ertit Tastan B, et al. Effects of Zn and ZnO nanoparticles on *Artemia salina* and *Daphnia magna* organisms: toxicity, accumulation and elimination. Sci Total Environ. 2020;711:134869. doi: 10.1016/j.scitotenv.2019.134869
[13] Djebbi E, Bonnet D, Pringault O, et al. Effects of nickel oxide nanoparticles on survival, reproduction, and oxidative stress biomarkers in the marine calanoid copepod *Centropages ponticus* under short-term exposure. Environ Sci Pollut Res. 2021;28(17):21978–21990. doi: 10.1007/s11356-020-11781-1
[14] Suganya G, Senthamarai R. Mathematical modeling and analysis of phytoplankton–zooplankton–nanoparticle dynamics. Math Model Comput. 2022;9(2):333–341. doi: 10.23939/mmc2022.02.333
[15] Yan N, Tsim SMJ, He X, et al. Direct visualization and quantification of maternal transfer of silver nanoparticles in zooplankton. Environ Sci Technol. 2020;54(17):10763–10771. doi: 10.1021/acs.est.0c03228
[16] Asai S, Sanges R, Lauritano C, et al. *De novo* transcriptome assembly and gene expression profiling of the copepod *Calanus helgolandicus* feeding on the PUA-producing diatom *Skeletonema marinoi*. Mar Drugs. 2020;18(8). doi: 10.3390/MD18080392
[17] Fan D, Li L, Li Z, et al. Biosynthesis of selenium nanoparticles and their protective, antioxidative effects in streptozotocin induced diabetic rats. Sci Technol Advanc Mater. 2020;21(1):505–514. doi: 10.1080/14686996.2020.1788907
[18] Mulay SV, Choi M, Jang YJ, et al. Enhanced fluorescence turn-on imaging of hypochlorous acid in living immune and cancer cells. Chem - A Eur J. 2016;22(28):9642–9648. doi: 10.1002/chem.201601270
[19] Saleh TA. Nanomaterials: classification, properties, and environmental toxicities. Environ Technol Innov. 2020;20:101067. doi: 10.1016/j.eti.2020.101067
[20] Itoh A, Kabe N, Kuwae S, et al. Multi-element profiling analyses of symbiotic *Zooxanthellae* and soft tissues in a giant clam (*Tridacna crocea*) living in the coral reefs and their intake process of Zn and Cd. Bull Chem Soc Jpn. 2017;90(5):520–526. doi: 10.1246/bcsj.20160404
[21] Gharbavi M, Johari B, Mousazadeh N, et al. Hybrid of niosomes and bio-synthesized selenium nanoparticles as a novel approach in drug delivery for cancer treatment. Mol Biol Rep. 2020;47(9):6517–6529. doi: 10.1007/s11033-020-05704-z
[22] Vineeth Kumar CM, Karthick V, Ganesh Kumar V, et al. The impact of engineered nanomaterials on the environment: release mechanism, toxicity, transformation, and remediation. Environ Res. 2022;212:113202. doi: 10.1016/j.envres.2022.113202
[23] Sai S, Mani R, Vijayakumar P, et al. Risk assessment of potential toxicity induced by bio and synthetic plastic microspheres in *Lates calcarifer*. Chemosph.

2022;298:134269. doi: 10.1016/j.chemosphere.2022.134269
[24] Gojkovic Ž, Garbayo I, Ariza JLG, et al. Selenium bioaccumulation and toxicity in cultures of green microalgae. Algal Res. 2015;7:106–116. doi: 10.1016/J.ALGAL.2014.12.008
[25] Bulteau AL, Chavatte L. Update on selenoprotein biosynthesis. Antioxid Redox Signal. 2015; 23 (10):775–794. doi: 10.1089/ars.2015.6391
[26] Chua Tan L, Nancharaiah YV, van Hullebusch ED, et al. Selenium: environmental significance, pollution, and biological treatment technologies. Biotechnol Adv. 2016;34(5):886–907. doi: 10.1016/j.biotechadv.2016.05.005
[27] Yabutani T, Inoi YJ, Kimura AM, et al. Speciation of chromium(VI) and selenium(IV and VI) by high-performance liquid chromatography-fluorometric detection using 2,3-diaminonaphthalene as a single derivatizing reagent. Bull Chem Soc Jpn. 2014;87 (12):1402–1406. doi: 10.1246/bcsj.20140109
[28] Karthikeyan P, Marigoudar SR, Nagarjuna A, et al. Toxicity assessment of cobalt and selenium on marine diatoms and copepods. Environ Chem Ecotoxicol. 2019;1:36–42. doi: 10.1016/j.enceco.2019.06.001
[29] Li Q, Chen T, Yang F, et al. Facile and controllable one-step fabrication of selenium nanoparticles assisted by L-cysteine. Mater Lett. 2010;64(5):614–617, 676. doi: 10.1016/j.matlet.2009.12.019
[30] Čempel D, Nguyen MT, Ishida Y, et al. L-arginine-stabilized highly uniform Ag nanoparticles prepared in a microwave-induced plasma-in-liquid process (MWPLP). Bull Chem Soc Jpn. 2018;91(3):362–367. doi: 10.1246/bcsj.20170327
[31] Water quality: determination of acute lethal toxicity to marine copepods (*Copepoda, Crustacea*). International Organization for Standardization (ISO) 14669:1999(E), 1999. ISO/TC 147/SC 5.
[32] Zhoua C, Vitielloa V, Casals E, et al. Toxicity of nickel in the marine calanoid copepod *Acartia tonsa*: nickel chloride versus nanoparticles. Aquat Toxicol. 2016;170:1–12. doi: 10.1016/j.aquatox.2015.11.003
[33] Wang Q, Li X, Yan T, et al. Detrimental impact of hypoxia on the mortality, growth, reproduction, and enzyme activities of planktonic mysid *Neomysis awatschensis*. Aquat Ecol. 2021;55(3):849–859. doi: 10.1007/s10452-021-09864-3
[34] Kumar A, Pandey AK, Singh SS, et al. Engineered ZnO and TiO_2 nanoparticles induce oxidative stress and DNA damage leading to reduced viability of *Escherichia coli*. Free Radic Biol Med. 2011;51(10):1872–1881. doi: 10.1016/j.freeradbiomed.2011.08.025
[35] Pakrashia S, Dalaia S, Prathna TC, et al. Cytotoxicity of aluminium oxide nanoparticles towards fresh water algal isolate at low exposure concentrations. Aquat Toxicol. 2013;132–133:34–45. doi: 10.1016/j.aquatox.2013.01.018
[36] Chaudière J, Ferrari-Iliou R. Intracellular antioxidants: from chemical to biochemical mechanisms. Food Chem Toxicol. 1999;37(9–10):949–962. doi: 10.1016/S0278-6915(99)00090-3
[37] Jang M-H, Lim M, Hwang YS. Potential environmental implications of nanoscale zero-valent iron particles for environmental remediation. Environ Health Toxicol. 2014;29:e2014022. doi: 10.5620/eht.e2014022
[38] Haas BJ, Papanicolaou A, Yassour M, et al. *De novo* transcript sequence reconstruction from RNA-seq using the trinity platform for reference generation and analysis. Nat Protoc. 2013;8(8):1494–1512. doi: 10.1038/nprot.2013.084
[39] Buchfink B, Xie C, Huson DH. Fast and sensitive protein alignment using DIAMOND. Nat Methods. 2014;12(1):59–60. doi: 10.1038/nmeth.3176
[40] Moriya Y, Itoh M, Okuda S, et al. KAAS: an automatic genome annotation and pathway reconstruction server. Nucleic Acids Res. 2007;35:W182–W185. doi: 10.1093/nar/gkm321
[41] Langmead B, Salzberg SL. Fast gapped-read alignment with bowtie 2. Nat Methods. 2012;9 (4):357–359. doi: 10.1038/nmeth.1923
[42] Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 2014;15(12):1–21. doi: 10.1186/s13059-014-0550-8
[43] Richards D, Renaud L, Agarwal N, et al. *De novo* hepatic transcriptome assembly and systems level analysis of three species of dietary fish, *Sardinops sagax, Scomber japonicus, and Pleuronichthys verticalis*. Genes (Basel). 2018;9(11):521–719. doi: 10.3390/genes9110521
[44] Vineeth Kumar CM, Karthick V, Inbakandan D, et al. Effect of selenium nanoparticles induced toxicity on the marine diatom *Chaetoceros gracilis*. Process Saf Environ Prot. 2022;163:200–209. doi: 10.1016/j.psep.2022.05.021
[45] Bielmyer GK, Jarvis TA, Harper BT, et al. Metal accumulation from dietary exposure in the sea urchin, *Strongylocentrotus droebachiensis*. Arch Environ Contam Toxicol. 2012;63(1):86–94. doi: 10.1007/s00244-012-7269755-6
[46] Jarvis TA, Miller RJ, Lenihan HS, et al. Toxicity of ZnO nanoparticles to the copepod *Acartia tonsa*, exposed through a phytoplankton diet. Environ Toxicol Chem. 2013;32(6):1264–1269. doi: 10.1002/etc.2180
[47] LeBel CP, Ischiropoulos H, Bondy SC. Evaluation of the probe 2′,7′-737 dichlorofluorescin as an indicator of reactive oxygen species formation and oxidative stress. Chem Res Toxicol. 1992;5(2):227–231. doi: 10.1021/tx00026a012
[48] Wang H, Joseph JA. Quantifying cellular oxidative stress by dichlorofluorescein assay using microplate reader. Free Radic Biol Med. 1999;27(5–6):612–742 616. doi: 10.1016/S0891-5849(99)00107-0
[49] Xie C, Zhang P, Guo Z, et al. Elucidating the origin of the surface functionalization - dependent bacterial toxicity of graphene nanomaterials: oxidative damage, physical disruption, and cell autolysis. Sci Total Environ. 2020;747:141546. doi: 10.1016/j.scitotenv.2020.141546
[50] Kim B-M, Rhee J-S, Jeong C-B, et al. Heavy metals induce oxidative stress and trigger oxidative stress-mediated heat shock protein (HSP) modulation in the intertidal copepod *Tigriopus japonicus*. Comp Biochem Physiol Part - C Toxicol Pharmacol. 2014;166:65–74. doi: 10.1016/j.cbpc.2014.07.005
[51] Rosli NA, Teow YH, Mahmoudi E. Current approaches for the exploration of antimicrobial activities of nanoparticles. Sci Technol Adv Mater. 2021;22(732 1):885–907. doi: 10.1080/14686996.2021.1978801
[52] Lee C, Jee YK, Won IL, et al. Bactericidal effect of zero-valent iron nanoparticles on *Escherichia coli*. Environ Sci Technol. 2008;42(13):4927–4933. doi: 10.1021/es800408u

[53] Kumar D, Roy R, Parashar A, et al. Toxicity assessment of zero valent iron nanoparticles on *Artemia salina*. Environ Toxicol. 2017;32(5):1617–1627. doi: 10.1002/tox.22389

[54] Zhou C, Carotenuto Y, Vitiello V, et al. *De novo* transcriptome assembly and differential gene expression analysis of the calanoid copepod *Acartia tonsa* exposed to nickel nanoparticles. Chemosph. 2018;209:163–172. doi: 10.1016/j.chemosphere.2018.06.096

[55] Tintinger GR, Theron AJ, Potjo M, et al. Reactive oxidants regulate membrane repolarization and store-operated uptake of calcium by formyl peptide-activated human neutrophils. Free Radic Biol Med. 2007;42(12):1851–1857. doi: 10.1016/j.freeradbiomed.2007.03.012

[56] Wang M, Jeong CB, Li Y, et al. Different transcriptomic responses of two marine copepods, *Tigriopus japonicus* and *Pseudodiaptomus annandalei*, to a low dose of mercury chloride ($HgCl_2$). Aquat Toxicol. 2017;187:124–131. doi: 10.1016/j.aquatox.2017.03.018

[57] Christen V, Treves S, Duong FHT, et al. Activation of endoplasmic reticulum stress response by hepatitis viruses up-regulates protein phosphatase 2A. Hepatol. 2007;46(2):558–565. doi: 10.1002/hep.21611

[58] Burden-Gulley SM, Pendergast M, Lemmon V. The role of cell adhesion molecule L1 in axonal extension, growth cone motility, and signal transduction. Cell Tissue Res. 1997;290(2):415–422. doi: 10.1007/s004410050948

[59] Lowery J, Kuczmarski ER, Herrmann H, et al. Intermediate filaments play a pivotal role in regulating cell architecture and function. J Biol Chem. 2015;290(28):17145–17153. doi: 10.1074/jbc.R115.640359

[60] Pieters T, van Roy F. Role of cell-cell adhesion complexes in embryonic stem cell biology. J Cell Sci. 2014;127(12):2603–2613. doi: 10.1242/jcs.146720

[61] Kim B-M, Rhee J-S, Lee K-W, et al. UV-B radiation-induced oxidative stress and p38 signaling pathway involvement in the benthic copepod *Tigriopus japonicus*. Comp Biochem Physiol Part - C Toxicol Pharmacol. 2015;167(792):15–23. doi: 10.1016/j.cbpc.2014.08.003

[62] Herrero Ó, Planelló R, Morcillo G. The ribosome biogenesis pathway as an early target of benzyl butyl phthalate (BBP) toxicity in *Chironomus riparius* larvae. Chemosph. 2016;144:1874–1884. doi: 10.1016/j.chemosphere.2015.10.051

[63] Dodson SI, Hanazato T. Commentary on effects of anthropogenic and natural organic chemicals on development, swimming behavior, and reproduction of *Daphnia*, a key member of aquatic ecosystems. Environ Health Perspect. 1995;103(Suppl. 4):7–11. doi: 10.1289/ehp.95103s47

[64] Gong Y, Zhang K, Geng N, et al. Molecular mechanisms of zooplanktonic toxicity in the okadaic acid-producing dinoflagellate *Prorocentrum lima*. Environ Pollut. 2021;279:116942. doi: 10.1016/j.envpol.2021.116942

[65] Xu Z, Li Y, Li M, et al. Transcriptomic response of *Daphnia magna* to nitrogen- or phosphorus-limited diet. Ecol Evol. 2021;11(16):11009–11019. doi: 10.1002/ece3.7889

[66] Carotenuto Y, Dattolo E, Lauritano C, et al. Insights into the transcriptome of the marine copepod *Calanus helgolandicus* feeding on the oxylipin-producing diatom *Skeletonema marinoi*. Harmful Algae. 2014;31:153–162. doi: 10.1016/j.hal.2013.11.002

[67] Zamora-Terol S, Novotny A, Winder M. Reconstructing marine plankton food web interactions using DNA metabarcoding. Mol Ecol. 2020;29(17):3380–3395. doi: 10.1111/mec.15555

[68] Lomartire S, Marques JC, Gonçalves AMM. The key role of zooplankton in ecosystem services: a perspective of interaction between zooplankton and fish recruitment. Ecol Indic. 2021;129:107867. doi: 10.1016/j.ecolind.2021.107867

[69] Thornton Hampton LM, Bouwmeester H, Brander SM, et al. Research recommendations to better understand the potential health impacts of microplastics to humans and aquatic ecosystems. Microplast Nanoplast. 2022;2(1). doi: 10.1186/s43591-022-00038-y

[70] Rasheed A, Li1 H, Tahir MM, et al. The role of nanoparticles in plant biochemical, physiological, and molecular responses under drought stress: a review. Front Plant Sci. 2022;13:1–15. doi: 10.3389/fpls.2022.976179

[71] Roncalli V, Niestroy J, Cieslak MC, et al. Physiological acclimatization in high-latitude zooplankton. Mol Ecol. 2022;31(6):1753–1765. doi: 10.1111/mec.16354

[72] Arriazu E, Pippa R, Odero MD. Protein phosphatase 2A as a therapeutic target in acute myeloid leukemia. Front Oncol. 2016;6(4):1–11. doi: 10.3389/fonc.2016.00078

[73] Yamada KM, Geiger B. Molecular interactions in cell adhesion complexes. Curr Opin Cell Biol. 1997;9(1):76–85. doi: 10.1016/S0955-0674(97)80155-X

[74] Breithaupt T, Thiel M. Chemical communication in crustaceans. Chem Commun Crustac. 2011:1–565. doi: 10.1007/978-0-387-77101-4

[75] Li D, Wang J. Ribosome heterogeneity in stem cells and development. J Cell Biol. 2020;219(6):1–14. doi: 10.1083/jcb.202001108

[76] Wang P, Wang X, Wang L, et al. Interaction of gold nanoparticles with proteins and cells. Sci Technol Adv Mater. 2016;16(3):034610. doi: 10.1088/1468-6996/16/3/034610

[77] Azevedo RB, Pic-Taylor A, Mazzeu JF. Integrated assessment of toxic effects of maghemite (γ-Fe_2O_3) nanoparticles in zebrafish. Aquat Toxicol. 2017. doi: 10.1016/j.aquatox.2017.08.004