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Transcriptomic analysis reveals the immune response mechanisms of sea cucumber *Apostichopus japonicus* under noise stress from offshore wind turbine

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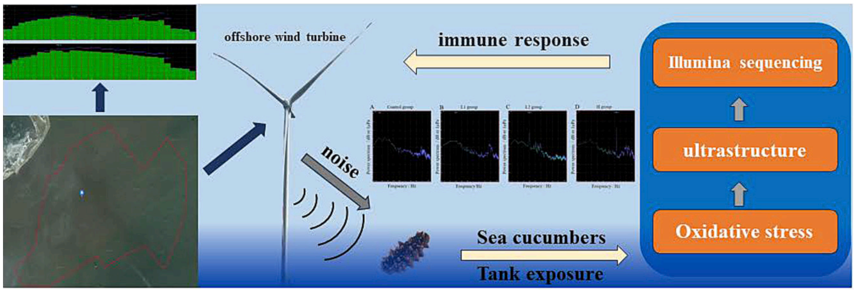
HIGHLIGHTS

- Characteristic assessment of noise at the Bodhi Island offshore wind farm
- Underwater noise suppresses protein synthesis and cellular apoptosis in sea cucumber intestines
- Underwater noise causes oxidative damage to the body cavity of sea cucumber
- Lysosomes and pancreatic secretion support sea cucumbers' nutrient and energy needs in adverse conditions
- Sea cucumbers' immune system responds more to low-frequency noise than high-frequency

GRAPHICAL ABSTRACT



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ABSTRACT

As an important form of renewable energy, offshore wind power can effectively reduce dependence on traditional energy sources and decrease carbon emissions. However, operation of wind turbines can generate underwater noise that may have negative impacts on marine benthic organisms in the surrounding area. Sea cucumbers are slow-moving invertebrates that inhabit the ocean, relying on their immune system to adapt to their environment. To evaluate the frequency range of characteristic noise produced by offshore wind turbines, we conducted a field survey. Additionally, we utilized sea cucumbers in simulated experiments to assess their response to the noise produced by offshore wind turbines. We established a control group, a low-frequency noise group simulating offshore wind turbine noise at 125 Hz and 250 Hz, and a high-frequency noise group at 2500 Hz, each lasting for 7 days. Results from measuring immune enzyme activity in the coelomic fluid suggest that noise can reduce the activity of superoxide dismutase enzymes, which may make sea cucumbers more susceptible to oxidative damage caused by free radicals. Exposure to low-frequency noise can have the effect of diminishing the activity of

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catalase, and this decrease in catalase activity could potentially increase the susceptibility of the sea cucumber's coelom to inflammation. In order to elucidate the hypothetical mechanism of immune response, intestinal tissue was extracted for transcriptome sequencing. The results showed that under 125 Hz low-frequency noise stress, the number of differentially expressed genes was the highest, reaching 1764. Under noise stress, sea cucumber's cell apoptosis and cell motility are reduced, interfering with lipid metabolism process and membrane synthesis. This research provides theoretical support for the environmental safety assessment of offshore wind power construction.

1. Introduction

Underwater noise generated by human activities is widely recognized as a significant threat to marine life. Examples of such activities encompass pile driving, deep-sea mining, seismic testing, and shipping noise (Howe et al., 2019; Miksis-Olds and Nichols, 2016). Extensive evidence has confirmed the detrimental impact of these noises on marine organisms (Jones, 2019; Popper et al., 2003; Talianni, 2020). The understanding of the hazards posed by underwater noise has expanded exponentially (Williams et al., 2015), covering a diverse range of taxonomic groups from marine mammals and sea turtles to fish and invertebrates (Duarte et al., 2021). Marine mammals, such as whales, heavily rely on acoustics for crucial activities like migration and mating (Ferguson et al., 2023). Exposure to noise significantly diminishes the foraging efficiency of marine mammals (Leduc et al., 2021). Similarly, coral fish exhibit reduced foraging behavior and diminished escape distance in response to rising noise levels (Popper and Hawkins, 2019). In the case of invertebrates, research has demonstrated that anthropogenic noise suppresses the biologically disruptive behavior of species like *Nephrops norvegicus* and Philippine clams (Solan et al., 2016). Soundscape studies have also explored various marine ecosystems, including rocky reefs, seagrass meadows, kelp/algae forests, oyster reefs, estuaries, and mangroves (Bertucci et al., 2015; Halliday et al., 2020; Shi et al., 2019). Anthropogenic noise disturbance has become inevitable in the marine soundscape, particularly due to its low-frequency characteristics that allow it to propagate over long distances underwater. Consequently, anthropogenic noise has emerged as a prominent component of contemporary ocean noise.

Amidst the drive for a low-carbon economy, there has been significant global attention on accelerating the transition to clean energy (Strunz, 2018). During the “14 th Five-Year Plan” period, offshore wind power has been actively promoted in China as a crucial component of the country's clean energy strategy. It serves as a pivotal means to achieve carbon peak and neutrality objectives. (Zhang et al., 2022a). Over the past few decades, countries worldwide have been planning a large-scale transition towards enhanced energy efficiency and adoption of safer and cleaner energy sources (Park and Kim, 2019). However, offshore engineering activities like pile driving, drilling, dredging, and increased shipping introduce factors such as sound and vibration into the seabed, causing alterations to the ocean's physical environment. Consequently, this can lead to various physiological changes in marine organisms exposed to these disturbances (Lindeboom et al., 2011; Roberts and Elliott, 2017). As anthropogenic low-frequency noise invades natural soundscapes and penetrates into the marine environment, it may disrupt the interactions between organisms and potentially cause harm (Wang et al., 2022). Low-frequency noise (LFN) refers to sound waves with frequencies between 0.1 Hz and 2 kHz (Lagrois et al., 2023). Low-frequency noise originates from various sources, with fishing vessels being a primary contributor among them (De Robertis and Handegard, 2013). As marine traffic continues to grow, the LFN emitted by these large vessels is almost ubiquitous in the world's oceans. Offshore wind power is another major contributor to LFN, and its presence is increasing with the implementation of offshore wind farms (Cao et al., 2019). Although oceanic LFN has been increasing with human activity, little is known about how this noise affects the marine organisms.

Natural sounds play a vital role in assisting animals with various aspects of their lives, including spatial orientation, finding food, guiding reproductive migrations, and locating habitats (Lillis et al., 2014; Maiditsch and Ladich, 2023; Simpson et al., 2016a). Consequently, noise pollution can disrupt animal behavior and have direct or indirect effects on their physiological state, ranging from mild stress to internal and external injuries, or even death (Erbe et al., 2016; Erbe et al., 2018; Fernandez et al., 2005; Nedelec et al., 2017). Over the past decade, research on the impact of ocean noise pollution has expanded beyond its initial focus on mammals capable of detecting sound, to include fish and invertebrates (Gotz and Janik, 2013; Nowacek et al., 2010; Patek et al., 2009; Soto et al., 2016; Williams et al., 2015). Species such as sea cucumbers, which have significant socio-economic value as part of marine ranching, are crucial to understanding the impact of ocean noise on invertebrates (Anderson et al., 2011; Cecilia and Monica, 1999; Eddy et al., 2017).

The noise generated by offshore wind turbines can alter the acoustic environment of large marine habitats, with both known and unknown effects on marine biota (Park and Do, 2022). Several studies in the field of behavioral ecology have demonstrated significant impacts of noise on invertebrates, including bivalves, cephalopods, and crustaceans (Di Franco et al., 2020; Walsh et al., 2017). Invertebrates can experience sub-lethal effects such as reduced feeding (Celi et al., 2015), slow or unsuccessful location of shelter (Walsh et al., 2017), and increased behavioral energy expenditure due to noise pollution (Edmonds et al., 2016; Wale et al., 2013). These studies all support the idea that organisms with weaker locomotor abilities are more susceptible to the effects of noise pollution. *Apostichopus japonicus* is a benthic invertebrate species that moves slowly (Ding et al., 2019) and is widely distributed in the Yellow and Bohai Seas as well as along the northwest coastal areas of the Pacific Ocean in China. These seas are also the main areas for wind power development in China, which pose a significant threat to the habitat of *A. japonicus* due to the strong overlap between the two. Furthermore, *A. japonicus* has a strong territorial behavior and may use wind turbines as substrates for their habitat (Hu et al., 2021). This could lead to chronic stress caused by noise pollution from offshore wind turbines, making them even more vulnerable to the sub-lethal effects of this type of pollution.

Currently, the physiological mechanisms underlying the effects of offshore wind turbines on marine invertebrates remain unclear. Previous research demonstrated that noise increases total hemocyte count levels in *spiny lobsters* and decreases the blood refractive index (Fitzgibbon et al., 2017). Will the underwater noise pollution of wind power characteristics affect the physiological state of benthic echinoderms such as sea cucumbers? It is worth noting that fish are more sensitive to noise pollution due to their possession of air-filled organs, which can rupture and cause damage to surrounding organs under conditions of vibration in the presence of noise (Halvorsen et al., 2012). In contrast, invertebrates lack air-filled organs and there is no evidence that they are sensitive to sound pressure. Thus, we must investigate the effects of the particle motion (PM) component of noise on these organisms. In this study, sea cucumbers was selected as a representative invertebrate species, and the effects of exposure to four different noise frequencies on these organisms were analyzed and studied. The objective was to understand the physiological mechanisms by examining their immune system and transcriptome response to characteristic noise from wind turbines. These findings will greatly contribute to the environmental safety assessment of offshore wind power construction, providing crucial theoretical support for this form of renewable energy development.

2. Material and methods

The Bodhi Island Offshore Wind Farm is located in the Bohai Bay of Lao Ting County, Tangshan City, Hebei Province, China (118° 75′ - 118° 88′ E, 39° 04′ - 39° 96′ N). Comprised of 75 wind turbines with a capacity of 4 MW each, the Bodhi Island Offshore Wind Power Farm is scheduled to be connected to the grid in June 2020. The focus of this experiment was to conduct a case study on the Bodhi Island Offshore Wind Farm and determine the frequency range of noise produced by its wind turbines. We then conducted simulated experiments in storage tanks to further investigate the effects of this noise.

2.1. Recording of offshore wind power noise sources

In June 2022, we recorded the characteristic noise near and far from the pile foundation of wind power underwater (Fig. 1). The recorded noise signals were converted into “wav” format using a converter (Lightning DAT format converter, China) and stored in a computer. The recorded noise audio was analyzed using audiotools.

2.2. Experimental animals and maintenance

The experimental animals used in this experiment were healthy sea cucumbers cultivated by Shandong Tonghe Ocean Science and Technology Co., Ltd. (Dongying, China). The cultivation experiment was conducted in the laboratory of Zhongke Tonghe (Shandong) Ocean Science and Technology Co., Ltd. (Dongying, China), from November 1st, 2022, to December 30th, 2023. Firstly, approximately 200 healthy sea cucumbers of similar size were temporarily kept in a large water tank (4 m × 1.5 m × 1.2 m) and acclimated for 14 days to provide samples for subsequent experiments. The temporary breeding conditions were as follows: salinity of 27–28, pH of 7.8–8.1, and the central air conditioning system maintained room and water temperature at 15 °C ± 0.4 °C. There was an aeration device at the bottom of the tank to ensure that dissolved oxygen in the water remained above 8 mg L^{-1}. During the temporary breeding period, sea cucumbers were fed twice a day at 10:00 and 22:00. The amount of food provided exceeded 5 % of the body weight of the sea cucumbers, and any remaining food was observed the next day to ensure sufficient feeding. The feed consisted of 70 % mud sediment, 20 % multiple-celled algae, and 10 % kelp powder. Throughout the process, we maintained a 12-h light-dark cycle. The sea water used in both the temporary breeding and subsequent experiments met the first-class conditions of the seawater quality standard.

2.3. Noise exposure protocol

The noise generation for the experiment was accomplished using SweepGen software. The programmed sounds were stored in a Secure-Digital Card and played through a standard power amplifier (SAST SA-9019) to an underwater loudspeaker (SAST F4 50W). To determine the frequency settings, we referred to spectral time series data ranging from 0 Hz to 500 Hz obtained from the Sinovel 3.0 MW SL3000 turbine tower and the Shanghai Electric 3.6 MW W3600 turbine tower. (Yang et al., 2018). We utilize Low-Frequency Group 1 (L1) and Low-Frequency Group 2 (L2) to describe the low-frequency noise characteristics of wind turbines at close distances and far distances, respectively. L1 has a peak frequency of 125 Hz, while L2 has a peak frequency of 250 Hz. In addition, we set a blank control and a high-frequency (H) control of 2500 Hz. Prior to the experiment, we calibrated the output noise using Audiotools, and the calibration results can be seen in Fig. 2. This calibration step ensured that the underwater received noise characteristics met our requirements, and the sounds were digitized using the SAST SA-9019 and stored on a laptop computer. Each group was exposed independently for 7 days.

2.4. Tank exposure

In December 2022, we conducted a Control Exposure Experiment (CEE) on four groups of sea cucumbers following the temporary breeding period. We used a white, transparent cylindrical tank with a diameter of 74 cm and a water depth of 10 cm to isolate the sea cucumbers from their surroundings. An aeration device was installed at the bottom of the tank to maintain dissolved oxygen concentration in the water. To prevent noise interference between the groups, only one group of sea cucumbers was exposed at a time while the others remained in the temporary breeding state. A fixed loudspeaker positioned below the water surface played audio with specific characteristics. Throughout the study, we maintained consistent lighting and feeding conditions as during the temporary breeding period. All animal experiments conducted in this study have adhered to and followed the National Research Council's Guide for the Care and Use of Laboratory Animals.

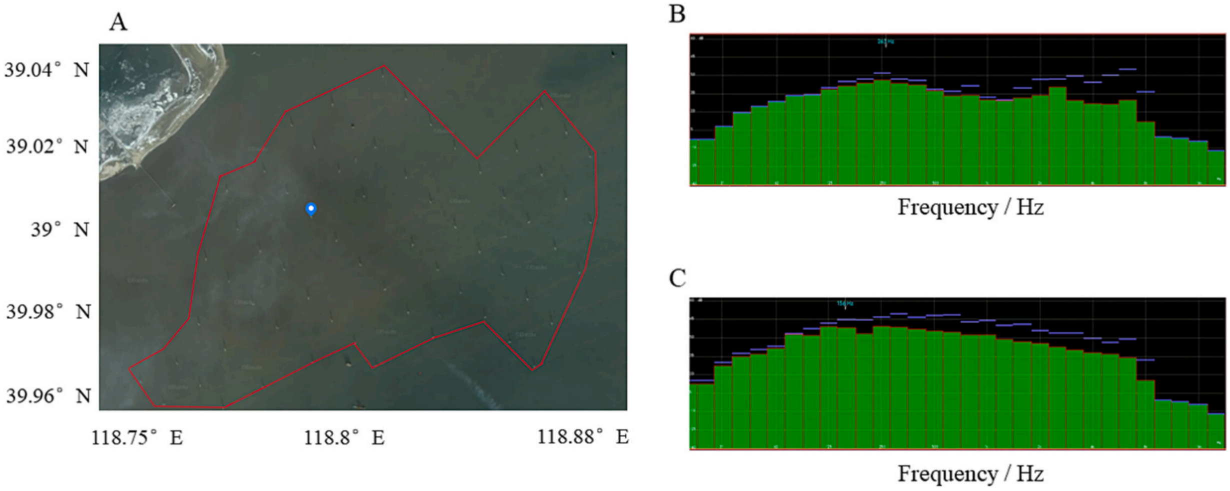

Fig. 1. The location of Bodhi Island Offshore Wind Farm in China. A: The location of the Bodhi Island offshore wind farm and the noise testing points. B: Acoustic data was measured below the water surface at a distance of 0 m from the wind turbine. C: Acoustic data was measured below the water surface at a distance of 50 m from the wind turbine.

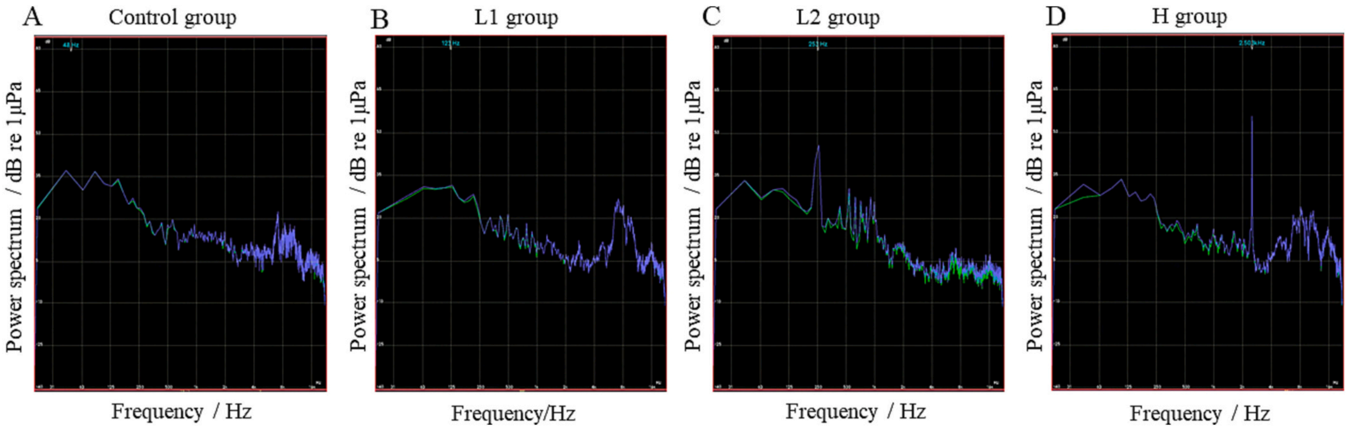

Fig. 2. Peak underwater sound spectrum of control group and different noise groups. A: Peak spectrum of control group underwater noise frequency experiment; B: Peak spectrum of Low Frequency Group 1 underwater noise frequency experiment; C: Peak spectrum of Low Frequency Group 2 underwater noise frequency experiment; D: Peak spectrum of high Frequency Group underwater noise frequency experiment.

2.5. Detection of indicators related to oxidative stress

After each exposure, the sea cucumbers were dissected on ice, and their coelomic fluid was collected. The coelomic fluid was rapidly frozen with liquid nitrogen and stored, then placed in a − 80 °C ultra-low temperature freezer for preservation. Before detection, the coelomic fluid was thawed and centrifuged at 3500 rpm for ten minutes at 4 °C, and the supernatant was collected for further use.

2.5.1. Superoxide dismutase (SOD) activity

The total activity of serum SOD (T-SOD) was measured using a standard assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) based on the WST-1 method. Through preliminary experiments, we diluted the coelomic fluid 2.5 times with saline to ensure that the inhibition rate was between 40 % and 60 %. This was done before detection. Measurement was performed using an enzyme-linked immunosorbent assay (ELISA) reader (Thermo Scientific, Waltham, MA, USA) at a wavelength of 450 nm. One unit (U) of SOD activity was defined as the amount of enzyme required to inhibit the reaction rate by 50 % under the assay conditions.

2.5.2. Catalase (CAT) activity

CAT activity was evaluated using the CAT assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) based on the ammonium molybdate method. Measurement was performed using an ELISA reader (Thermo Scientific, Waltham, MA, USA) at a wavelength of 405 nm. Under 37 °C conditions, one unit of CAT enzyme activity corresponds to the decomposition of 1 mmol hydrogen peroxide per milliliter of coelomic fluid per minute.

2.5.3. Malondialdehyde (MDA) activity

MDA activity was evaluated using the MDA assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) based on the thiobarbituric acid (TBA) method. Measurement was performed using an ELISA reader (Thermo Scientific, Waltham, MA, USA) at a wavelength of 532 nm.

2.6. Ultrastructural analysis

We collected intestinal tissues from each group of sea cucumbers immediately after exposure and fixed them with 4 % formaldehyde solution A mixture of 1: 2 volume ratio of formaldehyde-gelatin solution and 50 % silver nitrate solution was prepared by mixing them thoroughly in a clean plastic container. The slices were sequentially placed into xylene I for 20 min, xylene II for 20 min, 100 % ethanol I for 5 min, 100 % ethanol II for 5 min, 75 % alcohol for 5 min, and pure water for three minutes of rinsing. The slices were immersed in a 1 % sodium thiosulfate solution and incubated at room temperature for 15 min, then rinsed three times with pure water and dried. The tissue was circled with an immunohistochemical pen, and the AgNOR staining solution was dropped inside the circle to cover the tissue. The slide was placed in a light-shielded humid box at room temperature (25 °C) for 25–40 min of incubation. Then, the staining solution was poured off, and the slice was thoroughly rinsed with pure water. The slice was incubated at room temperature with 5 % sodium thiosulfate solution for 5 min, then rinsed three times with pure water. Next, it was stained with 0.01 % eosin solution for 1 min and dehydrated with a gradient of ethanol (95 %, 100 %). Finally, the slice was immersed in clean xylene for 10 min to become transparent and was mounted with neutral gum. Imaging analysis was performed using a microscope (ZEISS Axio Imager 2, DEU) at 40× magnification under oil immersion objective lens.

2.7. RNA isolation and Illumina sequencing

2.7.1. RNA isolation

Total RNA was extracted from the samples using the TRIzol reagent (Invitrogen) and treated with DNase I (TaKara) to eliminate genomic DNA. The quality of the RNA samples was assessed using the 2100 Bioanalyzer (Agilent) and ND-2000 (NanoDrop Technologies) methods to ensure that qualified samples were used for transcriptome sequencing (OD 260/280 = 1.8–2.2, OD 260/230 ≥ 2.0, RIN ≥ 6.5, 28 S: 18 S ≥ 1.0, > 1 μg).

2.7.2. Library construction and Illumina Hiseq Xten/NovaSeq 6000 sequencing

Intestinal tissue samples were collected from four sea cucumbers in each group and immediately frozen in sterile tubes with liquid nitrogen. The tubes were stored in a liquid nitrogen container. To selectively capture and enrich mRNA molecules containing the poly (A) tail, oligo (dT)-coated magnetic beads were used, forming A-T base pairs with the poly(A) tail. This process, known as oligo (dT) enrichment or mRNA isolation, is employed for transcriptome analysis. The enriched mRNA, with an average length of several kilobases, was then fragmented into small fragments approximately 300 bp in length using a fragmentation buffer. Reverse transcription was performed using random primers and reverse transcriptase to synthesize single-stranded cDNA from the mRNA template. The single-stranded cDNA was then converted into double-stranded cDNA through second-strand synthesis, forming a stable double-stranded structure. The sticky ends of the double-stranded cDNA were repaired to create blunt ends by adding an End Repair Mix. Subsequently, a Y-shaped adapter was ligated to the 3′ end of the cDNA after adding a single A base. The product after adapter ligation underwent purification and fragment selection, followed by PCR amplification using the selected fragments. The resulting library was further purified, and its effective concentration (each concentration being above 2 nm) was measured using qPCR to ensure its quality. Finally, high-throughput sequencing was performed on these libraries using the Illumina NovaSeq 6000 platform with a read length of PE 150.

2.8. Statistical analysis

The data are presented as means ± standard deviation (SD). IBM SPSS Statistics 24 software was used for data analysis. Before analysis, the Levene test was conducted to test the homogeneity of variance in the data. One-way analysis of variance (ANOVA) was conducted to test the differences in oxidative stress activity and gene expression before and after noise exposure using Welch's *t*-test, Tamhane's test, and LSD test. A value of $P < 0.05$ indicates a statistically significant difference.

3. Results

3.1. Oxidative stress related indicators

The effects of different treatment methods on the activity of SOD, content of MDA, and CAT in sea cucumbers are shown in Fig. 3. In the noise exposure experiment, the SOD enzyme activity in the control group was significantly higher than that in the exposure group ($df = 3$, $F = 12.58$, $P < 0.001$). The MDA activity in the H group was significantly lower than that in the L2 group ($df = 3$, $F = 4.86$, $P < 0.05$). CAT activity in the L1 group was significantly lower than that in the control group and H groups ($df = 3$, $F = 8.75$, $P < 0.01$). There was a certain gradient change in SOD activity and MDA activity in the low-frequency range.

3.2. Histopathology

The AgNOR staining results indicate that intestinal cell proliferation activity in sea cucumbers decreases with increasing noise frequency, as demonstrated by SME analysis (Fig. 4) and count statistics (Fig. 5). The proliferation activity of cells in the control group was significantly higher than that in the L2 group and the H group ($df = 3$, $F = 8.03$, $P < 0.01$).

3.3. Gene function annotation analysis

Fig. 6 displays the classification of the enriched gene set and the results of Gene Ontology (GO) enrichment analysis, which aimed to investigate the biological functions of all differentially expressed genes (DEGs). Based on sequence homology, both single genes and DEGs were categorized into multiple functional groups. Regarding the biological process ontology, the most prevalent terms were metabolic process, cellular process, and biological regulation. Inl;.’ the molecular function ontology, catalytic activity and binding were the most abundant terms. Within the cellular component ontology, membrane part, cell part, and membrane were the most frequently observed terms.

3.4. Identification of differentially expressed genes

To reveal the response mechanisms of sea cucumbers to different frequency noise stress, we performed differential gene expression analysis ($P < 0.05$) between the intestinal tissues of the control group in the environmental treatment and those of the L1, L2, and H groups. Compared with the control group, the L1 group had the largest number of DEGs, reaching 1764, including 825 upregulated DEGs and 939 downregulated DEGs. The L2 group had 949 DEGs, including 557 upregulated DEGs and 392 downregulated DEGs. The H group had 692 DEGs, consisting of 330 upregulated DEGs and 362 downregulated DEGs. In addition, the L1 group had a significantly higher number of DEGs than the L2 and H groups, with 1688 (878 upregulated and 810 downregulated) and 1659 (892 upregulated and 767 downregulated) DEGs, respectively (Fig. 7).

3.5. Gene ontology enrichment analysis of differentially expressed genes

To comprehensively understand the biological function of differentially expressed genes (DEGs), GO enrichment analysis was performed. The results revealed three major functional categories: Biological Processes (BP), Cellular Components (CC), and Molecular Functions (MFs). In the L1 group compared to the CK group, the three major functional categories that showed the most significant enrichment for DEGs were catalytic activity, metabolic process, and organic substance metabolic process ; In the L2 group compared to the CK group, the three major functional categories that showed the most significant enrichment for DEGs were endonuclease activity, synaptic membrane, and animal organ development ; In the H group compared to the CK group, the three major functional categories that showed the most significant enrichment for DEGs were organelle membrane, oxidoreductase activity acting on CH-OH group of donors, and endoplasmic reticulum membrane ; In the L1 group compared to the L2 group, the three major functional categories that showed the most significant enrichment for DEGs were lipid metabolic process, isomerase activity, and lipid catabolic process ; In the L1 group compared to the H group, the three major functional categories that showed the most significant enrichment for DEGs were lipid metabolic process, oxoacid metabolic process, and carboxylic acid metabolic process.

3.6. Kyoto encyclopedia of genes and genomes (KEGG) enrichment analysis of differentially expressed genes

Through the comparison of RNA-seq data from two low-frequency groups and one high-frequency group exposed to sound, we studied the regulatory mechanism of noise exposure on intestinal immunity in sea cucumber. We conducted a cluster analysis on the differentially expressed genes that we obtained. The results of our study showed that sea cucumber intestinal immunity is most significantly affected under low-frequency sound. We further analyzed the functions of DEGs using KEGG annotation (with a *P*-value threshold of 0.05 based on hypergeometric testing as the standard for pathway detection). By analyzing

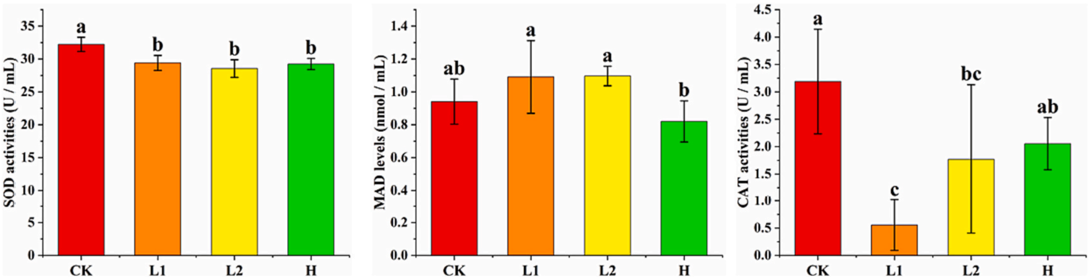

Fig. 3. The activity of SOD, content of MDA, and CAT in sea cucumbers at the end of the noise exposure experiment. CK: control group; L1: 125 Hz low-frequency group; L2: 250 Hz low-frequency group; H: 2500 Hz high-frequency group.

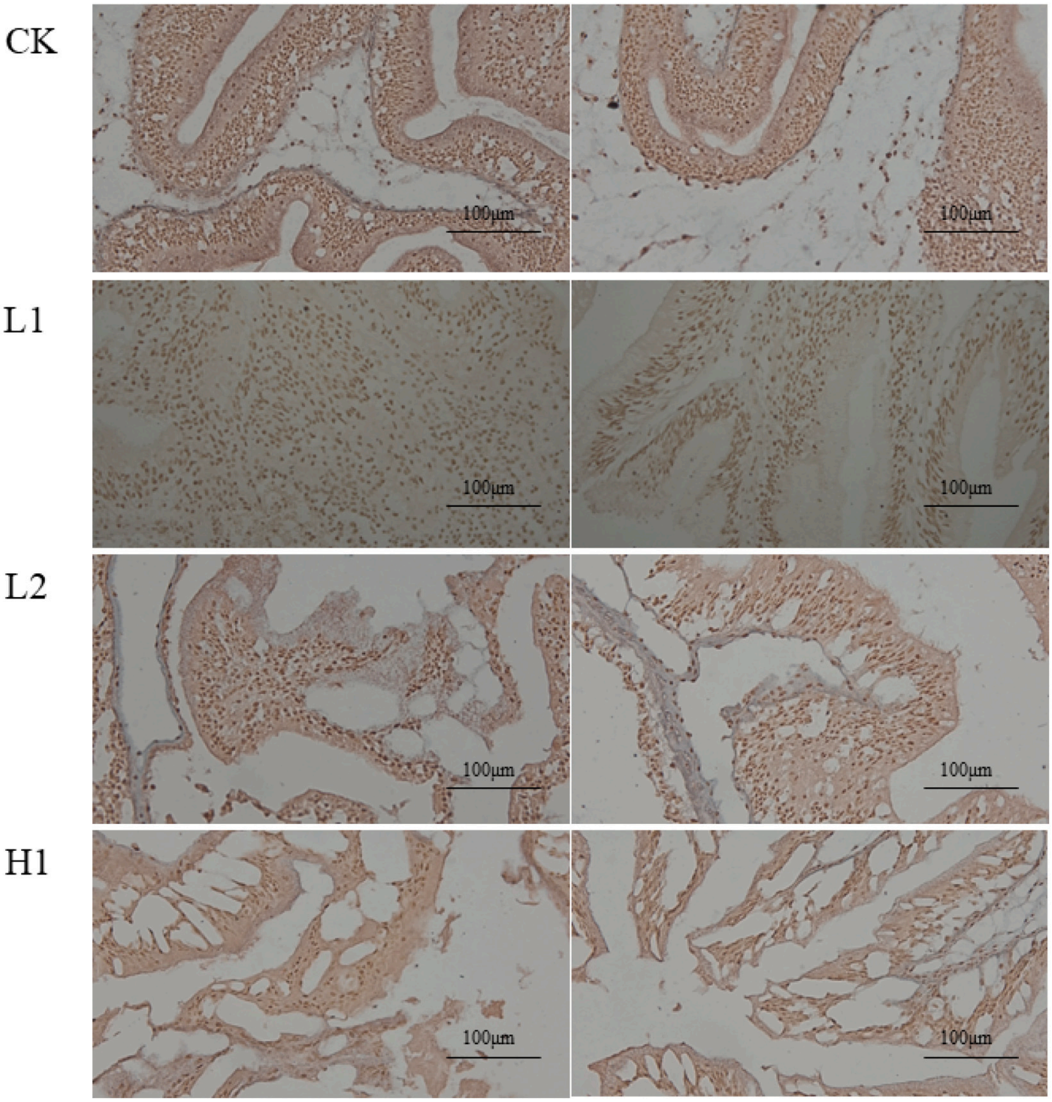

Fig. 4. The AgNOR staining of intestinal tissue of *A. japonicus* was observed under microscope in control group and different noise exposure group. The scale bar measures 100 μm.

the KEGG data, we identified significant pathways that differed between the low-frequency group and the H group. The results of our study showed that the most significantly changed pathways in the low-frequency group were pancreatic secretion, protein digestion and absorption, and glycerol lipid metabolism, whereas the most significant pathways in the high-frequency group were peroxisome and butanoate metabolism when compared to the CK group. The most significant pathways that differed between the low-frequency group and high-frequency group were valine, leucine and isoleucine degradation, and lysosome. In order to express our findings clearly and concisely, we analyzed the KEGG pathways that showed significant changes in both environmental concentration and high-concentration treatment groups separately (Fig. 8).

4. Discussion

4.1. Wind turbine noise causes oxidative damage

Sea cucumber coelomic fluid, which functions similarly to lymph, harbors a substantial population of immune cells responsible for immune responses. Serving as a vital element of the innate immune system in marine invertebrates, it is frequently employed as a significant parameter to assess the immune competence of organisms (Lin et al., 2011; Zhengqiang et al., 2019).

In this study, the noise group exhibited a decrease in SOD activity in the coelomic fluid, rendering the enclosed intestines more vulnerable to oxidative damage. The inhibition of CAT activity by low-frequency noise significantly increased the susceptibility of sea cucumbers to intestinal inflammation reactions. This is similar to the previous findings in the D-veliger larval phase research (De Soto et al., 2013). albeit the observed changes in oxidative stress markers were attributed to long-term exposure to noise in this study. Long-term exposure to noise also triggers an increase in cortisol levels in clownfish, whereas short-term exposure does not elicit significant changes in stress hormones (Mills et al., 2020). On the other hand, goldfish showed an increase in cortisol levels within 10 min of noise exposure (Smith et al., 2004), indicating that different species require varying durations to respond to acoustic stress. Moreover, marine mammals like gray whales exhibit notable alterations in stress hormones, including cortisol, progesterone, and testosterone, when exposed to low-frequency noise emitted by ships and other sources (Lemos et al., 2022). Although evidence suggests that animals' stress indicators and vital signals may diminish with chronic adaptation to sound (Berkhout et al., 2023; Slabbekoorn et al., 2010), it is evident that low-frequency noise has a widespread physiological impact on marine

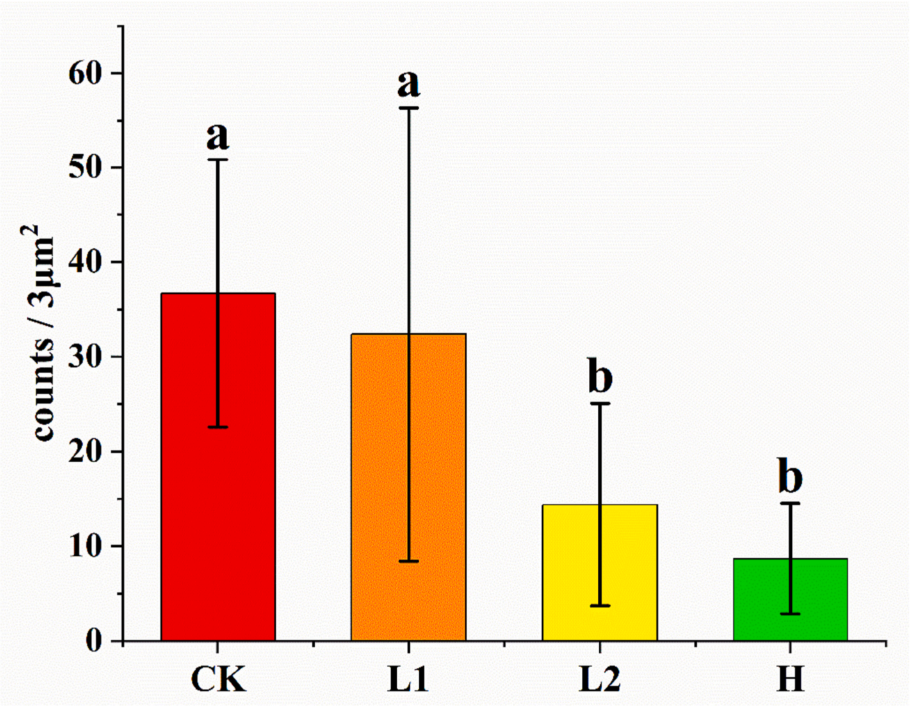

Fig. 5. Counting the brown particles of NORs under the microscope. Counting using grid sampling method for statistical analysis.

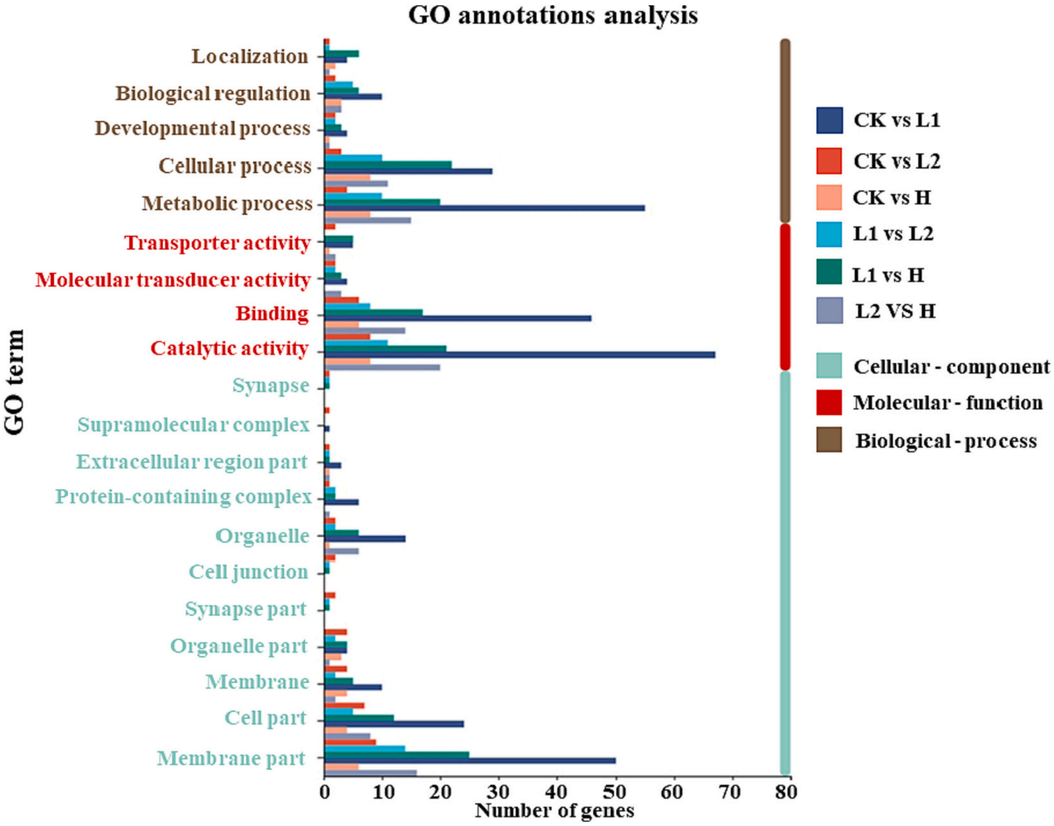

Fig. 6. The bar graph illustrates the statistics of GO classification for multiple gene sets, with different colors representing each gene set.

organisms.

This study examined the effects of high-frequency noise on sea cucumbers through observation. Surprisingly, similar outcomes were observed in the high-frequency group compared to the control group. Some research has indicated that high-frequency noise exposure can increase the activity of acellular coelomic fluid in sea cucumbers. However, under the same conditions, there was no significant change in total protein concentration (Vazzana et al., 2020). Currently, there are

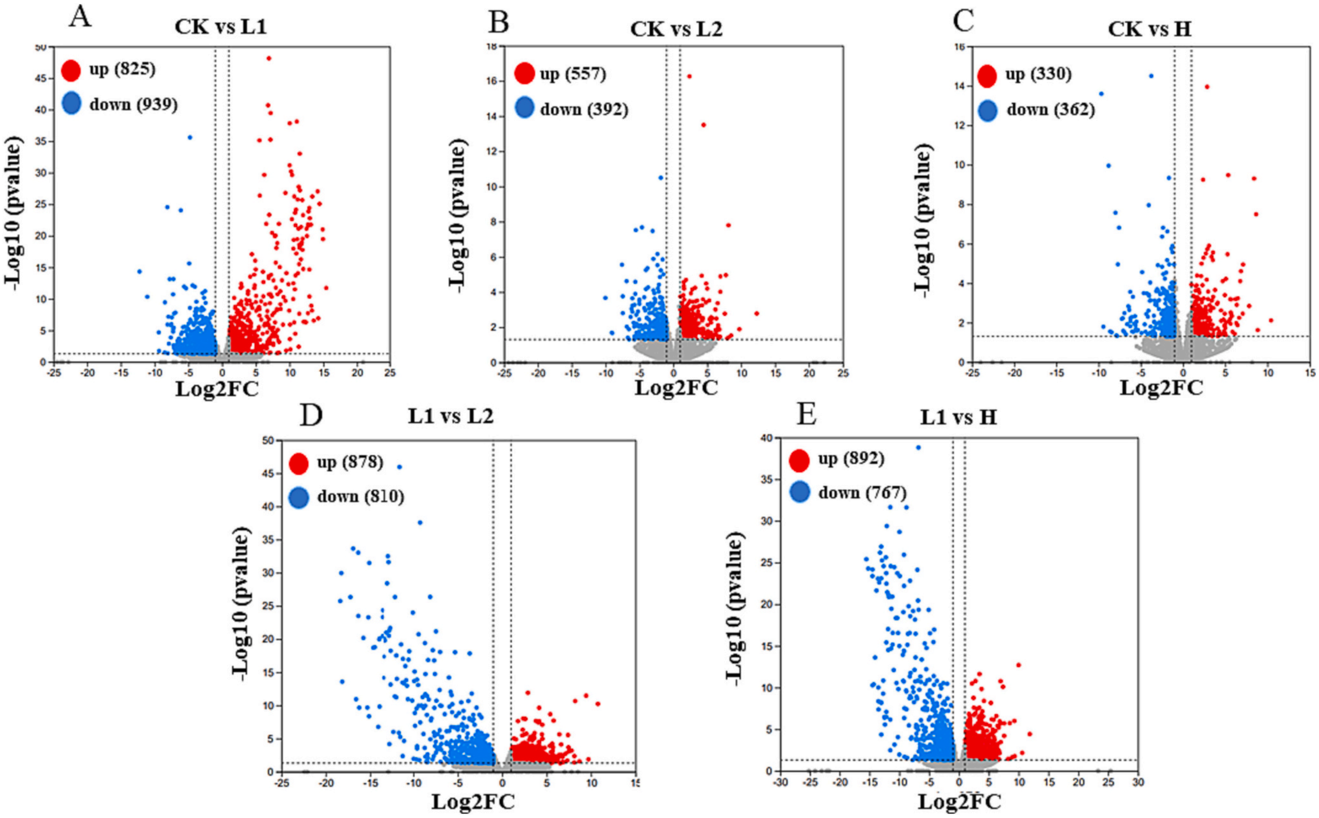

Fig. 7. Differential expression genes (DEGs) were identified in the intestine of sea cucumbers after noise exposure. Volcano plots for DEGs between (A) CK and L1, (B) CK and L2, (C) CK and H, (D) L1 and L2, and (E) L1 and H were generated. Red dots represent upregulated genes, and blue dots represent downregulated genes.

no further reports available describing the potential effects of high-frequency noise on benthic species that lack the ability to quickly escape noise. One possible reason for this is that water absorbs high-frequency noise more than low-frequency noise, resulting in a significant reduction of high-frequency noise over short distances (Hada et al., 2010). Additionally, it is also possible that invertebrates are less sensitive to high-frequency noise compared to mammals (Deng et al., 2014).

4.2. Cell proliferation and apoptosis

Apoptosis, as a fundamental biological phenomenon, plays an important role in the evolution of organisms, the maintenance of internal environment homeostasis, and the development of multiple systems (Gregory and Devitt, 2003; Krysko et al., 2006). Lutein has been found to reduce apoptosis activity in mice (Chen et al., 2016). Our transcriptome sequencing results indicate that the expression of genes related to cell growth and apoptosis is downregulated in the noise-exposed group, such as those involved in cell senescence, apoptosis, hydrolysis, redox regulation, and endoplasmic reticulum stress. Treatment of both the low-frequency and high-frequency groups led to a significant decrease in cell senescence and apoptosis. However, a study found that fish exposed to noise stress exhibited enhanced apoptosis and cell motility (Zhang et al., 2022b). This disparity may be attributed to the fact that fish exhibit more pronounced avoidance behavior and have increased metabolic rates in response to acoustic stress compared to sea cucumbers (Jhawar et al., 2020; Simpson et al., 2016b). When exposed to in situ pile driving stress, *Dicentrarchus labrax* showed a considerable decrease in oxygen consumption and lactate response (Debusschere et al., 2016). This could be due to the metabolic and oxidative stress related to the exposure to noise (Karimaian et al., 2017; Wale et al., 2019). Interestingly, our study found similar results at the histological level, in contrast to studies that use metabolic and oxidative stress as biomarkers to assess the effects of underwater noise on marine organisms (Gaspar et al., 2009; Watts, 2022). Based on our findings, we propose that underwater noise, being a potent stressor, may disrupt the cell apoptosis process in sea cucumber intestinal tissue.

4.3. Mechanism of the immune physiology impact of wind turbine noise on sea cucumbers

Lysosomes are acidic cell compartments containing a large number of hydrolytic enzymes. The hydrolytic enzymes within lysosomes can degrade components such as proteins and lipids, which can kill and degrade pathogens, playing a crucial role in the immune response. Food particles in the partially digested gut lumen of invertebrates are absorbed and intracellularly digested through phagocytosis or pinocytosis (Hartenstein and Martinez, 2019). Under exposure to noise, *Terapon jarbua* exhibit higher lysosomal activity, which is similar to the results obtained in our experimental study. During the experiment, sea cucumbers showed no difference in feeding time between the low and high frequency groups. However, a comparison of gut transcriptomes revealed that as noise frequency increased, the expression of lysosomal genes in sea cucumbers was upregulated. At the same time, the expression levels of genes related to autoimmune thyroid disease were also upregulated. The increase in noise frequency promotes the gut immune function of sea cucumbers.

Amino acids, as organic compounds, play crucial physiological roles in regulating metabolism, providing energy, and supporting the immune system (Broer, 2022). Exposure to low-frequency noise environment leads to an upward trend in carbohydrate and amino acid metabolism in the *Tegillarca granosa* to meet the energy demands associated with feeding (Peng et al., 2016). Our experimental results are consistent with this finding. In the low-frequency noise group, genes related to carbohydrate digestion and absorption were upregulated. Notably, in the

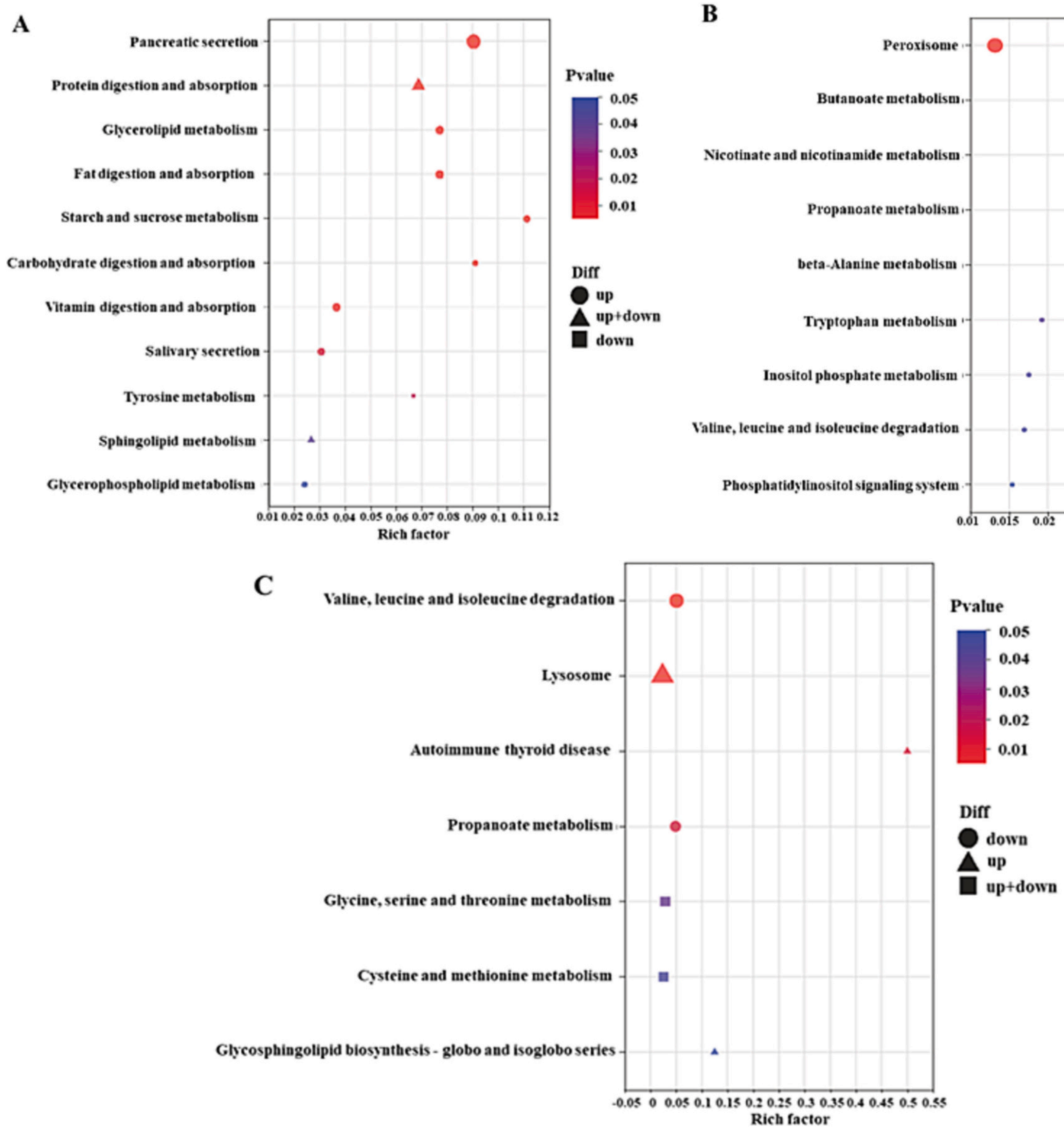

Fig. 8. The KEGG pathways enriched in DEGs between the CK group and low-frequency and high-frequency groups, with a *P*-value of <0.05. A: CK vs low-frequency group. B: CK vs high-frequency group. C: low-frequency group vs high-frequency group.

comparison between the high and low-frequency groups, genes related to the degradation of valine, leucine, and isoleucine were downregulated. Underwater low-frequency noise may stimulate sea cucumbers to produce more nutrients to meet the energy demands underwater. However, as the noise frequency increases, the energy demand will decrease, resulting in the inhibition of muscle growth and energy metabolism (Zeitz et al., 2019).

Pancreatic secretion plays a positive regulatory role in food digestion and absorption in the body's digestive system (Hasegawa et al., 1993). Secretin plays a role in lipid breakdown through protein kinase A activation and hormone sensitive lipase phosphorylation, as demonstrated in both in vitro and in vivo studies (Sekar and Chow, 2014). In this study, exposure to low-frequency noise resulted in upregulation of genes associated with pancreatic secretion in sea cucumbers. Additionally, the expression levels of genes related to fat digestion and absorption were also upregulated. Our experimental results are consistent with previous findings, which observed a significant increase in both pro-secretory and pancreatic fluid levels in insect cells infected with rod-shaped viruses (Asmann et al., 2004). Underwater low-frequency noise may promote pancreatic secretion in sea cucumbers, leading to increased absorption and utilization of nutrients such as fats. Additionally, genes related to carbohydrate absorption were upregulated in the low-frequency noise group, which is consistent with our analysis results.

Peroxisomes are important cell organelles that contain various enzyme molecules capable of catalyzing a wide range of metabolic reactions (Nazarko, 2017). Peroxisomes are involved in a variety of physiological processes, including muscle growth, energy metabolism, maintaining redox balance, and protecting cells from harmful oxides (Zalckvar and Schuldiner, 2022). Peroxisomes have important physiological functions for dna protective activity in aquatic invertebrate immune response (Abbas et al., 2019). In this study, exposure to high-frequency noise resulted in downregulation of genes related to peroxisomes in sea cucumbers, which is consistent with our results from analyzing oxidative stress markers. Exposure to high-frequency noise significantly altered the gene expression of peroxisomes in the coelomic fluid of sea urchin (Vazzana et al., 2020). The gene expression of peroxisomes in sea cucumbers was significantly suppressed under the influence of high-frequency noise, indicating that high-frequency noise may affect the oxidative and toxin metabolism of sea cucumbers.

5. Conclusion

In summary, the impact of noise on oxidative stress in sea cucumbers is mainly from low-frequency noise. Although high-frequency noise also leads to a reduction in enzyme activity, its effect is weaker than that of low-frequency noise. Low-frequency noise is more likely to induce intestinal inflammation and reduce the antioxidant capacity of sea cucumbers. Continuous exposure to underwater noise leads to decreased intestinal cell vitality, as well as the suppression of protein synthesis and cellular apoptosis in sea cucumbers. This study reports for the first time the transcriptome information of the gut of sea cucumbers after exposure to noise at different frequencies. Upregulation of lysosomal, pancreatic secretion, and fat digestion and absorption genes under low-frequency noise ensures the nutritional and energy needs of sea cucumbers exposed to adverse conditions, while also activating their intestinal immune system. As frequency increases from low to high, carbohydrate absorption and amino acid degradation decrease while peroxisome expression is suppressed, leading to inhibition of muscle growth and energy metabolism. Overall, these findings provide valuable insights into the regulatory mechanisms of sea cucumbers under underwater noise exposure and highlight the importance of considering the effects of different frequency ranges when assessing the impacts of noise pollution on marine organisms.

CRediT authorship contribution statement

Xiaochen Cheng: Investigation, Formal analysis, Writing – original draft, Writing – review & editing. **Libin Zhang:** Conceptualization, Supervision, Funding acquisition. **Zhaoming Gao:** Investigation. **Kehan Li:** Investigation. **Jialei XU:** Provision of site facilities .**Weijian Liu:** Supervision, conceptualization. **Xiaoshang Ru:** Conceptualization, Methodology, Supervision, Funding acquisition.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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References

Abbas, M.N., Kausar, S., Cui, H.J., 2019. The biological role of peroxiredoxins in innate immune responses of aquatic invertebrates. Fish Shellfish Immunol. 89, 91–97. https://doi.org/10.1016/j.fsi.2019.03.062.

Anderson, P.A., Berzins, I.K., Fogarty, F., Hamlin, H.J., Guillette, L.J., 2011. Sound, stress, and seahorses: the consequences of a noisy environment to animal health. Aquaculture 311, 129–138. https://doi.org/10.1016/j.aquaculture.2010.11.013.

Asmann, Y.W., Dong, M., Miller, L.J., 2004. Functional characterization and purification of the secretin receptor expressed in baculovirus-infected insect cells. Regul. Pept. 123, 217–223. https://doi.org/10.1016/j.regpep.2004.04.027.

Berkhout, B.W., Budria, A., Thieltges, D.W., Slabbekoorn, H., 2023. Anthropogenic noise pollution and wildlife diseases. Trends Ecol. Evol. 39, 181–190. https://doi.org/10.1016/j.pt.2022.12.002.

Bertucci, F., Parmentier, E., Berten, L., Brooker, R.M., Lecchini, D., 2015. Temporal and spatial comparisons of underwater sound signatures of different reef habitats in Moorea Island, French Polynesia. Plos One 10 (9), e0135733. https://doi.org/10.1371/journal.pone.0135733.

Broer, S., 2022. Review amino acid transporters as modulators of glucose homeostasis. Trends Endocrinol. Metab. 33, 120–135. https://doi.org/10.1016/j.tem.2021.11.004.

Cao, J.F., Zhu, W.J., Wu, X.B., Wang, T.G., Xu, H.R., 2019. An aero-acoustic noise distribution prediction methodology for offshore wind farms. Energies 12, 18. https://doi.org/10.3390/en12010018.

Cecilia, Holmlund, Monica, Hammer, 1999. Ecosystem services generated by fish populations. Ecol. Econ. 29, 253–268. https://doi.org/10.1016/S0921-8009(99)00015-4.

Celi, M., Filiciotto, F., Vazzana, M., Arizza, V., Maccarrone, V., Ceraulo, M., et al., 2015. Shipping noise affecting immune responses of European spiny lobster (Palinurus elephas). Can. J. Zool. 93, 113–121. https://doi.org/10.1139/cjz-2014-0219.

Chen, D., Liu, J., Lu, L., Huang, Y., Wang, Y., Wang, M., et al., 2016. Emodin attenuates TNF-α-induced apoptosis and autophagy in mouse C2C12 myoblasts though the phosphorylation of Akt. Int. Immunopharmacol. 34, 107–113. https://doi.org/10.1016/j.intimp.2016.02.023.

De Robertis, A., Handegard, N.O., 2013. Fish avoidance of research vessels and the efficacy of noise-reduced vessels: a review. ICES J. Mar. Sci. 70, 34–45. https://doi.org/10.1093/icesjms/fss155.

De Soto, N.A., Delorme, N., Atkins, J., Howard, S., Williams, J., Johnson, M., 2013. Anthropogenic noise causes body malformations and delays development in marine larvae. Sci. Rep. 3, 2831. https://doi.org/10.1038/srep02831.

Debusschere, E., Hostens, K., Adriaens, D., Ampe, B., Botteldooren, D., De Boeck, G., et al., 2016. Acoustic stress responses in juvenile sea bass Dicentrarchus labrax induced by offshore pile driving. Environ. Pollut. 208, 747–757. https://doi.org/10.1016/j.envpol.2015.10.055.

Deng, Z.D., Southall, B.L., Carlson, T.J., Xu, J.S., Martinez, J.J., Weiland, M.A., et al., 2014. 200 kHz commercial sonar systems generate lower frequency side lobes audible to some marine mammals. PloS One 9 (4), e95315. https://doi.org/10.1371/journal.pone.0095315.

Di Franco, E., Pierson, P., Di Iorio, L., Calò, A., Cottalorda, J., Derijard, B., et al., 2020. Effects of marine noise pollution on Mediterranean fishes and invertebrates: a review. Mar. Pollut. Bull. 159, 111450 https://doi.org/10.1016/j.marpolbul.2020.111450.

Ding, K., Zhang, L.B., Zhang, T., Yang, H.S., Brinkman, R., 2019. The effect of melatonin on locomotor behavior and muscle physiology in the sea cucumber Apostichopus japonicus. Front. Physiol. 10, 221. https://doi.org/10.3389/fphys.2019.00221.

Duarte, C.M., Chapuis, L., Collin, S.P., Costa, D.P., Devassy, R.P., Eguiluz, V.M., et al., 2021. The soundscape of the Anthropocene ocean. Science 371 (6529), eaba4658. https://doi.org/10.1126/science.aba4658.

Eddy, T.D., Lotze, H.K., Fulton, E.A., Coll, M., Ainsworth, C.H., de Araujo, J.N., et al., 2017. Ecosystem effects of invertebrate fisheries. Fish Fish. 18, 40–53. https://doi.org/10.1111/faf.12165.

Edmonds, N.J., Firmin, C.J., Goldsmith, D., Faulkner, R.C., Wood, D.T., 2016. A review of crustacean sensitivity to high amplitude underwater noise: data needs for effective risk assessment in relation to UK commercial species. Mar. Pollut. Bull. 108, 5–11. https://doi.org/10.1016/j.marpolbul.2016.05.006.

Erbe, C., Reichmuth, C., Cunningham, K., Lucke, K., Dooling, R., 2016. Communication masking in marine mammals: a review and research strategy. Mar. Pollut. Bull. 103, 15–38. https://doi.org/10.1016/j.marpolbul.2015.12.007.

Erbe, C., Williams, R., Parsons, M., Parsons, S.K., Hendrawan, I.G., Dewantama, I.M.I., 2018. Underwater noise from airplanes: an overlooked source of ocean noise. Mar. Pollut. Bull. 137, 656–661. https://doi.org/10.1016/j.marpolbul.2018.10.064.

Ferguson, E.L., Clayton, H.M., Sakai, T., 2023. Acoustic indices respond to specific marine mammal vocalizations and sources of anthropogenic noise. Front. Mar. Sci. 10, 1025464. https://doi.org/10.3389/fmars.2023.1025464.

Fernandez, A., Edwards, J.F., Rodriguez, F., De LM, A.E., Herraez, P., Castro, P., et al., 2005. "Gas and Fat Embolic Syndrome" Involving a Mass Stranding of Beaked Whales (Family Ziphiidae) Exposed to Anthropogenic Sonar Signals. Vet. Pathol. 42, 446–457. https://doi.org/10.1354/vp.42-4-446.

Fitzgibbon, Q.P., Day, R.D., McCauley, R.D., Simon, C.J., Semmens, J.M., 2017. The impact of seismic air gun exposure on the haemolymph physiology and nutritional condition condition of spiny lobster, *Jasus edwardsii*. Mar. Pollut. Bull. 125, 146–156. https://doi.org/10.1016/j.marpolbul.2017.08.004.

Gaspar, E.B., Mortara, R.A., Andrade, L.O., da Silva, C.V., 2009. Lysosomal exocytosis: an important event during invasion of lamp deficient cells by extracellular amastigotes of Trypanosoma cruzi. Biochem. Biophys. Res. Commun. 384, 265–269. https://doi.org/10.1016/j.bbrc.2009.04.114.

Gotz, T., Janik, V.M., 2013. Acoustic deterrent devices to prevent pinniped depredation: efficiency, conservation concerns and possible solutions. Mar. Ecol. Prog. Ser. 492, 285–302. https://doi.org/10.3354/meps10482.

Gregory, C.D., Devitt, A., 2003. Innate immunity and apoptosis: CD14-dependent clearance of apoptotic cells. Apoptosis Autoimmun. 118, 111–132. https://doi.org/10.1002/3527601457.

Hada, E., Aaronson, N.L., Miller, K., 2010. Attenuation of high-frequency sound waves by concentric nitrogen bubble curtains in salt water (vol 128, pg 15, 2010). J. Acoust. Soc. Am. 128, 3809. https://doi.org/10.1121/1.3518269.

Halliday, W.D., Pine, M.K., Insley, S.J., 2020. Underwater noise and Arctic marine mammals: review and policy recommendations. Environ. Rev. 28, 438–448. https://doi.org/10.1139/er-2019-0033.

Halvorsen, M.B., Casper, B.M., Matthews, F., Carlson, T.J., Popper, A.N., 2012. Effects of exposure to pile-driving sounds on the lake sturgeon, Nile tilapia and hogchoker. Proc. R. Soc. B Biol. Sci. 279, 4705–4714. https://doi.org/10.1098/rspb.2012.1544.

Hartenstein, V., Martinez, P., 2019. Phagocytosis in cellular defense and nutrition: a food-centered approach to the evolution of macrophages. Cell Tissue Res. 377, 527–547. https://doi.org/10.1007/s00441-019-03096-6.

Hasegawa, H., Okabayashi, Y., Koide, M., Kido, Y., Okutani, T., Matsushita, K., et al., 1993. Effect of islet hormones on secretin-stimulated exocrine secretion in isolated perfused rat pancreas. Dig. Dis. Sci. 38, 1278–1283. https://doi.org/10.1007/bf01296079.

Howe, B.M., Miksis-Olds, J., Rehm, E., Sagen, H., Worcester, P.F., Haralabus, G., 2019. Observing the oceans acoustically. Front. Mar. Sci. 6, 426. https://doi.org/10.3389/fmars.2019.00426.

Hu, F.Y., Ding, P., Yu, Y.S., Wen, B., Cui, Z.P., Yang, M.F., et al., 2021. Effects of artificial reefs on selectivity and behaviors of the sea cucumber Apostichopus japonicas: new insights into the pond culture. Aquacult. Rep. 21 https://doi.org/10.1016/j.aqrep.2021.100842.

Jhawar, J., Morris, R.G., Amith-Kumar, U.R., Raj, M.D., Rogers, T., Rajendran, H., et al., 2020. Noise-induced schooling of fish. Nat. Phys. 16, 488–493. https://doi.org/10.1038/s41567-020-0787-y.

Jones, N., 2019. Ocean uproar: saving marine life from a barrage of noise. Nature 568, 158–162. https://doi.org/10.1038/d41586-019-01098-6.

Karimaian, A., Majidinia, M., Bannazadeh Baghi, H., Yousefi, B., 2017. The crosstalk between Wnt/β-catenin signaling pathway with DNA damage response and oxidative stress: implications in cancer therapy. DNA Repair 51, 14–19. https://doi.org/10.1016/j.dnarep.2017.01.003.

Krysko, D.V., D'Herde, K., Vandenabeele, P., 2006. Clearance of apoptotic and necrotic cells and its immunological consequences. Apoptosis 11, 1709–1726. https://doi.org/10.1007/s10495-006-9527-8.

Lagrois, D., Kowalski, C., Sénécal, J.-F., Martins, C.C.A., Chion, C., 2023. Low-to-mid-frequency monopole source levels of underwater noise from small recreational vessels in the St. Lawrence Estuary Beluga Critical Habitat. Sensors 23, 1674. https://doi.org/10.3390/s23031674.

Leduc, A., Nunes, J., de Araujo, C.B., Quadros, A.L.S., Barros, F., Oliveira, H.H.Q., et al., 2021. Land-based noise pollution impairs reef fish behavior: a case study with a Brazilian carnival. Biol. Conserv. 253, 108910 https://doi.org/10.1016/j.biocon.2020.108910.

Lemos, L.S., Haxel, J.H., Olsen, A., Burnett, J.D., Smith, A., Chandler, T.E., et al., 2022. Effects of vessel traffic and ocean noise on gray whale stress hormones. Sci. Rep. 12, 18580. https://doi.org/10.1038/s41598-022-14510-5.

Lillis, A., Eggleston, D.B., Bohnenstiehl, D.R., 2014. Soundscape variation from a larval perspective: the case for habitat-associated sound as a settlement cue for weakly swimming estuarine larvae. Mar. Ecol. Prog. Ser. 509, 57–70. https://doi.org/10.3354/meps10917.

Lin, T., Xing, J., Jiang, J., Tang, X., Zhan, W., 2011. β-Glucan-stimulated activation of six enzymes in the haemocytes of the scallop Chlamys farreri at different water temperatures. Aquaculture 315, 213–221. https://doi.org/10.1016/j.aquaculture.2011.02.034.

Lindeboom, H.J., Kouwenhoven, H.J., Bergman, M.J.N., Bouma, S., Brasseur, S., Daan, R., et al., 2011. Short-term ecological effects of an offshore wind farm in the Dutch coastal zone; a compilation. Environ. Res. Lett. 6, 035101 https://doi.org/10.1088/1748-9326/6/3/035101.

Maiditsch, I.P., Ladich, F., 2023. Effects of noise on acoustic and visual signalling in the croaking gourami: differences in adaptation strategies in fish. Bioacoustics 32, 181–198. https://doi.org/10.1080/09524622.2022.2086174.

Miksis-Olds, J.L., Nichols, S.M., 2016. Is low frequency ocean sound increasing globally? J. Acoust. Soc. Am. 139, 501–511. https://doi.org/10.1121/1.4938237.

Mills, S.C., Beldade, R., Henry, L., Laverty, D., Nedelec, S.L., Simpson, S.D., et al., 2020. Hormonal and behavioural effects of motorboat noise on wild coral reef fish. Environ. Pollut. 262 https://doi.org/10.1016/j.envpol.2020.114250.

Nazarko, T.Y., 2017. Pexophagy is responsible for 65% of cases of peroxisome biogenesis disorders. Autophagy 13, 991–994. https://doi.org/10.1080/15548627.2017.1291480.

Nedelec, S.L., Radford, A.N., Pearl, L., Nedelec, B., McCormick, M.I., Meekan, M.G., et al., 2017. Motorboat noise impacts parental behaviour and offspring survival in a reef fish. Proc. R. Soc. B Biol. Sci. 284, 20170143. https://doi.org/10.1098/rspb.2017.0143.

Nowacek, D.P., Thorne, L.H., Johnston, D.W., Tyack, P.L., 2010. Responses of cetaceans to anthropogenic noise. Mammal Rev. 37 https://doi.org/10.1111/j.1365-2907.2007.00104.x.

Park, J.-K., Do, Y., 2022. Wind turbine noise behaviorally and physiologically changes male frogs. Biology 11, 516. https://doi.org/10.3390/biology11040516.

Park, J., Kim, B., 2019. An analysis of South Korea's energy transition policy with regards to offshore wind power development. Renew. Sustain. Energy Rev. 109, 71–84. https://doi.org/10.1016/j.rser.2019.04.031.

Patek, S.N., Shipp, L.E., Staaterman, E.R., 2009. The acoustics and acoustic behavior of the California spiny lobster (Panulirus interruptus). J. Acoust. Soc. Am. 125, 3434–3443. https://doi.org/10.1121/1.3097760.

Peng, C., Zhao, X., Liu, S., Shi, W., Han, Y., Guo, C., et al., 2016. Effects of anthropogenic sound on digging behavior, metabolism, Ca^{2+}/Mg^{2+} ATPase activity, and metabolism-related gene expression of the bivalve Sinonovacula constricta. Sci. Rep. 6, 24266. https://doi.org/10.1038/srep24266.

Popper, A.N., Hawkins, A.D., 2019. An overview of fish bioacoustics and the impacts of anthropogenic sounds on fishes. J. Fish Biol. 94, 692–713. https://doi.org/10.1111/jfb.13948.

Popper, A.N., Fewtrell, J., Smith, M.E., Mccauley, R.D., 2003. Anthropogenic sound: effects on the behavior and physiology of fishes. Mar. Technol. Soc. J. 37, 35–40. https://doi.org/10.4031/002533203787537050.

Roberts, L., Elliott, M., 2017. Good or bad vibrations? Impacts of anthropogenic vibration on the marine epibenthos. Sci. Total Environ. 595, 255–268. https://doi.org/10.1016/j.scitotenv.2017.03.117.

Sekar, R., Chow, B.K.C., 2014. Lipolytic actions of secretin in mouse adipocytes. J. Lipid Res. 55, 190–200. https://doi.org/10.1194/jlr.M038042.

Shi, Y., Yang, Y.X., Tian, J.W., Sun, C., Zhao, W., Li, Z.L., et al., 2019. Long-term ambient noise statistics in the northeast South China Sea. J. Acoust. Soc. Am. 145, EL501–EL507. https://doi.org/10.1121/1.5110740.

Simpson, S.D., Radford, A.N., Holles, S., Ferarri, M.C.O., Chivers, D.P., Mccormick, M.I., et al., 2016a. Small-Boat Noise Impacts Natural Settlement Behavior of Coral Reef Fish Larvae. Springer New York. https://doi.org/10.1007/978-1-4939-2981-8_129.

Simpson, S.D., Radford, A.N., Nedelec, S.L., Ferrari, M.C.O., Chivers, D.P., McCormick, M.I., et al., 2016b. Anthropogenic noise increases fish mortality by predation. Nat. Commun. 7, 10544. https://doi.org/10.1038/ncomms10544.

Slabbekoorn, H., Bouton, N., van Opzeeland, I., Coers, A., ten Cate, C., Popper, A.N., 2010. A noisy spring: the impact of globally rising underwater sound levels on fish. Trends Ecol. Evol. 25, 419–427. https://doi.org/10.1016/j.tree.2010.04.005.

Smith, M.E., Kane, A.S., Popper, A.N., 2004. Noise-induced stress response and hearing loss in goldfish (Carassius auratus). J. Exp. Biol. 207, 427–435. https://doi.org/10.1242/jeb.00755.

Solan, M., Hauton, C., Godbold, J.A., Wood, C.L., Leighton, T.G., White, P., 2016. Anthropogenic sources of underwater sound can modify how sediment-dwelling invertebrates mediate ecosystem properties. Sci. Rep. 6 https://doi.org/10.1038/srep20540.

Soto, N.A.D., Gkikopoulou, K., Hooker, S., Isojunno, S., Thomas, L., 2016. From physiology to policy: a review of physiological noise effects on marine fauna with implications for mitigation. In: Fourth International Conference on the Effects of Noise on Aquatic Life.

Strunz, S., 2018. Speeding up the energy transition. Nat. Sustain. 1, 390–391. https://doi.org/10.1038/s41893-018-0120-2.

Talianni, K., 2020. The soundscape of Anthropocene. Science. https://doi.org/10.2218/airea.5037.

Vazzana, M., Mauro, M., Ceraulo, M., Dioguardi, M., Papale, E., Mazzola, S., et al., 2020. Underwater high frequency noise: biological responses in sea urchin Arbacia lixula (Linnaeus, 1758). Comp. Biochem. Physiol. A Mol. Integr. Physiol. 242, 110650 https://doi.org/10.1016/j.cbpa.2020.110650.

Wale, M.A., Simpson, S.D., Radford, A.N., 2013. Size-dependent physiological responses of shore crabs to single and repeated playback of ship noise. Biol. Lett. 9, 20121194. https://doi.org/10.1098/rsbl.2012.1194.

Wale, M.A., Briers, R.A., Hard, M.G.J., Bryson, D., Diele, K., 2019. From DNA to ecological performance: effects of anthropogenic noise on a reef-building mussel. Sci. Total Environ. 689, 126–132. https://doi.org/10.1016/j.scitotenv.2019.06.380.

Walsh, E.P., Arnott, G., Kunc, H.P., 2017. Noise affects resource assessment in an invertebrate. Biol. Lett. 13, 20170098 https://doi.org/10.1098/rsbl.2017.0098.

Wang, S.V., Wrede, A., Tremblay, N., Beermann, J., 2022. Low-frequency noise pollution impairs burrowing activities of marine benthic invertebrates. Environ. Pollut. 310, 119899 https://doi.org/10.1016/j.envpol.2022.119899.

Watts, C., 2022. Lysosomes and lysosome-related organelles in immune responses. FEBS Open Bio 12, 678–693. https://doi.org/10.1002/2211-5463.13388.

Williams, R., Wright, A.J., Ashe, E., Blight, L.K., Bruintjes, R., Canessa, R., et al., 2015. Impacts of anthropogenic noise on marine life: publication patterns, new discoveries, and future directions in research and management. Ocean Coast. Manag. 115, 17–24. https://doi.org/10.1016/j.ocecoaman.2015.05.021.

Yang, C.M., Liu, Z.W., Lü, L.-G., Yang, G.B., Huang, L.F., Jiang, Y., 2018. Observation and comparison of tower vibration and underwater noise from offshore operational wind turbines in the East China Sea Bridge of Shanghai. J. Acoust. Soc. Am. 144, EL522–EL527. https://doi.org/10.1121/1.5082983.

Zalckvar, E., Schuldiner, M., 2022. Beyond rare disorders: a new era for peroxisomal pathophysiology. Mol. Cell 82, 2228–2235. https://doi.org/10.1016/j.molcel.2022.05.028.

Zeitz, J.O., Kading, S.C., Niewalda, I.R., Machander, V., Dorigam, J.C.D., Eder, K., 2019. Effects of leucine supplementation on muscle protein synthesis and degradation pathways in broilers at constant dietary concentrations of isoleucine and valine. Arch. Anim. Nutr. 73, 75–87. https://doi.org/10.1080/1745039X.2019.1583519.

Zhang, H., Wang, Q.W., Huang, J.B., 2022a. China's policy for the marine engineering equipment industry and potential challenges: an appraisal of the new developments under the 14th five-year plans. Front. Mar. Sci. 9, 1014959. https://doi.org/10.3389/fmars.2022.1014959.

Zhang, Y., Liu, C.H., Liu, J.H., Liu, X.M., Tu, Z.H., Zheng, Y.P., et al., 2022b. Multi-omics reveals response mechanism of liver metabolism of hybrid sturgeon under ship noise stress. Sci. Total Environ. 851, 158348 https://doi.org/10.1016/j.scitotenv.2022.158348.

Zhengqiang, C., Dongdong, Z., Jing, X., Wenbin, Z., 2019. Effects of temperature on haemocyte and granulocyte counts and expressions of immunity-related genes in haemocytes of scallop Chlamys farreri after vibrio anguillarum infection. J. Ocean Univ. China 18, 1163–1173. https://doi.org/10.1007/s11802-019-4180-3.