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Effects of artificial electric fields on the physiological state of *Amphistegina lessonii*: Insights from oxidative stress biomarkers and gene expression

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ABSTRACT

Anthropogenic energy infrastructure, including subsea power cables and renewable devices, introduces unexpected artificial electricity into marine ecosystems, potentially acting as ecological stressors on benthic community. The physiological responses of benthic organisms to electric stimulation remain poorly understood. Benthic foraminifera, single-cell organisms widely used as bioindicators in marine environments, are sensitive to environmental stressors. This proof-of-concept study aims to evaluate the effects of different electric current densities on the benthic foraminiferal species *Amphistegina lessonii* based on single-cell transcriptomic and biochemical (i.e. proteins and enzymes) analyses in short-term exposure experiments. Electrical stimulation leads to the upregulation of mitochondrial metabolic (*COQ3, PPOX,* and *ATP6*) genes, as well as TCA cycle pathways. The biomarkers p-p38 MAPK and p-PKC, which are associated with cellular responses to stressful stimuli, are significantly enhanced at 0.86 μA/cm^2, suggesting that this electric density induced a significant physiological response. These responses indicate that electrical stimulation may enhance mitochondrial energy production, potentially increasing reactive oxygen species (ROS) generation. Following cellular responses to stressful stimuli, antioxidant defence is upregulated as reflected by redox-related (*GPX, GGT1*) genes. This antioxidant activity is further supported by the levels of multiple biomarkers (GST, GPx, and Se-GPx). This study demonstrates significant metabolic and physiological changes in *A. lessonii* and its adaptive capability to ensure cell survival under electrical stimulation. These findings also highlight the combined use of cellular biomarkers and single-cell transcriptomic analysis as a promising approach for assessing environmental stress in benthic foraminifera-based biomonitoring.

1. Introduction

Many marine organisms can detect electromagnetic fields in the environment, and, at the same time, utilise such electric and magnetic attractions in their life activities such as movement, orientation, and feeding (Gill et al., 2014). Some animals are electroreceptive, possessing specialised organs capable of perceiving weak electric fields generated by both biological and non-biological sources (Gill et al., 2014). The introduction of energy, particularly in the form of electric and electromagnetic fields, can act as a stressor that alters the natural sensory capabilities of marine organisms (Otremba et al., 2019). Indeed, the introduction of energy is included in Descriptor 11 of the Marine Strategy Framework Directive (MSFD) to characterise the marine environment and assess its achievement of a Good Environmental Status. Sources of energy input in the marine environment include wind turbines, offshore operations, marine renewable energy devices, and subsea electrical cables that produce both electric and electromagnetic fields (Directive 2008/56/EC, 2008).

Benthic organisms, particularly those in proximity to the power cables on the seabed, are more exposed to artificial energy inputs. Previous

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studies have investigated the effects of anthropogenic electromagnetic fields on behaviour, physiology, and development of early life stages (e.g. Albert et al., 2020; James et al., 2026). However, our understanding of how benthic organisms interact with artificial electric fields in the marine environment remains quite limited, and the impact at the cellular level is still unknown, highlighting the need for further investigation.

Benthic foraminifera, single-celled organisms with a test (i.e. shell), are increasingly applied as bioindicators in environmental biomonitoring, because of their high abundance and diversity in marine environments, a widespread distribution, short-life and reproductive cycles (Martins et al., 2016). A first study for electrical current stimulation with constant and pulsed direct currents has been conducted to evaluate the short-term impact of artificial electric fields on the viability of the symbiont-bearing foraminiferal species *Amphistegina lessonii* (Rebecchi et al., 2023). This pioneering study revealed that constant direct current stimulation had a more acute effect on viability, affecting pseudopodial activity even at low current values and for shorter exposure times than pulsed direct current. These findings provide valuable insights for establishing the highest potential tested electrical density to assess the stress induced by artificial electric fields. In addition, this study has enabled the development of an electric prototype for electrochemical experiments or other applications (Lattanzi et al., 2024; Rebecchi et al., 2025). For a better understanding of the anthropogenic electromagnetic impact, it is essential to investigate the physiological responses of marine organisms to artificial electric fields. In this context, the application of cellular biomarkers provides an additional perspective by identifying short- and long-term responses to a multiplicity of chemical and physical stressors (El-SiKaily and Shabaka, 2024; Hagger et al., 2006). Exposure to various environmental stressors, such as thermal stress, UV radiation exposure, and pollution, stimulates specific cellular responses, leading to oxidative stress and the production of reactive oxygen species (ROS) (Lesser, 2006). While ROS play natural roles in metabolism, signalling, and cellular processes, they also have the potential to induce harmful effects on lipids, proteins, and nucleic acids, causing lipid peroxidation and genotoxic effects (Regoli and Giuliani, 2014). Marine organisms maintain ROS homeostasis through antioxidant enzymes, such as glutathione peroxidase (GPx), glutathione selenium peroxidase (Se-GPx), and glutathione S-transferase (GST), which scavenge ROS and prevent oxidative stress (Chainy et al., 2016). In addition to antioxidant defences, several proteins like mitogen-activated protein kinase (MAPK) and protein kinase C (PKC) are commonly used as biomarkers associated with environmental stressors due to their roles in general stress responses (Trapp et al., 2014).

The cellular biomarkers have been utilised to investigate the biochemical changes of benthic foraminifera in response to heavy metal exposure, showing that mercury activates GSH enzymes, the MAPK pathway (e.g., p-p38), as well as HSP70 (Ciacci et al., 2022). The antioxidant capacity against peroxyl radicals has also been evaluated in *A. lessonii* to assess the water quality (Prazeres et al., 2012). Thus, cellular biomarkers offer the advantage of detecting stress effects and enable the early identification of impacted areas and their causes, making them a reliable diagnostic tool for environmental health. Moreover, the application of transcriptomics provides valuable insights into molecular alterations of biological processes (Volkova and Geras'kin, 2018). In this context, the mechanisms underlying TiO_2 nanoparticle (NP) cytotoxicity and subsequent detoxification were investigated through single-cell transcriptome analyses in *Ammonia veneta* (Ishitani et al., 2023). Titanium dioxide NPs exposure leads to the upregulation of *protoporphyrinogen III oxidase (PPOX)*, which produces ROS in mitochondria. The resulting porphyrin molecules chelate metal ions, inducing ROS in acidic vesicles through the Fenton reaction. The ROS in mitochondria and vesicles are quenched by *GPX* and *sulfite oxidase* (*SUOX)*. With transcriptomics, it is possible to infer how pollutants induce stress and how organisms cope with that.

The present study aims to document the physiological response of *A. lessonii*, a foraminiferal species, to electric stimulation through short-term exposures (24 h) to different electric current densities. Laboratory setups using pulsed direct current cannot fully replicate the complex electromagnetic environments of subsea cables. Therefore, this work should be considered a proof-of-concept physiological investigation under controlled current densities. These intensities were specifically selected within a sublethal range, as determined by previous viability tests, to ensure foraminiferal survival while enabling cellular analysis. Five biomarkers showing antioxidant defences and stress were applied to investigate foraminiferal response to electric fields, integrating the related metabolic pathways revealed by transcriptomics. These results provide insight into the understanding of the future effects of artificial electric fields on marine benthos.

2. Materials and methods

2.1. Individual collection and experimental setup

Individuals of *A. lessonii* were collected from rock pebbles in the Gulf of Aqaba-Eilat (Red Sea, Israel) between June and September 2022 and transported to the University of Urbino (Italy). In the laboratory, adult individuals (approximately 300–600 μm of test diameter) were placed in 100 mm glass Petri dishes filled with artificial seawater (prepared in accordance with the composition indicated in ASTM D1141-98) at a salinity of 40. They were acclimated at 25 °C under a 12 h light-dark cycle for several days. Only healthy specimens of *A. lessonii*, displaying a distinct golden-brown colouration and noticeable pseudopodial activity, were selected for the experiment.

Living foraminiferal specimens were electrically stimulated using an in-house stimulus generator (Lattanzi et al., 2024). This electric prototype is made up of an Arduino nano board, an open-source electronic board, used to generate a low-intensity current stimulation between two platinum electrodes (anode and cathode). These electrodes were placed within a six-well plate, with each well containing 9.6 ml of artificial seawater. Living individuals of *A. lessonii* specimens were selected and randomly placed in a six-well plate and stimulated for 24 h with pulsed direct current values of 0 (control), 1 μA, and 3 μA (equivalent to 0, 0.29 and 0.86 $\mu A/cm^2$ of electric density, respectively) for the antioxidant and protein analyses (Fig. 1). These current densities were selected based on the previous experiment, in which they were identified as non-lethal and associated with the highest survival rates of *A. lessonii* (Rebecchi et al., 2023). For transcriptome analysis, specimens were stimulated for 24 h with pulsed direct currents of 0 (control) and 0.86 $\mu A/cm^2$ only, as this current density showed the most significant biochemical responses in terms of antioxidant enzyme activity and protein modulation (Fig. 1). Based on the applied current density and seawater conductivity (~5 S/m), the electric field strength (E) was estimated using formula $E = J/\sigma$. The current densities of 0.29 and 0.86 $\mu A/cm^2$ correspond to estimated electric field strengths of 0.58 and 1.72 mV/m, respectively.

2.2. Protein assays

The total protein content was determined using the methodology of Lowry et al. (1951), and the procedure is detailed in (Ciacci et al., 2022). To optimise protein extraction and characterisation for *A. lessonii*, the protein assay was quantified following the protocol of (Betti et al., 2021). A total of 600 individuals of *A. lessonii* were utilised, with batches of 50 individuals for each condition processed in four replicates (Fig. 1). Samples were treated with a homemade lysis buffer and subjected to sonication, boiling, and centrifugation. Supernatants were prepared and loaded onto SDS-polyacrylamide gels for electrophoresis and western blotting.

The proteins phospho-p38 (MAPK) and protein kinase C (p-PKC) were used to evaluate protein modification and activation induced by oxidative stress. Protein samples were analysed using SDS-

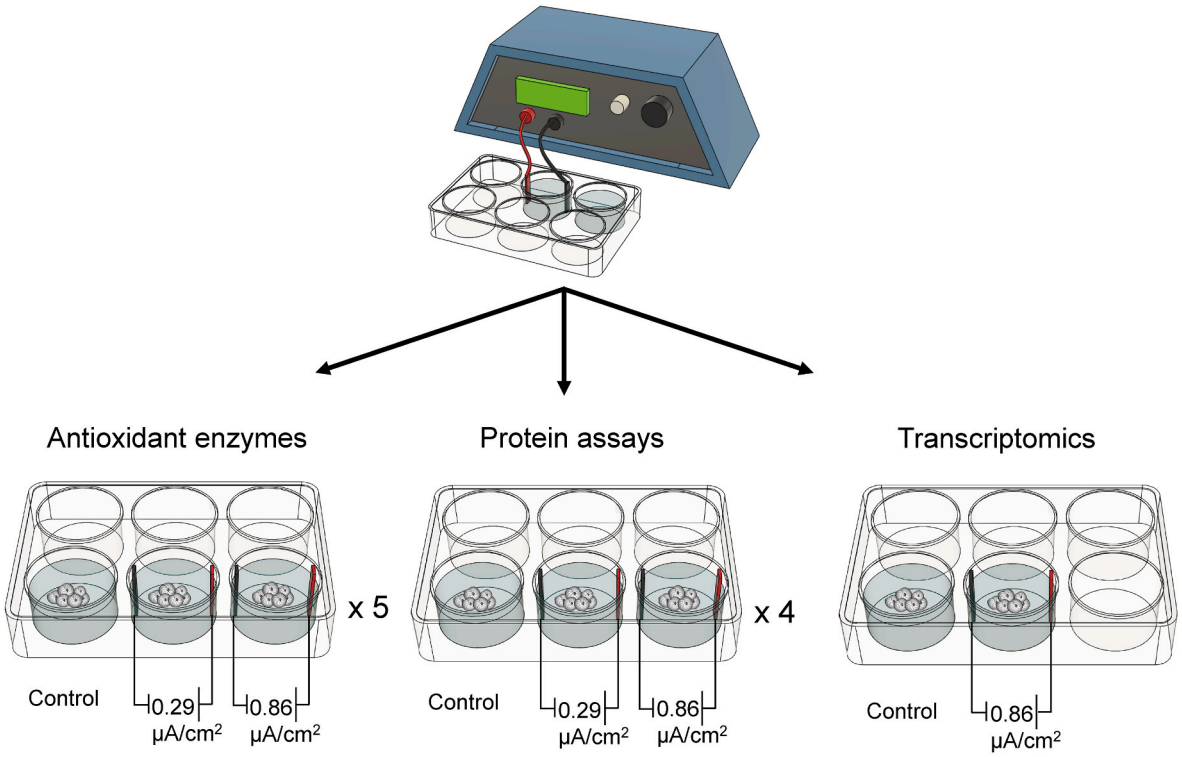

Fig. 1. Experimental design overview. Living foraminiferal specimens placed in a six-well plate were stimulated using an in-house stimulus generator for 24 h with pulsed direct current values of 0 (control), 0.29 and 0.86 µA/cm^2 for the antioxidant enzymes and protein analyses. Each condition was performed in five replicates for antioxidant enzyme analyses and four replicates for protein assay. For transcriptome analysis, specimens were stimulated for 24 h with pulsed direct currents of 0 (control) and 0.86 µA/cm^2.

polyacrylamide gel electrophoresis, with different gel percentages for phospho-p38 MAPK and Protein kinase C (p-PKC) (1:1000) as primary antibodies, and horseradish peroxidase-conjugated goat anti-rabbit IgG (1:3000) was used as a secondary antibody. Nitrocellulose membranes were stripped for 30 min at 50 °C using stripping buffer (62.5 mM Tris·HCl, pH 6.7, containing 10 mM β-mercaptoethanol and 2% SDS) and reprobed with anti-actin antibodies (1:1000 dilution) as a loading control. Western blot films were digitised (Chemidoc-Biorad), and band optical densities were quantified using a computerised imaging system (Quantity One software) that also calibrates the MWs of the sample bands using both LWM and HMW standards. Relative optical densities (arbitrary units) were normalised for the control band in each series.

2.3. Transcriptome analysis

Three specimens for each condition, either electrically stimulated or control, were isolated and used for direct single-cell cDNA amplification (Fig. 1) with the SMART-Seq HT kit (Clontech Laboratories, Inc., Mountain View, CA, USA) following the manufacturer's instructions. The quality and quantity of cDNAs were measured with a Qubit fluorometer (ThermoFisher Scientific, Waltham, MA, USA) using the 1x dsDNA HS system. Library samples were prepared with the Illumina DNA Prep kit (Illumina Inc., San Diego, CA, USA) following the manufacturer's instructions. Sequencing was carried out with 150-bp single-end reads on a Novaseq instrument (Illumina Inc.) by Macrogen Japan. All raw sequences in the present study have been deposited in GenBank repository under SRR31988627-SRR31988632.

All reads were quality-filtered with the FASTX-Toolkit 0.0.13 (Hannon, 2010) and those with fewer than 50 bases or that included ambiguous barcodes and showed poor quality (Q score <20) were removed. All clean reads were combined and assembled into reference contigs with Trinity 2.8.0 (Grabherr et al., 2011). The Ex90N50 for each condition was calculated using contig_ExN50_statistic.pl in Trinity 2.8.0 (Table S1). Quality-filtered sequences for each condition were mapped to this contig by using HISAT2 (Kim et al., 2019). Reference contigs were applied for Open Reading Frame (ORF) calling using TransDecoder v. 5.5.0 (Haas et al., 2013), and all ORFs were functionally annotated with Trinotate v.3.2.0 (Bryant et al., 2017) through a homology search to known sequences deposited at NCBI/SwissProt using blast + v. 2.10.0 (Camacho et al., 2009). The gene expression level of all reads against all ORFs was estimated with 100 bootstraps for an average fragment length of 200 and a standard deviation of 20 using Kallisto 0.46.0 (Bray et al., 2016). Transcript abundances and bootstrap values were analysed in R v.4.0.2 using the package tximport v.1.16.1 (Soneson et al., 2016). Metabolic pathway with the electric stimulus was reconstructed from KEGG annotation.

2.4. Antioxidant activity assays

The antioxidant activity assays for the GST, GPx, and Se-GPx were measured using the method described in Ciacci et al. (2022). A total of 750 individuals of *A. lessonii* were utilised, with each foraminiferal sample containing 50 individuals homogenised in 500 µL of 20 mM Tris–HCl buffer (pH 7.6), containing 0.5 M sucrose and 0.15 M NaCl. Each condition was tested in five replicates (Fig. 1). The homogenate was mechanically homogenised with Teflon, sonicated for 45 s at 100W, placed in an ultrasonic bath for 10 min, and then centrifuged at 13, 000×*g* for 90 min at 0-4 °C. The resulting supernatant was used to determine enzyme activity through spectrophotometry. GST activity was evaluated with CDNB (1-chloro-2,4-dinitrobenzene) as a substrate. The reaction mixture (1 mL) contained 125 mM K-phosphate buffer, pH 6.5, 1 mM CDNB, and 1 mM GSH. The formation of S-2,4-dinitrophenyl glutathione conjugate was evaluated by monitoring the increase in absorbance at 340 nm.

GPx catalyses the oxidation of glutathione by cumene hydroperoxide. In the presence of GSR and NADPH, the oxidised form of glutathione

is immediately converted to the reduced form with concomitant oxidation of NADPH to NADP+. The decrease in absorbance at 340 nm was measured. The Se-GPx activity was measured using H_2O_2 as the substrate for the oxidation of GSH. The molar extinction coefficients (ε) were set at 9.6 μmol/cm for GST and 6.2 μmol/cm for GPx and Se-GPx.

2.5. Statistical analysis

Differences in the mean enzyme (i.e., GST, GPx, and Se-GPx) values between control and treatment samples were analysed using a one-way analysis of variance (ANOVA). Post-hoc analysis was performed using Tukey's honestly significant difference (HSD) tests. The confidence level was set at 99% ($\alpha = 0.01$). For proteins (i.e., p-p38 and PKC), differences between control and treatment samples were assessed using the nonparametric Kruskal-Wallis H test. Post-hoc analysis, following significant results, was performed using the Dunn test with a confidence level of 95% ($\alpha = 0.05$). Multidimensional scaling (MDS) of transcriptome data was carried out in R v.4.0.2 (R Core Team, 2021) to visualise overall patterns among electrically stimulated and control specimens. Differential gene expression between the control and electrically stimulated conditions was statistically tested for significance using the likelihood ratio test with DESeq2 v. 1.28.1 (Love et al., 2014).

3. Results

3.1. Effects on p-p38 MAPK phosphorylation and p-PKC

The amount of detected p-p38 was higher in samples stimulated at 0.86 μA/cm^2 (1.68 ± 0.06) than those stimulated at 0.29 μA/cm^2 (1.07 ± 0.07) and control (1.06 ± 0.04) (Fig. 2, Table S2). Post-hoc Dunn's test showed a significant ($p < 0.01$) difference between the control and 0.86 μA/cm^2 and between 0.29 and 0.86 μA/cm^2 ($p < 0.02$), whereas no difference was observed between the control and 0.29 μA/cm^2.

The amount of detected p-PKC was higher after stimulation at 0.86 μA/cm^2 (1.78 ± 0.06) compared to stimulation at 0.29 μA/cm^2 (1.16 ± 0.05) and control (1.05 ± 0.05; Fig. 2, Table S2). Post-hoc Dunn's test showed a significant ($p < 0.01$) difference between the control and 0.86 μA/cm^2, while no significant differences were found between 0 and 0.29 μA/cm^2 and 0.29–0.86 μA/cm^2.

3.2. Gene expression

All reads of three control and three electric stimulated specimens were assembled into reference contigs, accounting for 388,934 contigs, from which 194,712 ORFs were obtained, and 85,353 ORFs out of them were annotated. The MDS analysis showed that expression profiles between control and electrically stimulated replicates were different (Fig. 3).

Comparative gene expression pattern was used to understand the metabolic response to electric stimulus. Deseq2 revealed significantly different gene expressions for 3870 ORFs ($p < 0.05$) between control and electrically stimulated replicates. While significantly down-regulated genes were also identified, they were associated with the cytoskeleton, glycolysis, and general signalling pathways. These were not further investigated due to their indirect involvement in the metabolic processes examined here. We focused on ten most significant upregulated genes and 19 related genes involved in electron and ROS production and quenching, and reconstructed the metabolic pathways stimulated by electron stimulation (Fig. 4, Table S3). Ubiquinol, the electron donor in mitochondria, was inferred to be synthesised through *COQ7* and *COQ3*. The related electron transport chain was upregulated, as shown by high expressions of *SDH3*, *NDUFS7*, and *QCR7,* resulting in ATP synthesis, as shown by significant expression of *ATP6L*. Another electron donor in the electron transport chain, cytochrome *c*, was highly synthesised through the porphyrin metabolism and ROS (H_2O_2) was accordingly produced with upregulation of *PPOX*. The glutamate, the precursor of porphyrin metabolism, was produced through the tricarboxylic acid (TCA) cycle and metabolised into glutathione, which quenches ROS with significant upregulations of *GST* and *GPX*. Our transcriptome analyses unveiled the metabolic mechanisms of electron stimulation.

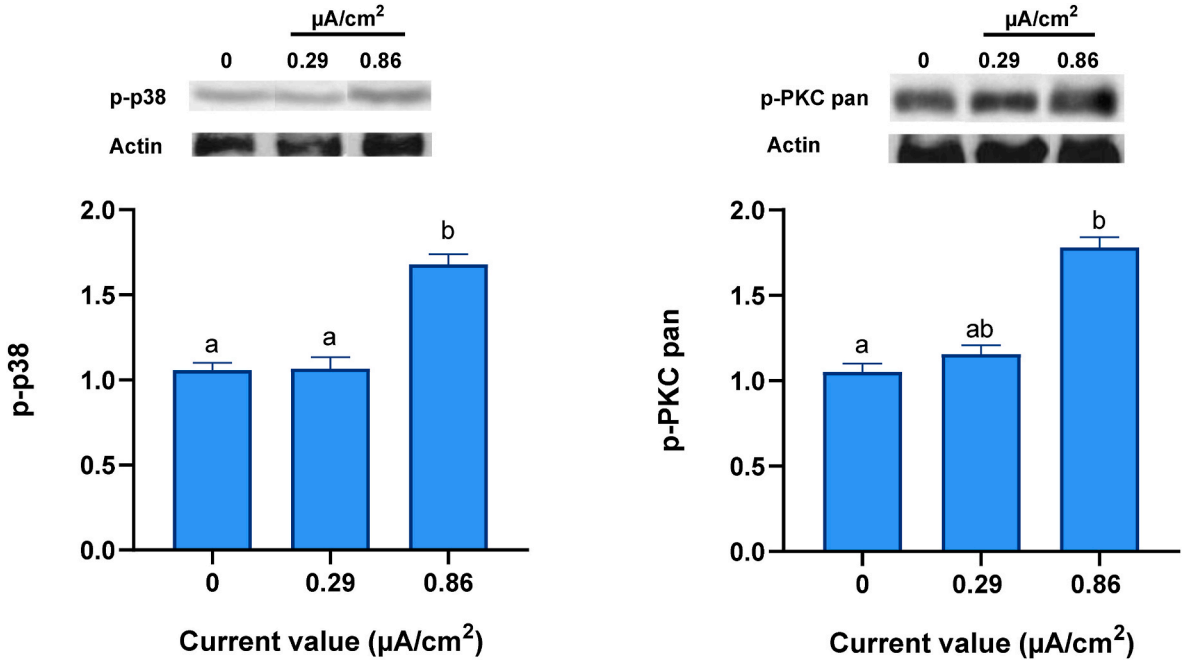

Fig. 2. Amounts of p-p38 and p-PKC in *Amphistegina lessonii* in samples stimulated for 24 h with an electric current density of 0.29 and 0.86 μA/cm^2 and control samples (no electric current). Data are reported as mean ± standard deviation (n = 4). Letters denote significant differences between groups. Representative Western blot analysis of p-p38 and p-PKC is shown; actin was used as the loading control. A stronger band indicates a higher protein expression level, while a weaker band indicates a lower expression.

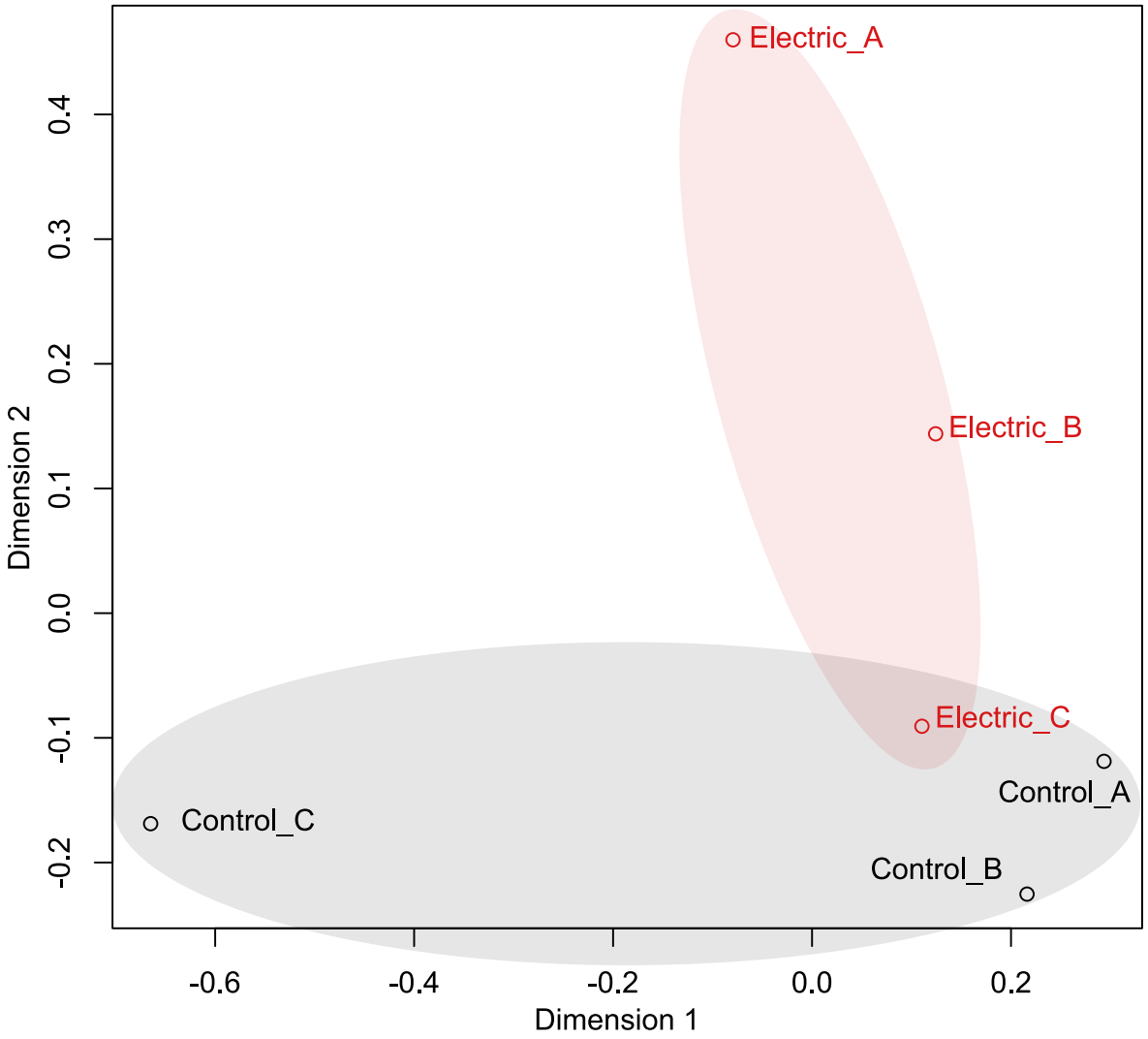

Fig. 3. Multi-dimensional scaling plot of transcriptome data. Red circles represent electrically stimulated specimens (Electric_A, B, C), while black circles denote control specimens (Control_A, B, C). Shaded areas indicate the clustering of samples for each experimental condition. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.3. Enzymatic biomarkers

The GST level exhibited significant ($p < 0.001$) higher values in samples stimulated at 0.86 μA/cm^2 (8.70 ± 0.84 nmol/mg protein) compared to samples stimulated at 0.29 μA/cm^2 (4.38 ± 0.41 nmol/mg protein) and control samples (3.39 ± 0.25 nmol/mg protein; Fig. 5, Table S4). In addition, a significant difference was detected between 0 and 0.29 μA/cm^2 ($p < 0.03$). The GPx had significantly higher ($p < 0.0001$) levels in samples stimulated at 0.86 μA/cm^2 (20.56 ± 1.67 nmol/mg protein) than control (11.20 ± 0.19 nmol/mg protein) and 0.29 μA/cm^2 (12.89 ± 0.76 nmol/mg protein; Fig. 5, Table S4), while no significant difference occurred between 0 and 0.29 μA/cm^2. The Se-GPx level was significantly higher ($p < 0.0001$) in samples stimulated at 0.86 μA/cm^2 (33.66 ± 2.99 nmol/mg protein) than in control (3.71 ± 0.25 nmol/mg protein) and at 0.29 μA/cm^2 (6.89 ± 1.70 nmol/mg protein; Fig. 5, Table S4), while no significant difference occurred between 0 and 0.29 μA/cm^2.

4. Discussion

This proof-of-concept investigates the short-term effects of different electrical stimulations on the larger benthic foraminifera *A. lessonii*, using transcriptomics and multiple biomarkers. This study represents the first direct assessment of the cellular-level impacts of electrical stimulation, focusing on oxidative stress within cells and the cellular mechanisms activated to mitigate potential damage. Two electric current densities based on previous experiments ensured high viability (Rebecchi et al., 2023). Although both current values were relatively low, our results revealed that stimulation at 0.86 μA/cm^2 can induce a cellular stress response, as demonstrated by changes in both protein synthesis and gene expression patterns.

Protein analysis reveals a significant increase in p-p38 levels in *A. lessonii* at a current density of 0.86 μA/cm^2, indicating that this low electric current is sufficient to trigger its stress cascade. Consequently, the observed protein-level response is likely attributable to oxidative stress. The p-p38 belongs to MAPKs, a family of serine/threonine kinases that mediates signal transduction pathways involved in cellular responses to various extracellular stimuli, including stress conditions (Johnson and Lapadat, 2002). This signalling cascade is activated by different upstream kinases and regulates a wide array of cellular processes, including apoptosis, cell cycle progression, differentiation, and gene expression (Zarubin and Han, 2005). This cascade is highly sensitive to even minor fluctuations in the cellular environment, ensuring precise responses to external cues (González-Rubio et al., 2019). Several studies have reported that MAPK pathway is activated by several stress stimuli, including different environmental pollutants (Châtel et al.,

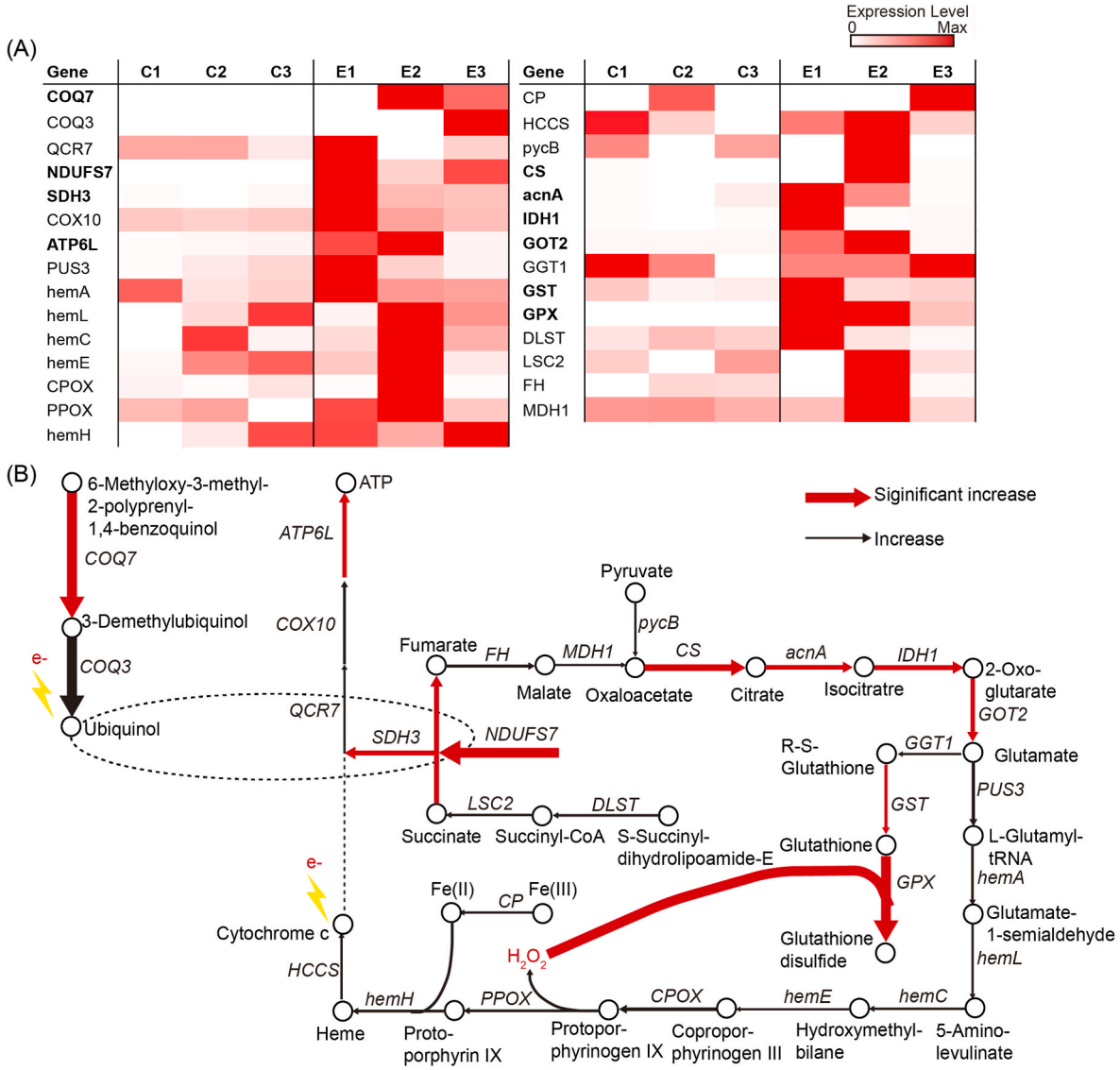

Fig. 4. Molecular response of *Amphistegina lessonii* stimulated for 24 h with electric current density. (A) Heatmap showing differential expression of selected genes in foraminifers electrically stimulated or control conditions. (B) Schematic metabolic changes occurring in foraminiferal cells in response to the electric stimulation. Red and black arrows show significant upregulation and upregulation, respectively. Dashed lines show the related metabolism with electron donors highlighted with yellow lightning. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

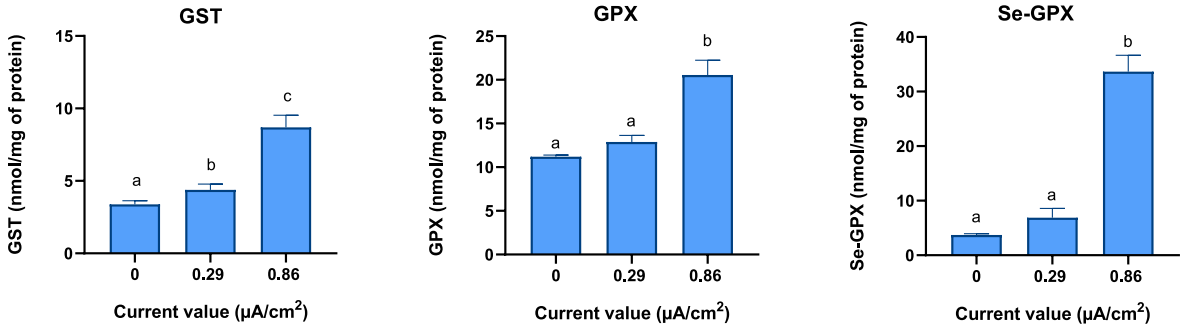

Fig. 5. Total levels of glutathione S-transferase (GST), glutathione peroxidase (GPx), and selenium-glutathione peroxidase (Se-GPx) in *Amphistegina lessonii* specimens stimulated for 24 h with an electric current density of 0.29 and 0.86 μA/cm^2 and control samples (no electric current). Data are reported as mean ± standard deviation (n = 5). Letters denote significant differences between groups.

2010; Park et al., 2020) and metal exposure (Hao et al., 2009) in diverse organisms. Similarly to our data, a significant increase in p-p38 was observed in *A. lessonii* exposed to $HgCl_2$ (Ciacci et al., 2022).

Along with protein p-p38, an increase in p-PKC levels in *A. lessonii*

was found at 0.86 μA/cm^2. The PKC represents a family of enzymes within the serine/threonine kinase group, known for their roles in cellular signalling pathways, including responses to oxidative stress (Newton, 2018). These lipid-sensitive enzymes are involved in numerous cellular processes, including responses to extracellular signals mediated by G protein-coupled receptors, cell differentiation, proliferation, survival, and apoptosis (Newton, 2018). PKC activation in responses to ROS varies according to isoform, subcellular localisation, and the intensity of oxidative stress (Cosentino-Gomes et al., 2012). These mechanisms highlight the significance of PKC in mediating cellular adaptation or damage under oxidative conditions. Similar PKC-mediated stress responses have been extensively described in marine mussel *Mytilus galloprovincialis*, where PKC activation contributes to immune and oxidative stress signalling to bacterial challenges (Canesi et al., 2006), heat-killed *Vibrio* species in mussel haemocytes (Ciacci et al., 2010) and in response to tetrabromobisphenol A (Canesi et al., 2005). These studies align with our results, in which external factors, such as electric current, also lead to PKC activation, reinforcing its significance in stress response mechanisms. The activation of both p-p38 and p-PKC thus suggests that electrical stimulation induces oxidative stress in *A. lessonii*, initiating early signalling pathways that trigger downstream antioxidant defence.

Transcriptomic analysis further provides insight into the source of this stress, showing that electrical stimulation alters the metabolic and oxidative balance. Specifically, the porphyrin metabolism pathway has been activated, leading to upregulation of all related genes and a significant increase in *protoporphyrinogen III oxidase (PPOX)* expression. The increased activity of *PPOX* is suggested to contribute to H_2O_2 production, potentially linking porphyrin metabolism to oxidative stress. Hydrogen peroxide (H_2O_2), a primary ROS, is involved in redox signalling within mitochondria at physiological levels. However, its production increases significantly during oxidative stress, causing mitochondrial membrane depolarisation and genotoxic effects, highlighting the critical need for its rapid neutralisation to maintain cellular homeostasis (Sies, 2017). High expression of *PPOX,* which stimulated ROS production in mitochondria, was also reported in *A. veneta* during TiO_2 NP exposure, suggesting that this pathway may be a primary source of ROS in response to stressful stimuli (Ishitani et al., 2023). Moreover, transcriptome data also reveal the upregulation of genes involved in cytochrome *c* and ubiquinol synthesis. Cytochrome *c* is the electron donor of the electron transport chain (ETC) in mitochondria and, under stress conditions, can activate the apoptotic pathway (Ow et al., 2008). Similarly, ubiquinol, another electron donor of the ETC, is also highly synthesised via *polyprenyl dihydroxybenzoate methyltransferase (COQ3)*. Ubiquinol plays a vital role in proton pumping into the intermembrane space, generating the proton gradient essential for ATP synthesis. These results suggest that electrical stimulation may influence mitochondrial energy production efficiency, a crucial process for cells with high energy demands (Nolfi-Donegan et al., 2020). It can be hypothesised that these electron donors are possibly charged by the electric stimulus, potentially enhancing electron transfer, which in turn could enhance ATP synthesis and ROS production. However, direct measurements of mitochondrial membrane potential or ATP levels would be required to confirm this mechanism.

To counterbalance ROS production, *A. lessonii* activated antioxidant enzymes. Protein data show higher GST, GPx, and Se-GPx levels in foraminiferal specimens exposed to electrical stimulation at 0.86 μA/cm^2, demonstrating the activation of antioxidant response. Glutathione S-transferases (GST) are crucial enzymes that neutralise ROS by catalysing the conjugation of reduced glutathione (GSH) to xenobiotic compounds (Kumar and Trivedi, 2018). These enzymes are ubiquitous in all life forms, including animals, plants, protozoa, fungi, and bacteria (Sherratt and Hayes, 2001). GST expression is commonly upregulated in marine invertebrates, where its activity serves as a potential biomarker for environmental stressors (Won et al., 2011). High GST levels have been documented in rotifers exposed to heavy metals (Lee et al., 2019), in copepods after exposure to several different compounds (see reference in Lauritano et al., 2021), as well as in polychaete exposed to copper (Won et al., 2012).

GPx enzymes are also essential for reducing H_2O_2 into water and oxygen, contributing to neutralising ROS within the cytosol and plasma membrane (Brigelius-Flohé and Maiorino, 2013). GPx enzymes have been identified across all domains of life, highlighting their evolutionary and functional significance (Margis et al., 2008). This activity is commonly selenium-dependent in metazoans, mediated by Se-GPx enzymes (Flohé et al., 2022). The first direct investigation of GSH-related enzymes, including GST, GSR, GPx, and Se-GPx, in foraminiferal species demonstrated that *A. lessonii* exhibited a significant increase in GST and GPx activity when exposed to Hg treatment, a response consistent with the results of the present study (Ciacci et al., 2022). The increased GPx activity in response to electric stimulation aligns with transcriptomic upregulation of the *glutathione peroxidase (GPX)*. In *A. lessonii*, this gene possesses the *GSHPx* motif (PF00255 in the Pfam database) and its expression increases under oxidative stress. This finding confirms the consistency between protein and gene expression data. Several studies have reported that exposure to environmental stressors, such as the combined effects of temperature and pH (González Durán et al., 2018), heavy metals (Kim et al., 2011), and pollutants (Alves de Almeida et al., 2007; Lee et al., 2017), upregulates the expression of various GPX in diverse organisms. Moreover, elevated *GPX* expression has been observed in the foraminiferal species *A. veneta* during 24 h of exposure to titanium nanoparticles (Ishitani et al., 2023). These findings emphasise the adaptive antioxidant responses in foraminifers, which may enhance their resilience to oxidative challenges induced by stressors. Furthermore, genes associated with the tricarboxylic acid (TCA) cycle (such as *CS*, *acnA*, and *IDH1*) and glutamate synthesis were also upregulated. Glutamate, produced via the TCA cycle, is used for glutathione synthesis. Glutathione, the precursor for both *GST* and *GPX*, is synthesised from glutamate via high expression of *gamma-glutamyl transpeptidase (GGT1)*. *GGT1* plays a critical role in regulating glutathione metabolism, essential for reducing hydrogen peroxide (H_2O_2) and maintaining redox homeostasis, as its upregulation enhances cellular resistance to oxidative stress (Mitrić and Castellano, 2023). Similarly, an enhanced TCA cycle, leading to improved ATP production, has been observed in scallops as a mechanism to counteract heat stress (Dong et al., 2022), as well as in rockfish and crucian carp in response to high temperatures (Jiang et al., 2019; Song et al., 2019). Our results suggest that electrical stimulation simultaneously activates both enzymatic detoxification (GST, GPx) and metabolic pathways (TCA, GSH synthesis) to maintain redox homeostasis.

Based on the biomarker and transcriptome profiles, we hypothesise that electrons from the environment potentially stimulate ubiquinol and cytochrome *c*, resulting in high ATP production. Also, this overload is suggested to lead to ROS production as a result of mitochondrial respiration. To compensate for exhausted electron donors such as ubiquinol and cytochrome *c*, the cell is inferred to upregulate its biosynthetic pathways, and H_2O_2 is likely produced via porphyrin metabolism. Simultaneously, the cell activates antioxidant defences, including glutathione-dependent pathways, to mitigate oxidative damage. This model highlights the sensitivity of *A. lessonii* to low electrical stimulation and their ability to respond to electrical stress through coordinated metabolic and antioxidant responses.

When compared with environmental measurements, electric fields documented near subsea power cables generally range from a few μV/m to low mV/m, with variations depending on cable configuration, burial depth, and vicinity to the source (Gill et al., 2014; Taormina et al., 2018). In this context, it is crucial to distinguish between direct and indirect emissions: while primary electric fields are commonly contained by cable shielding, organisms are primarily affected by induced electric fields (iEF). These iEFs arise from the interaction of tidal currents or the movement of the organisms themselves with the cable's magnetic field (Gill et al., 2014). The field strengths applied (0.58–1.72

mV/m) reflect the intensities reported in the marine environment. For instance, modelling for AC cables buried at 1 m depth indicates an electric field of approximately 0.76 mV/m at the seabed surface, while DC configurations typically induce electric fields around 0.19 mV/m under standard conditions (Normandeau Associates Inc et al., 2011). However, an important limitation of the present work is that our laboratory setup does not reproduce the spatial distribution, persistence, or electromagnetic complexity of *in situ* subsea infrastructure. This study utilised a confined pulsed stimulus to study the cellular responses under controlled conditions rather than a direct simulation of environmental exposure. While these results show that low-intensity electrical stimulation triggers oxidative and metabolic pathways in *A. lessonii*, ecological interpretation requires caution. Additionally, the present study focuses on short-term exposure and does not determine potential long-term adaptation or cumulative physiological effects. Future research should integrate spatial and long-term exposure studies to better estimate real marine conditions and the chronic effects of such stressors on benthic communities, including investigations across a broader range of marine invertebrates.

5. Conclusions

This study provides the first insights into the physiological responses of the larger benthic foraminifera *A. lessonii* to electrical stimulation, employing a multidisciplinary approach that integrates biomarkers and single-cell transcriptomic analysis. As a proof-of-concept investigation, our findings suggest that electric stimulation induces early stress signalling (p-p38 MAPK and p-PKC), which activates antioxidant enzymes (GST, GPx, and Se-GPx) involved in defence mechanisms against ROS. Transcriptomic analysis reveals the upregulation of redox-related genes such as *GPX, GGT1*, and metabolic genes linked to mitochondrial function, including *COQ3, PPOX, and ATP6*, as well as pathways of the TCA cycle, providing new perspectives on the metabolic mechanisms potentially associated with electrical stimulation. The data suggest that the enhanced mitochondrial activity may support ATP synthesis, but could also increase ROS production, which can compromise cellular integrity. In response, antioxidant defence systems are activated to maintain cellular homeostasis under stress. Overall, these results indicate that electric currents have a significant influence on mitochondrial metabolism, potentially affecting electron transport and oxidative stress regulation in benthic foraminifera. This study further contributes to our understanding of the biological processes likely induced by electric current and the resilience of benthic foraminifera exposed to an artificial electric field. Ultimately, the integration of cellular biomarkers with transcriptomic data offers a promising tool for the evaluation of stress and environmental impacts in marine ecosystems, reinforcing the potential of benthic foraminifera as sensitive bioindicators.

CRediT authorship contribution statement

Federica Rebecchi: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **Yoshiyuki Ishitani:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **Caterina Ciacci:** Writing – review & editing, Methodology, Investigation. **Michele Betti:** Writing – review & editing, Methodology, Investigation. **Davide Lattanzi:** Writing – review & editing, Methodology. **Sigal Abramovich:** Writing – review & editing, Resources. **Yurika Ujiié:** Writing – review & editing, Formal analysis. **Fabrizio Frontalini:** Writing – review & editing, Supervision, Conceptualization.

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Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2026.107958.

Data availability

The data are available in the Supplementary Materials.

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