



Ocean warming and acidification reveal transcriptional plasticity in the gastropod *Hexaplex trunculus*

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ABSTRACT

Coastal ecosystems, particularly in the rapidly warming Mediterranean Sea, are increasingly impacted by climate change driven by ocean warming and acidification. Calcifying marine invertebrates are especially vulnerable, yet their molecular responses remain poorly understood. Gene expression dynamics in the ecologically and commercially important gastropod *Hexaplex trunculus* were investigated under simulated scenarios combining elevated temperature and reduced pH. High-throughput RNA sequencing was used to generate a *de novo* assembly of the foot transcriptome, followed by differential gene expression analyses to characterize molecular responses to individual and interactive stressors under high-emission (RCP 8.5) projections. The foot tissue - central to locomotion, adhesion, and environmental interaction - exhibited pronounced transcriptional changes in genes associated with calcium signaling, ion transport, aerobic respiration, muscle contraction, and trans-membrane transport. Ocean acidification emerged as the dominant driver of transcriptional change, inducing substantial shifts in metabolic demand and ion regulatory pathways, which might suggest increased energetic costs and potential functional constraints. The results demonstrate marked transcriptional plasticity in *H. trunculus* under combined climate stressors. By providing foundational transcriptomic resources for this species and mechanistic insight into its response to projected environmental changes, this study advances the understanding of molecular processes in vulnerable Mediterranean gastropods and informs ecological assessment efforts in rapidly warming marine ecosystems.

1. Introduction

Coastal ecosystems are undergoing rapid, multifactorial changes, primarily due to the steadily rising temperatures and ocean acidification (OA), both known to be triggered by climate change (Henson et al., 2017). The ocean has absorbed the majority of the Earth's warming over the past 50 years, with more than 90% of the heat gain occurring within its waters, leading to significant impacts on marine species' physiology, abundance, distribution and trophic interactions and also affecting marine resources and human societies (Venegas et al., 2023). The Mediterranean Sea is one of the most susceptible regions to climate change, as its temperature is rising two to three times faster in comparison to other ocean regions due to its bioclimatic peculiarities and semi-enclosed nature (Vargas-Yáñez et al., 2008; Schultz et al., 2024). Furthermore, anthropogenic pressures have exponentially increased the atmospheric concentration of carbon dioxide by 50% since the

pre-industrial period (Gingerich, 2019), with approximately 20-30% of those emissions being absorbed by the ocean, leading to a decrease of the ocean pH (Friedlingstein et al., 2022). In addition to OA, the saturation states of calcium carbonate minerals in seawater (e.g. aragonite and calcite) are reduced and this may have direct and indirect consequences especially for calcifying organisms such as marine gastropods (Zeebe and Wolf-Gladrow, 2001; Doney et al., 2020; Chatzinikolaou et al., 2017, 2021a). There are evident shifts in species distribution to higher latitudes and to deeper locations, advances in spring phenology, declines in calcification, and increases in the abundance of warm-water species as a response to climate change (Poloczanska et al., 2016).

The impact of climate change on the distribution of marine invertebrates is less emphasized in conservation assessments in comparison to fish and other vertebrates, although calcifying species such as bivalves and gastropods are extremely vulnerable to acidification (Doney et al., 2020). Molluscs are the most represented group in

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international agreements on the conservation of the Mediterranean Sea (e.g. CITES, Bern and Barcelona conventions, EU Habitats' Directive) (Schultz et al., 2024), therefore it is very important to increase scientific knowledge on the impact of climate change on these organisms. In the Mediterranean Sea, molluscs are facing reduction in distribution range, population fragmentation and potential local extinctions under mild and high emission scenarios (Gallagher and Albano, 2023). Furthermore, commercial molluscs are already overexploited and their extinction from the Mediterranean will imply substantial financial losses and a profound cultural change in coastal communities (Gallagher and Albano, 2023).

The gastropod *Hexaplex trunculus* (Linnaeus, 1758) (banded dye-murex) is a widely distributed sublittoral species (Lahbib et al., 2010), which is well-adjusted to varying physical environmental conditions characterising transitional coastal systems (e.g. rock pools, lagoons) where temperature and pH fluctuations occur naturally (Wahl et al., 2016). *H. trunculus* has been already widely used as an ecological indicator for the pollution of the marine environment by organotin compounds (e.g. Tributyltin, TBT) (Abidli et al., 2012; Anastasiou et al., 2016). It is also an edible species with important commercial value in several countries (e.g. Italy, Tunisia, Portugal and Spain) (Vasconcelos et al., 2006; Abidli et al., 2009; Lahbib et al., 2012). *H. trunculus* reproduces by egg capsules through direct development without an extended planktonic life-stage, therefore its dispersion zone and its ability to colonise new areas is limited (Lahbib et al., 2012). The impacts of ocean warming and acidification on the shell morphology and structure, as well as on the feeding behaviour of *H. trunculus* have been previously studied. Low pH reduced the density and increased the porosity of *H. trunculus* shells (Chatzinikolaou et al., 2021b), while it also increased the time and effort individuals spent in foraging (Chatzinikolaou et al., 2019). Transcriptome analysis under projected climatic scenarios is expected to shed light on the ways an organism adjusts its gene activity in response to stress, revealing cellular pathways that respond to environmental stress with implications for management approaches (Ahi et al., 2025). This contribution is particularly relevant for *H. trunculus*, given its pivotal ecological role as a predator, its established utility as a pollution bioindicator and its economic importance as a harvested species.

Advancements in high-throughput sequencing have rendered it possible to obtain comprehensive, genome-scale insights into organismal responses to environmental perturbations. In the current research, an experimental design that combines low pH and increased temperature treatments based on the RCP8.5 scenario expected by the end of the century has been implemented, in order to provide a robust framework for identifying interactive stress effects and potential synergistic responses. The aim of the present study was to gain insights into the transcriptional plasticity of *H. trunculus* through the generation and characterisation of a baseline transcriptome, followed by the identification of differentially expressed genes mapped to Gene Ontology (GO) terms responsive to acidification, warming, and their synergistic effects. In addition, the present study addresses the existing gap in the availability of raw RNA-seq data for *H. trunculus* focusing on comprehensive transcriptomes under low pH and increased temperature stressors.

2. Materials and methods

2.1. Experimental setup and treatments

Individuals of the gastropod *H. trunculus* were collected from shallow (<5m) soft bottom habitats in Elounda, Crete, Greece (N 35°16'2.4", E 25°43'27.2"). A written permission was obtained from the relevant authorities (Ministry of the Environment and Energy, protocol number 179388/390, 5/3/2019). The organisms were maintained under laboratory conditions with pH decreasing at a rate of 0.05 units every 10 days (total duration 50 days) and temperature increasing at a rate of 0.6 °C every 10 days (total duration 40 days), until reaching the desired

experimental conditions (−0.3 pH units and +3 °C respectively). *H. trunculus* were maintained under optimum conditions following standard aquarist practices and fed *ad libitum* three times per week with freshly opened mussels. The gastropods were equally separated into four different experimental treatments: a) Control (C) (normal pH = 8.1 and ambient temperature, b) Acid (A) (low pH = 7.8 and ambient temperature, c) Warm (W) (normal pH = 8.1 and increased temperature +3 °C) and d) Acid and Warm (AW) (low pH = 7.8 and increased temperature +3 °C) (Fig. 1). The experimental treatments represent the RCP8.5 “high GHG emission” scenario as this is described in the Climate Change 2023: Synthesis Report (Calvin et al., 2023), according to which a reduction of pH by 0.3 units and an increase of temperature by 3 °C is expected by the end of the century. Temperature during the experimental period was following the ambient seasonal fluctuations as they were monitored by the PODEIDON System and confirmed daily on-site using a 3420 WTW multimeter during the 14-month experiment.

A semi-closed experimental system was used to ensure controlled experimental conditions (see Suppl. File 1). An advanced electronic platform GHL Profilux 4 has been installed for the continuous monitoring and controlling of temperature and pH (on the National Bureau of Standards scale – pH NBS) in the experimental tanks through submerged electrodes. A heating-cooling system (TECO TK-500) equipped with water pumps (Hydor Centrifugal Universal Pump 1200 L/h) was installed in each tank to adjust the desired temperature levels. The low pH treatments were adjusted using a bubbling CO₂ system attached to CO₂ bottles (40L) through a manometer (Mufan) which was also connected to the GHL controller. The pH electrodes were calibrated twice every month with 5.00, 7.00, and 10.00 WTW NBS buffers. A wave maker pump (Sicce Voyager NANO), an air lift system and additional air stones have been used to ensure a sufficient level of water circulation. The deviation of the set temperature values was ± 0.3 °C and ± 0.01 for the pH (controller's capacity).

Salinity (40 ppt), oxygen (7 mg/L), photoperiod (12 h L/12h D) and light intensity (50 lux) were regularly monitored to ensure that they were maintained stable. Nitrogenous waste products were also assessed twice a week by using a Hanna HI83303 Multiparameter photometer and a high-water quality was ensured within the system (NO₂ < 0.1 mg/

	 low pH (7.8)	 normal pH (8.1)
ambient	A1 A2 A3 A4  49.6 - 52.2 mm	C1 C2 C3 C4 C5 47.0 - 50.0 mm
warm (+3 °C)	AW1 AW2 AW3 AW4  48.0 - 56.7 mm	W1 W2 W3 W4 46.8 - 53.6 mm

Fig. 1. Experimental treatments. Control (C): normal pH = 8.1 and ambient temperature, Acid (A): low pH = 7.8 and ambient temperature, Warm (W): normal pH = 8.1 and increased temperature +3 °C, Acid and warm (AW): low pH = 7.8 and increased temperature +3 °C. Numbers 1-5 next to capitals (C, A, W, AW) indicate replicate numbers. Shell length ranges are also indicated in blue for each treatment. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

l, $\text{NO}_3^- < 0.5 \text{ mg/l}$, $\text{NH}_3/\text{NH}_4^+ < 0.2 \text{ mg/l}$). Total alkalinity (TA) was measured every two weeks according to the Standard Operating Procedure (SOP 3b) described in [Dickson et al. \(2007\)](#) using an open-cell titration (Metrohm Dosimat 765; 0.1 mol/kg in HCl). Measured parameters (pH and TA) were used to calculate the additional parameters of the seawater carbonate system using CO2SYS program with the dissociation constants of [Mehrbach et al. \(1973\)](#) as refitted by [Dickson and Millero \(1987\)](#).

A group of 4–5 individuals of similar size per treatment ([Fig. 1](#)) were randomly sampled for the transcriptome analysis after the gastropods were maintained under the experimental conditions for a total period of 14 months. Specimens were anesthetized using a rising concentration of MgCl_2 starting at 1.5% and gradually reaching 3.5% according to the European Directive 2010/63 EU on the protection of animals used for scientific purposes. The shell was mechanically removed, the mantle was retracted and the muscular foot was isolated using fine forceps and scalpels. Tissue samples were collected from the gastropod foot and stored in RNA later (Qiagen, Duerren, Germany) at -80°C until transcriptome analysis. All the research complies with applicable laws on sampling from natural populations and animal experimentation (including the ARRIVE guidelines).

2.2. Sampling, RNA extraction, and sequencing

Foot tissue was subjected to RNA extraction with a method combining cetyltrimethylammonium bromide (CTAB) ([Doyle and Doyle, 1987](#)) and NucleoZOL (Macherey-Nagel GmbH & Co. KG, Duren, Germany) following the protocol of [Jordon-Thaden et al. \(2015\)](#). RNA quantity was assessed with Nano-Drop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE) and quality was evaluated on 1% agarose gel as well as on the DNAnalyzer RNAchip (Agilent 2100 Bioanalyzer, Agilent Technologies, Santa Clara, California, USA). Samples were sent to Novogene (Novogene UK Company LTD, Kristien Bateman Unit 25 Science Park Milton Road) for library preparation and Illumina paired-end sequencing. In brief, after quality control, mRNA was enriched via oligo(dT) beads (eukaryotes) and rRNA was removed using Ribo-Zero rRNA kit. After mRNA fragmentation, RNA was reverse-transcribed to cDNA using random hexamers, and second-strand synthesized with RNase H, DNA Pol I, dNTPs, and Illumina buffer. The ds-cDNA library was further completed via end repair, A-tailing, adaptor ligation, size selection, and PCR enrichment. All raw sequencing reads have been submitted to the publicly accessible BioProject database NCBI under the accession number PRJNA1378232.

2.3. Sequencing read analysis and transcript abundances

Sequence reads were submitted to quality control running FASTQC (version 0.11.9). Pre-processing of reads was carried out to remove adapter contamination and low-quality reads applying Trimmomatic v0.33 (Trimmomatic, RRID:SCR 011848) software ([Bolger et al., 2014](#)). Processed data from RNA samples were assembled using Trinity v2.12.0 ([Grabherr et al., 2011](#)) while the relative abundance of each transcript/isoform obtained by aligning all samples to the predicted transcriptome was achieved using RSEM ver. 1.2.17 ([Li and Dewey, 2011](#)). BUSCO v5.8.0 ([Manni et al., 2021](#)) analysis was performed within the European Galaxy platform (UseGalaxy.eu) for assessing completeness of the *de novo* assembled transcriptome ([Afgan et al., 2018](#)).

2.4. Transcriptome functional annotation

The functional annotation of assembled transcripts was performed using searches for nucleotide similarity against the non-redundant nucleotide database of the National Center for Biotechnology Information (NCBI) ([Sayers et al., 2025](#)) with the BLASTN program (NCBI BLAST + suite) ([Camacho et al., 2009](#)). The attribution of putative gene identities and functional descriptions was conducted on the basis of the

top-scoring hit, along with a cut-off E value of 10^{-5} , as well as the highest percentage identity, and the most extensive alignment coverage.

2.5. Differential expression analysis and Gene Ontology (GO) enrichment

The obtained expression matrix was used to assess differential expression levels for all possible combinations. Differentially expressed transcripts and their statistical significance were identified using DESeq2 in Rstudio (version 1.4.17) designed to be executed with SAR-Tools 1.7.4 ([Varet et al., 2016](#)). Gene-level differential expression analysis was conducted by modelling the raw read counts for each gene as a function of the experimental group. This design tests whether belonging to one group *versus* another is associated with a change in expression for each gene. Within this framework, DESeq2 fits a generalized linear model with a negative binomial response for each feature, and the resulting coefficients are interpreted as log2 fold changes between groups, which are subsequently subjected to statistical testing to obtain P values and multiple-testing-adjusted P values. Transcripts were considered to be differentially expressed according to pre-specified thresholds for adjusted P value of less than 0.05. A transcript was considered to be significantly differentially expressed if $p_{\text{adj.}} < 0.05$ and $\log_2\text{fold change} > |5|$. Principal Component Analysis (PCA) on Variance Stabilizing Transformation (VST) was performed on transformed counts for differentially expressed genes (DEGs) with 95% confidence ellipses. A correlation heatmap for pairwise similarity of normalized gene expression profiles across all DEGs was generated using the R heatmap package (version 4.5.2) ([Kolde, 2025](#)).

Functional enrichment analysis of DEGs was performed using g:Profiler (g:GOST) ([Kolberg et al., 2023](#)) in order to identify over-represented GO terms. Gene lists were queried against the GO category Biological Process (BP), using the default cumulative hypergeometric testing framework implemented by the tool. Multiple testing correction was carried out using the built-in g:Profiler adjustment procedure, and significantly enriched GO terms were defined based on an adjusted p-value cut-off ($p < 0.05$), yielding functionally coherent sets of genes associated with the experimental conditions. Driver GO terms were identified through a two-step filtering algorithm applied to enrichment results, offering a more efficient and robust alternative to conventional clustering approaches ([Chaar et al., 2025](#)). Initially significantly enriched terms are organized into sub-ontologies according to their semantic relationships, and then the key gene sets that drive the enrichment of other significant functions within each group are determined. This approach employs a greedy search strategy with iterative recalculation of hypergeometric p-values, allowing multiple leading terms to be retained within a component rather than selecting only the most statistically significant term ([Chaar et al., 2025](#)). Upset plots were generated applying the R package upSetR ([Conway et al., 2017](#)) to identify transcripts exclusively expressed in each treatment or commonly expressed between treatments.

3. Results

3.1. RNA extraction and sequencing

Total RNA extraction yielded satisfactory quality, as assessed by 1% agarose gel electrophoresis and RNA Pico chip analysis (Agilent RNA 6000 Pico Kit) using an Agilent 2100 Bionalyzer. Standard integrity metrics (e.g., RIN values) could not be obtained due to a hidden break in molluscan 28S rRNA, which causes the 28S subunit to co-migrate with the 18S subunit, resulting in a single peak ([Natsidis et al., 2019](#)). This affects RNA Integrity Number (RIN) estimation which relies on detecting distinct 18S and 28S peaks. All obtained sequence reads passed the quality control and on average 92% passed the Q30 threshold. The average guanine-cytosine (GC) content amounted to 44% and the Q30 average value was 93% (Suppl. File 2).

3.2. Transcriptome assembly

Transcriptome assembly resulted in 765,481 transcripts. BUSCO analysis against the eukaryota_odb10 lineage dataset, which contains 255 universal single-copy orthologs derived from 70 eukaryotic genomes (dataset updated on 2024-01-08), identified 192 out of 255 BUSCO groups to be complete (Completeness, C: 75.3%), thus most of the essential eukaryotic genes were found to be present in the assembled transcriptome. A small portion of fragmented genes (F: 16.1% corresponding to 41 genes) were detected, which pinpoints to regions of lower assembly or annotation quality. A total of 22 genes were missing (missing, M: 8.6%) indicating a good completeness and expectation for high-quality transcriptome assemblies (Seppey et al., 2019; Simão et al., 2015).

3.3. Differential expression analysis

Differential expression analysis revealed a comparable number of transcripts which were either up-regulated or down-regulated in response to the studied experimental conditions (see Suppl. File 3). A sample-to-sample correlation heatmap was generated with heatmap using hierarchical clustering (Fig. 2A). Biological replicates within the same experimental treatment form tight, distinct clusters along the main diagonal, reflecting high reproducibility and strong intra-group consistency (diagonal correlations ≈ 1.0). Lower similarity observed between blocks of different experimental groups (light blue to white off-diagonal cells), demonstrates clear transcriptomic differences between them. The dendrogram was generated by hierarchical clustering based on the correlation matrix, revealing groups of similar expression profiles and suggesting biological variability rather than technical artifacts (Sun et al., 2025). No sample appears as a clear outlier. More specifically, higher similarity appeared in the comparison between Control and Warm (W) treatments, while lower similarity was shown in the comparisons between Acid (A) and all the other treatments.

The application of selective Variance Stabilizing Transformation (VST) DEG-based Principal Component Analysis (PCA) (Fig. 2B)

revealed acidification-driven separation for DEGs between treatments, which was highly significant (PERMANOVA: $F = 1214.1$, $R^2 = 0.996$, $p < 0.001$). Non-overlapping 95% confidence ellipses confirm strong multivariate separation. The Acid (A) group showed the greatest dispersion along PC1, which accounted for 12% of the total variance. The Control (C) and Warm (W) groups clustered closely in the upper-left corner of the plot, while the Acid-Warm (AW) group was positioned separately in the lower-left region along PC2, which explained 11% of the variance.

Individuals exposed to reduced pH at ambient temperature (A) had 160 up-regulated and 205 down-regulated genes in respect to control treatment, while individuals exposed to elevated temperature at normal pH (W) had 142 up-regulated and 211 down-regulated genes. Exposure to the combined stressors of low pH and high temperature (Acid-Warm) resulted in 144 up-regulated and 189 down-regulated genes (Fig. 3A). Hence, both single and combined stressors activated transcriptional responses, with the reduced pH (A) having a slightly higher number of up-regulated transcripts than the other two treatments.

3.4. Gene Ontology (GO) enrichment

Heatmap analysis identified five major gene clusters (c1–c5), each displaying distinct expression patterns that separated the three treatments (Fig. 3B). GO annotation followed by functional enrichment analysis showed associations between specific clusters and the five major biological processes (BP), namely muscle system process, biological regulation and response, cellular component organization and biogenesis, transport, and metabolic process (Fig. 3C). Flow thickness is proportional to the number of transcripts mapped between each BP group and cluster. More specifically, muscle system process is mainly expressed in cluster 1 which is up-regulated for the Acid (A) treatment and down-regulated for Acid-Warm (AW). The BPs biological regulation and response, as well as cellular component organization and biogenesis, are mainly expressed in cluster 2 which is down-regulated for the Warm (W) treatment. The majority of transcripts reflect metabolic processes mainly observed in clusters 1 and 2 (although present in all

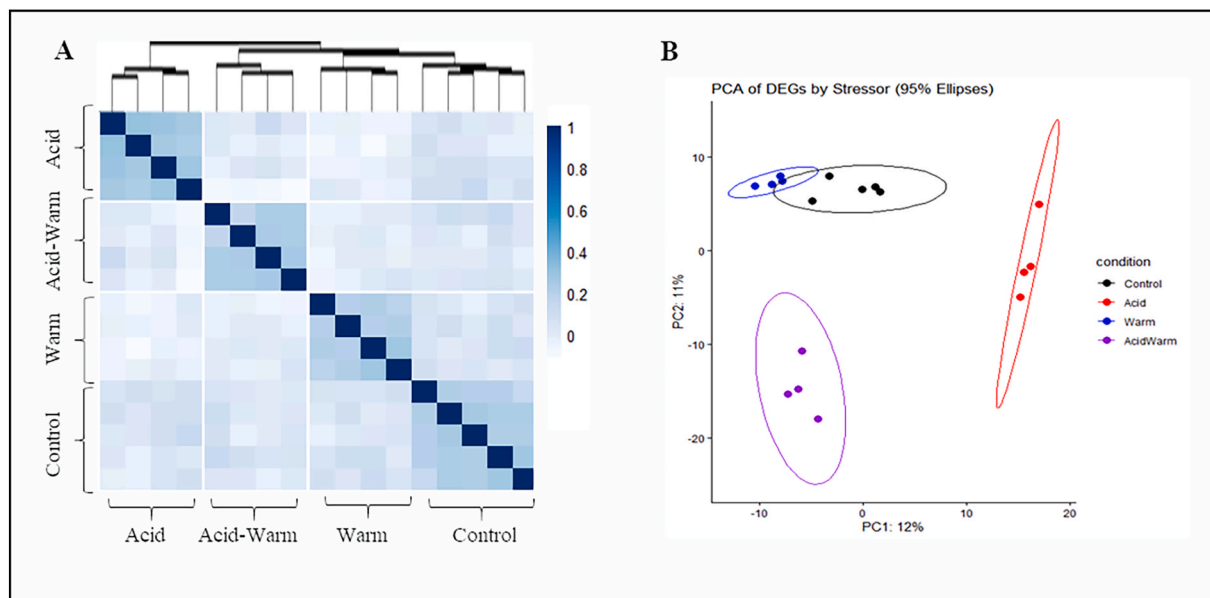


Fig. 2. A) The heatmap displays pairwise similarity (correlation coefficients) of normalized gene expression profiles across all differentially expressed genes (DEGs). Rows and columns correspond to samples grouped for treatments (brackets). Each square represents the similarity between a pair of samples, with darker blue colours indicating higher similarity (correlation coefficient closer to 1) and lighter colours indicating lower similarity. Dendrogram generated by hierarchical clustering based on the correlation matrix enables visualization of sample-to-sample relationships and clustering consistency within treatments. B) Principal Component Analysis (PCA) of Variance Stabilizing Transformation (VST)-transformed counts for DEGs summarizing the main axes of variation across samples with 95% confidence ellipses indicating grouping by treatment. Treatments are Acid (A): red, Control (C): black, Warm (W): blue and Acid-Warm (AW): purple. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

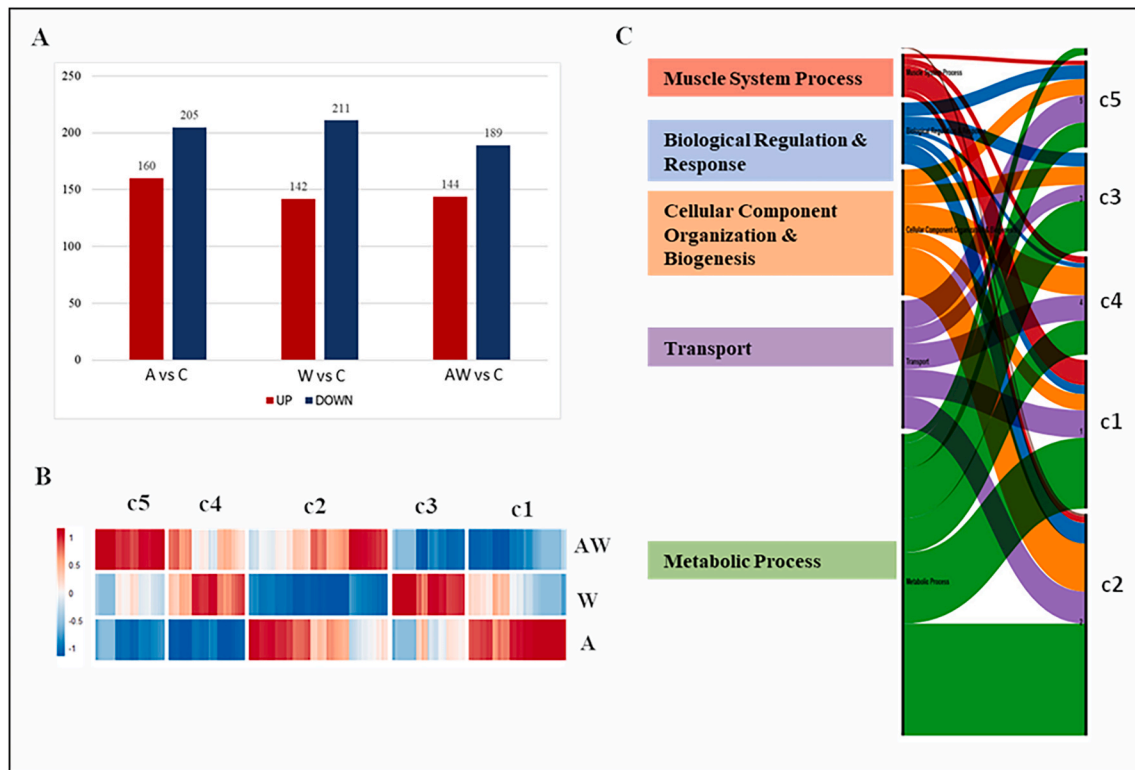


Fig. 3. A) Bar chart displaying the total number of differentially expressed genes (DEGs) identified under the comparisons of the experimental treatments Acid (A), Warm (W), and Acid-Warm (AW) against the Control (C) samples. Red bars (“Up”) indicate the number of up-regulated genes when comparing to the control, while blue bars (“Down”) represent down-regulated genes when comparing to the control. Values above each bar denote the exact count of DEGs observed per category. B) Heatmap illustrating all DEGs for each treatment (A, W, AW) divided into 5 major gene clusters (c1-c5). Red colours indicate up-regulated genes when comparing to the control, while blue colours down-regulated genes. C) Alluvial plot displaying the relationships between Gene Ontology categories of five major biological processes (BP) and the expression clusters c1-c5. Flow thickness is proportional to the number of transcripts mapped between process group and cluster. BPs are muscle system process (red), biological regulation and response (blue), cellular component organization and biogenesis (orange), transport (purple) and metabolic process (green). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

clusters) which are up-regulated for Acid (A) and down-regulated for Warm (W). These results indicate that treatment-specific and overlapping molecular responses may affect distinct gene networks and functions.

Gene count data for the major BP categories were plotted as violin plots for the three treatments as illustrated in Fig. 4. Each violin subplot compares gene expression distributions for treatments Acid (A), Acid-Warm (AW) and Warm (W) with vertical whiskers extending to outliers. All medians are positive (>0 log fold change), indicating significant over-representations. The higher data frequencies (density) were observed in muscle system process for AW, in biological regulation and response for A, in cellular component organization and biogenesis for W, in metabolic process for W and in transport for AW and W. Significant differences ($p < 0.05$) were observed for several BPs and treatment comparisons pairs, indicating that gene activity in these categories is sensitive to experimental treatments. For muscle system process, expression values were broadly comparable between treatments, with overlapping distributions and statistically significant differences detected only for the comparison A versus W ($p = 0.046$). Regarding the category biological regulation and response, AW exhibited a significant trend towards higher median gene expression compared to A ($p = 0.025$). The category cellular component organization and biogenesis, also exhibited a significantly higher median gene expression of AW compared to W ($p = 0.00036$) and of A compared to W ($p = 0.026$). The category metabolic process showed significant differences for all comparisons between treatments (A vs AW $p = 0.0058$; A vs W $p = 7.8e^{-07}$; AW vs W $p = 0.0089$). In the transport category, statistically significant differences were observed between A and AW ($p = 0.032$), and between A mean and W mean ($p = 0.021$), whereas

expression distributions for AW mean and W mean were not significantly different ($p = 0.74$).

The Upset diagram generated to identify transcripts commonly and exclusively expressed in each treatment, illustrated the overlap of DEGs among the three treatments (Fig. 5A). The most abundant transcripts were the down-regulated ones uniquely expressed in the low pH and ambient temperature treatment (A) which amounted to 153 in the normal pH and higher temperature treatment (W) they were 156, and in the combined low pH and higher temperature (AW) treatment they were 132. Shared gene expression patterns were identified with 89 up-regulated and 10 down-regulated DEGs common to all treatments. Gene Ontology (GO) enrichment analysis for Biological Processes (BP) of DEGs was performed and indicated significantly over- and under-regulated DEGs in the three treatments when compared to the control. The main GO driver terms identified in the present study were associated with ATP synthesis, mitochondrial processes, ion transport, phosphorylation, respiration, homeostasis, muscle system processes, and biosynthetic processes, and were strongly affected in response to either acidification, warming or combined stressors (Fig. 5B). More specifically, it was observed that most GO terms related to muscle functionality and calcium activity were up-regulated in the low pH treatment (A) and/or in the warm (W) treatment compared to the control. Also, aerobic respiration was up-regulated in the low pH treatment (A) and in the warm treatments (W and AW). In addition, an increased gene ratio was observed for the cyclic-nucleotide phosphodiesterase (PDE) activity, which is related to the modulation of intracellular cAMP levels and can influence neuronal activity and signaling pathways (Calhoun and Gillette, 1983). This GO term was down-regulated in the low pH treatment (A) and up-regulated in the warm (W) treatment (Fig. 5B).

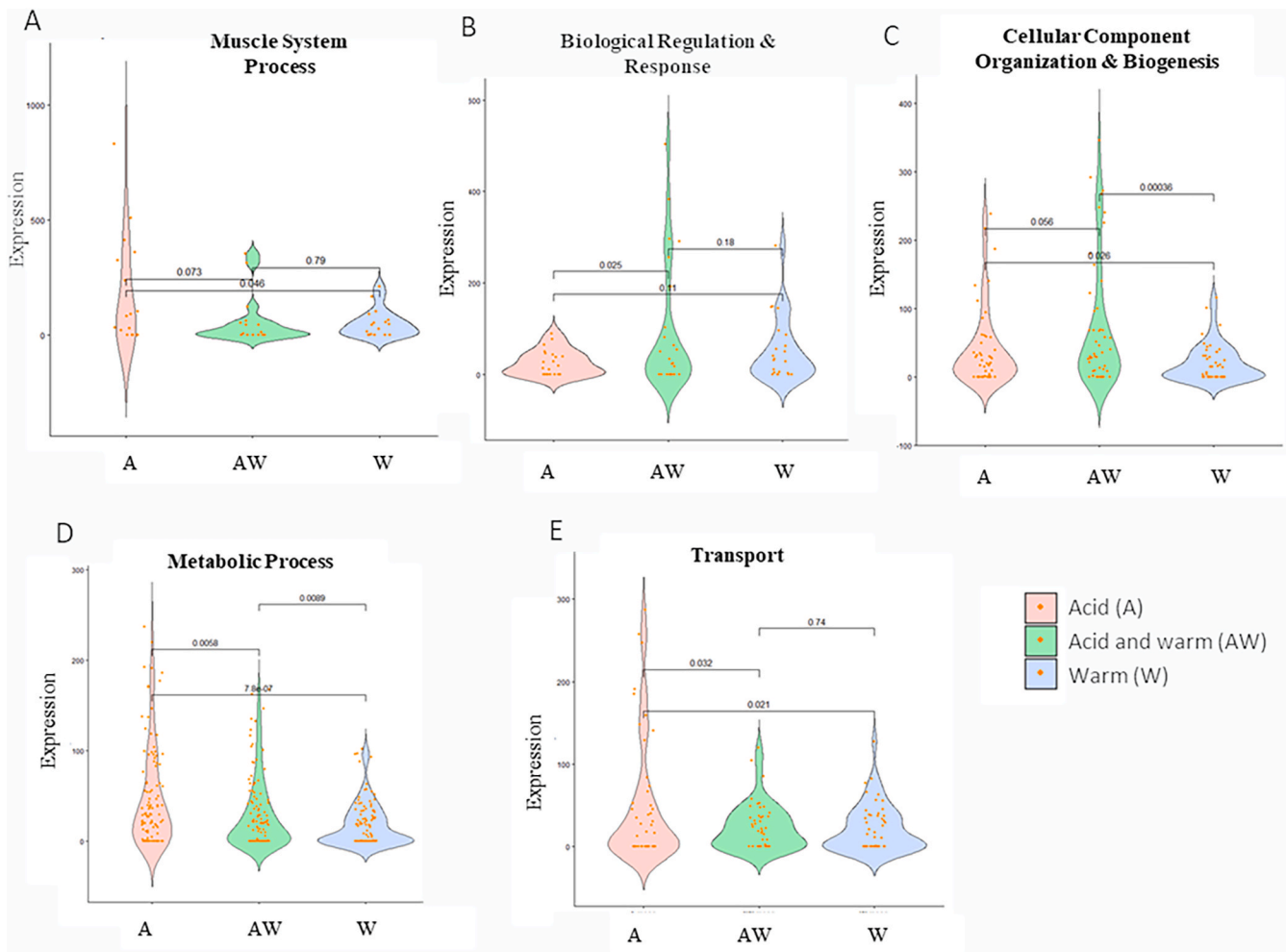


Fig. 4. Violin plots summarizing gene expression distributions for the five major Gene Ontology (GO) biological process (BP) categories: **A)** muscle system process, **B)** biological regulation & response, **C)** cellular component organization & biogenesis, **D)** metabolic process, and **E)** transport. Mean expression values for genes in each category are shown across the three experimental treatments: Acid (A, pink), Warm (W, light blue) and Acid-Warm (AW, green), against the Control (C). Orange dots represent individual gene measurements. Horizontal bars mark statistical comparisons between treatments, with p-values from two-sided t-tests annotated above each comparison. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

To further investigate the transcriptome dynamics in relation to environmental parameters the samples exposed to low pH and ambient temperature (A) and the samples exposed to normal pH and warm temperature (W) were compared to the samples which were exposed to the combined stressors of low pH and warm temperature (AW). The Upset diagram (Fig. 6A) indicated 167 down-regulated and 51 up-regulated transcripts uniquely expressed in the low pH and ambient temperature treatment (A), while 186 were down-regulated and 30 up-regulated in normal pH and higher temperature (W). In addition, 147 up-regulated and 20 down-regulated shared DEGs were found between treatments A and W were compared to the combined stressors treatment (AW).

Gene Ontology (GO) enrichment analysis for Biological Processes (BP) of DEGs was performed and indicated significantly over- and under-regulated DEGs in the two treatments (A and W) when compared to AW (Fig. 6B). Common GO terms included aerobic and cellular respiration (down-regulated in both A and W) and proton transmembrane transport which was up-regulated in A and down-regulated in W. All the GO terms related to calcium activity were significantly up-regulated in the warm (W) treatment when this was compared with the treatment combining increased temperature and low pH (AW). In addition, an increased gene ratio was also observed in this comparison for the cyclic-nucleotide phosphodiesterase (PDE) activity, which was up-regulated in the

warm (W) treatment.

4. Discussion

The gastropod *H. trunculus* (Linnaeus, 1758) was selected as the model species for the present study, due to its wide distribution and abundance in Mediterranean sublittoral habitats (Lahbib et al., 2010), as well as its flexibility to fluctuating environmental conditions characterising transitional coastal systems (Wahl et al., 2016). Nevertheless, *H. trunculus* is confronted with the challenges of intensive ocean warming and acidification. Up until today, no comprehensive *de novo* transcriptome assemblies or RNA-seq studies existed for *H. trunculus*. So far, targeted gene expression analysis has been published only with a focus on imposex from TBT pollution quantifying retinoid X receptor (RXR) transcription in penis/penis-forming tissues via qPCR (Abidli et al., 2013). In contrast, several RNA-seq studies have been published on other molluscs investigating the plasticity of their transcriptomes in response to altered environmental factors (Sun et al., 2025; Schultz et al., 2024). However, raw RNA-seq data availability remains limited to niche contexts like xenobiotic metabolism studies, with no published environmental stressor transcriptomes. This gap underscores the novelty of our multi-stressor profiling in advancing molecular understanding of *H. trunculus* environmental plasticity.

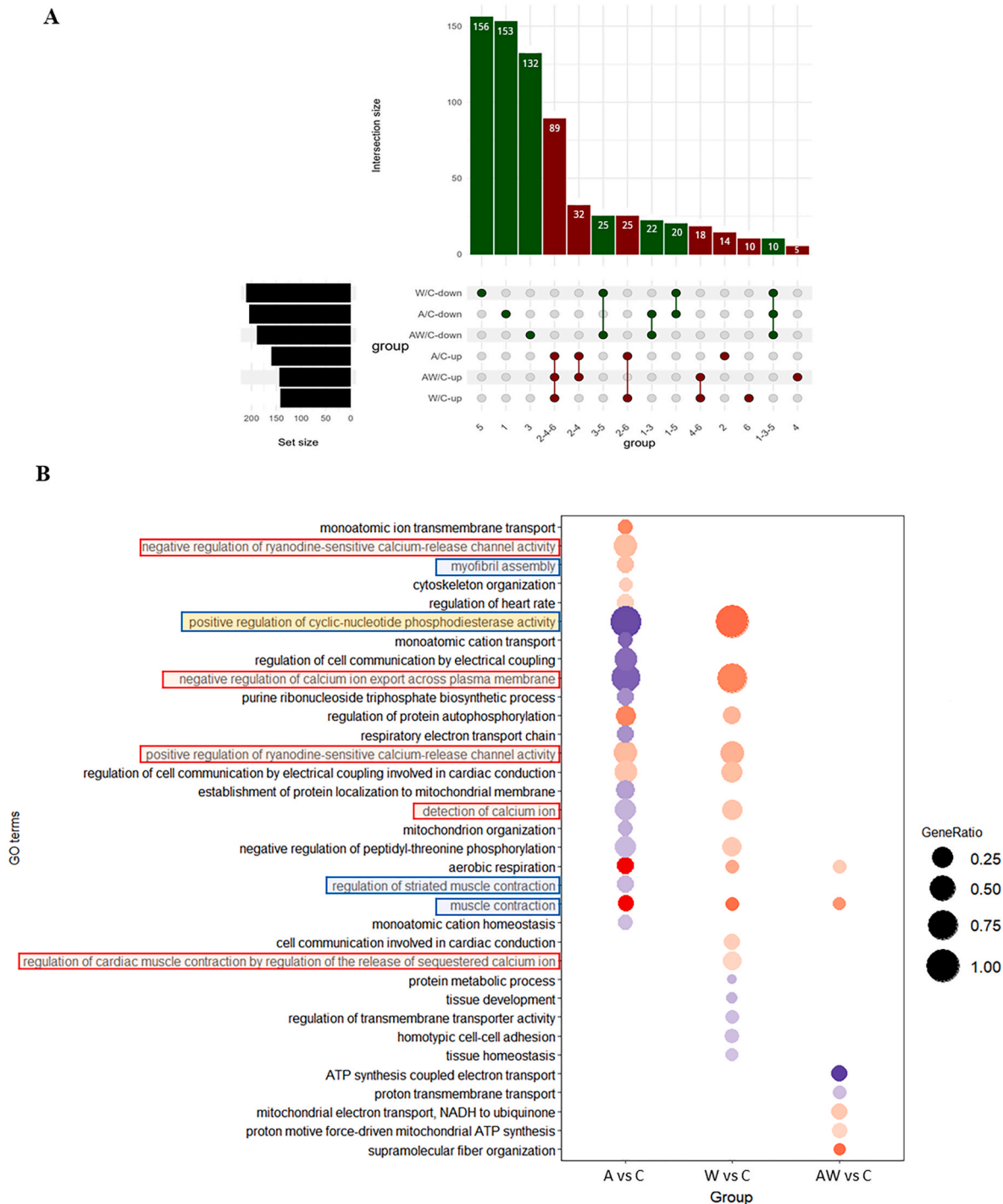
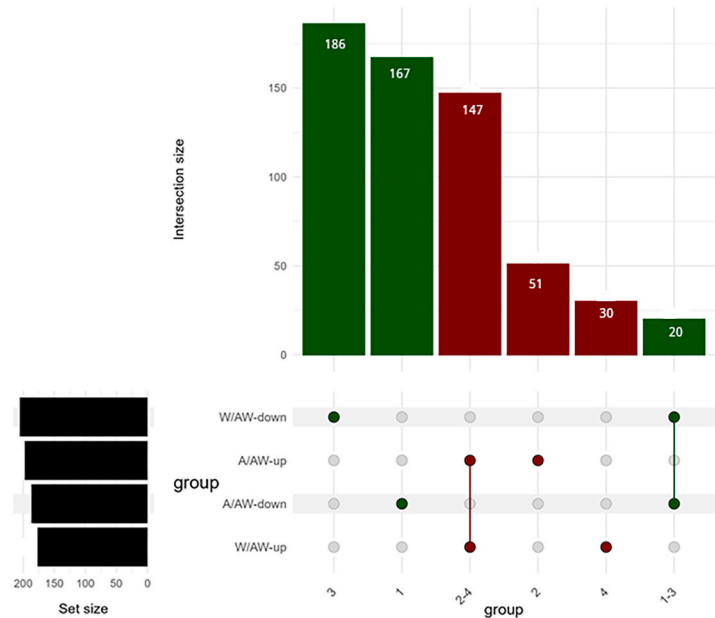


Fig. 5. A) Upset diagram illustrating the number of differentially expressed genes (DEGs) found in the different experimental treatments Acid (A), Warm (W) and Acid-Warm (AW) in comparison to the Control (C). Down-regulated DEGs in green and up-regulated DEGs in red. B) Gene Ontology (GO) biological process (BP) enrichment analysis displaying significant driver terms of differentially expressed genes (DEGs) for each treatment (A, W and AW) in comparison to the Control (C). Red bubbles indicate enriched using up-regulated DEGs and purple bubbles indicate enriched using down-regulated DEGs. Bubble size is proportional to the gene ratio. The colour intensity encodes enrichment significance, with darker colours corresponding to higher statistical significance (adjusted p-value < 0.05). The GO terms marked in red boxes are relevant to calcium ions, the ones in blue boxes are relevant to muscle activity and the one highlighted in yellow refers to cyclic-nucleotide phosphodiesterase (PDE). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

A 14-month experiment on *H. trunculus* was set up to investigate its transcriptome dynamics in relation to low pH and increased temperature conditions mimicking RCP8.5 end-century projections (pH −0.3, +3 °C). Continuous monitoring of experimental conditions, monthly calibrations, and rigorous water quality minimized confounds over the chronic exposure period, enabling detection of responses that might be

absent in short-term studies. On average 46 million reads for each sample were obtained (Suppl. File 2) ensuring sufficient read counts for reference transcriptome assembly, as well as for robust gene expression analysis (ENCODE Consortium, 2016). The obtained reads within the present work permit a wider view of gene expression rather than only a snapshot of highly expressed genes where only 5 – 25 million reads per

A



B

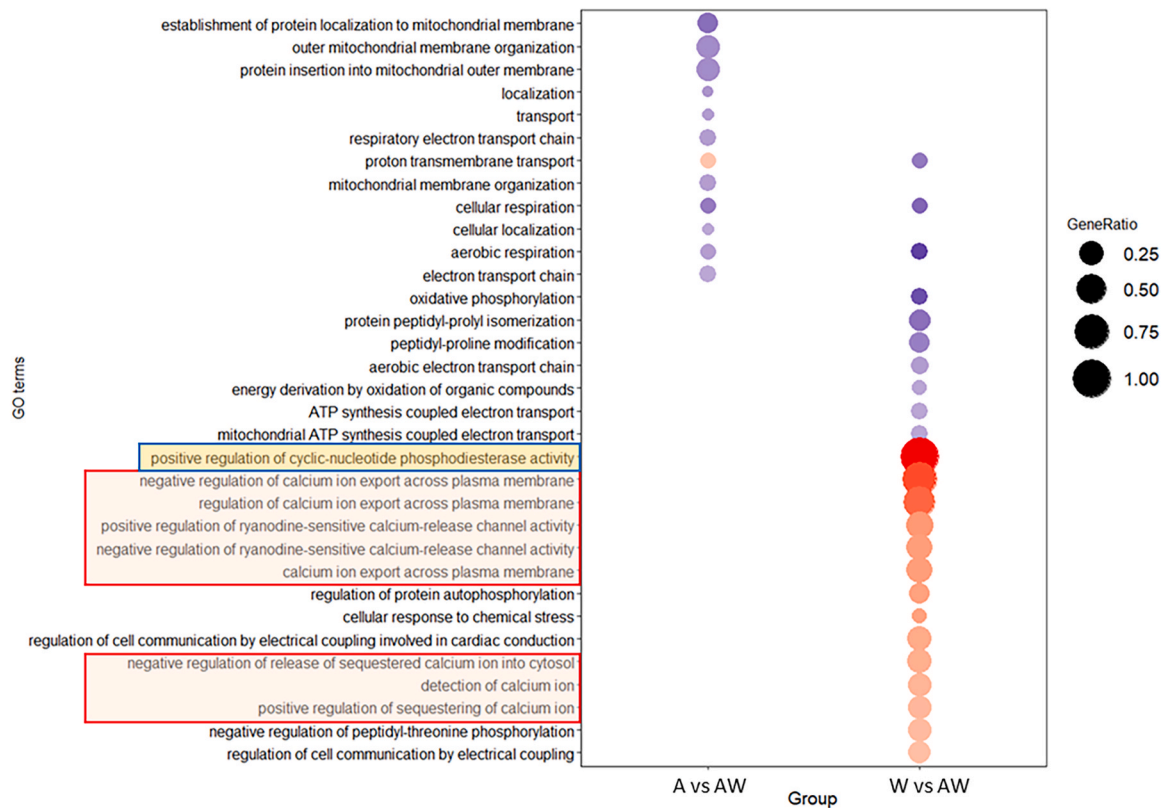


Fig. 6. A) Upset diagram illustrating the number of differentially expressed genes (DEGs) found in Warm (W) and Acid (A) treatments in comparison to the Acid-Warm (AW) treatment. Down-regulated DEGs in green and up-regulated DEGs in red. B) Gene Ontology (GO) biological process (BP) enrichment analysis displaying significant differentially expressed genes (DEGs) for Warm (W) and Acid (A) treatments in comparison to the Acid-Warm (AW) treatment. Red bubbles indicate enriched using up-regulated DEGs and purple bubbles indicate enriched using down-regulated DEGs. Bubble size is proportional to the gene ratio. The colour intensity encodes enrichment significance, with darker colours corresponding to higher statistical significance (adjusted p-value < 0.05). The GO terms marked in red boxes are relevant to calcium ions and the ones in blue boxes are relevant to muscle activity. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

sample are required (Liu et al., 2014). The BUSCO assessment of the generated *H. trunculus* reference transcriptome in the current study evidenced a good level of completeness for mollusc RNAseq experiments with the detection of 75.3% BUSCO completeness. Similarly, in the bivalve *Chamelea galina* 77.2% BUSCO completeness was reported (Carducci et al., 2022).

The contemporaneous specimens used in the current experiment might account for some limitations when considering the realistic effects of future climatic conditions on organisms that have evolved over successive generations. Therefore, the aim of the present experimental design is mainly focused on capturing transcriptomic responses, while additional future work on growth and relevant physiological processes would provide additional insight into further studying transgenerational changes. The differential expression analysis identified a number of transcripts that were up-regulated and down-regulated depending on the experimental conditions. The heatmap analysis showed that replicates within each treatment formed distinct clusters reflecting strong intra-group consistency, while clear transcriptomic differences were observed between treatments (Fig. 2A). The VST-transformed DEG-based PCA analysis (Fig. 2B) confirmed the clear clustering of biological replicates within the same treatment and mirrored the significance of treatment separation with the acidification (A) showing the greatest divergence. The close clustering of the Warm (W) and Control (C) groups indicated that the factor of increased temperature alone was not responsible for a considerable impact. Acidification notably reprograms *H. trunculus* DEGs, while warming elicits a subtler heat shock response. This is also supported by the fact that a higher number of up-regulated transcripts was found in the reduced pH treatment (A) in comparison to the other two treatments. Robust qualitative transcriptomic response to ocean acidification has also been shown in other mollusc transcriptomic studies (Strader et al., 2020). For example, exposure of larvae of the Pacific oyster *Crassostrea gigas* to low pH resulted in differential expression of extracellular matrix formation genes, relevant to shell matrix calcification proteins (De Wit et al., 2018), whereas in the pearl oyster *Pinctada fucata* (mantle sample) differential expression was relevant to calcium binding proteins (Li et al., 2016). The study of *Mytilus coruscus* mantle transcriptome, showed also a strong response to acute acidification, where most DEGs were up-regulated indicating the activation of biomineralization-related processes (Liao et al., 2023).

Five distinct clusters were identified for *H. trunculus* in the groups A, W and AW underscoring transcriptional divergence between treatments (Fig. 3B). Cluster 1 comprises of genes mainly up-regulated in group A and respective transcripts were mainly mapped to muscle system process (Fig. 3C), possibly indicating a stress response to the increased energy demands of metabolic compensation and enhanced locomotion for foraging or escape. Indeed, both adults and juveniles of *H. trunculus* spent more time and effort for foraging and were less successful in reaching their food under low pH conditions (Chatzinikolaou et al., 2019). Accordingly, the GO term metabolic process contains mainly genes of cluster 1 (higher expression in A) and 2 (higher expression in A and AW) indicating a higher metabolic demand under low pH. Statistically significant shifts were largely restricted to comparisons involving the A group, thereby supporting a model in which acidification is the most critical condition. Similarly, DEGs isolated from *M. coruscus* mantle were mainly up-regulated in pathways related to cell processes and metabolism under low pH (Liao et al., 2023). However, a review published by Strader et al. (2020) indicated that metabolic genes are more often down-regulated in bivalves following acute or short-term exposure to low pH, as a way to conserve energy while cells try to decrease the influx in hydrogen ions. Nevertheless, the results of the present study indicate that this is not the case when molluscs are subjected in chronic exposure to acidified conditions, since a long-term metabolic depression could become lethal for the organisms. Metabolic responses are complex and species specific – and often even population specific – and are also based on the length of exposure to stressors (Strader et al., 2020).

Previous studies indicated that transgelin-like proteins were

involved in biomineralization in several bivalve species (Jiang et al., 2020), while tyrosinases were involved in the formation of the shell matrix and periostracum (Sleight et al., 2016; Aguilera et al., 2014). In the present study (see Suppl. File 3), the DEGs analysis revealed the presence of tyrosinases (tyr-3 and serpin B6) in *H. trunculus* under the warm treatment (W) (versus C or AW), as well as the presence of transgelins in all treatments (versus AW). However, the key GO terms in relation to shell formation, such as shell calcification (GO:0031215), biomineral tissue development (GO:0031214) or chitin biosynthetic process (GO:0006031) were not found to be enriched, which may be anticipated since expression analysis was conducted using foot and not mantle tissue. In molluscs, the foot is a muscular ventral structure specialised for locomotion and attachment (Denny, 1980), and thus is expected to primarily express genes related to muscle contraction, ciliary transport and metabolic support, (Zhang et al., 2021). Shell formation occurs via the extrapallial epithelium and the mantle, where matrix proteins such as Pif, Perlustrin and chitin synthases drive the deposition of CaCO₃ (Marin et al., 2012; Chen et al., 2018). The absence of these terms as key GOs aligns with the observed enrichment in processes such as muscle system, biological regulation and response, cellular component organization and biogenesis metabolic process and transport, reflecting foot-specific stress responses such as energy reallocation and ion homeostasis. In most cases significant differences were observed in treatments of low pH (A and AW). These outcomes were further supported by investigating the driver terms of differentially expressed genes for each treatment (A, W and AW) in comparison to the Control (C) (Fig. 5B).

Interestingly, two GO terms related to calcium activity (i.e. detection and ion export) (Smeets et al., 2015) were down-regulated for *H. trunculus* in the low pH treatment (A) (Fig. 5B), indicating that gastropods maintained for a long-term under acidified conditions might be less effective in calcium management. On the contrary, four calcium related GO terms were up-regulated in the warm (W) treatment suggesting a more efficient calcium management when the temperature was increased. Therefore, the combination of those two stressors compensated each other and no up- or down-regulations related to calcium activity were observed in the combined treatment (AW). Shell formation and biomineralization in marine molluscs is dependent on the availability and effective regulation of calcium, as calcium ions are taken up from the environment and transported to the extrapallial space where CaCO₃ is deposited during shell growth (Yarra et al., 2021). Low pH also induced differential expression of genes regulating ion transport and acid-base homeostasis, including calcium binding that is likely to maintain calcification, in adults of the pearl oyster *Pinctada fucata*, while elevated temperature alleviated the negative effects of acidification (Li et al., 2016). Acute acidification promoted chitin synthesis and inhibited chitin degradation in *M. coruscus* to maintain the integrity of the shell and mitigate the impairments caused by acidification (Liao et al., 2023).

Calcium ions do not only participate in shell formation, but they are also essential for the regulation of muscle contraction in molluscs. Unlike vertebrate skeletal muscles, many molluscan muscles use a myosin-linked regulatory system in which Ca²⁺ binds directly to myosin regulatory components to initiate contraction, indicating that calcium binding is required for contractile and ATPase activity (Kendrick-Jones et al., 1970). In the present study this is further supported by the fact that two GO terms related to muscle functionality (i.e. contraction and assembly) were up-regulated in the low pH treatment (A), highlighting the fact that acidified conditions also affected muscle activity in *H. trunculus*.

Additional genes that were differentially expressed in the present study were related to aerobic respiration, transmembrane cell transport and cyclic-nucleotide phosphodiesterase (PDE) activity. Aerobic respiration was up-regulated in all treatments but mainly in the low pH treatment (A). Transcriptomic analyses in the blue mussels *Mytilus edulis* revealed metabolic depression of aerobic energy pathways under ocean

acidification (Strader et al., 2020). The increased gene ratio observed for the cyclic-nucleotide phosphodiesterase (PDE) activity, which was down-regulated in the low pH treatment (A) and up-regulated in the warm (W) treatment, is related to the modulation of intracellular cAMP levels and can influence neuronal activity and signalling pathways (Calhoun and Gillette, 1983). PDE activity is stimulated by Ca^{2+} ions, since calcium increases the rate at which PDE degrades cAMP (Calhoun and Gillette, 1983). At the same time PDE's activity is pH-dependent and typically declines under acidic conditions due to protonation of catalytic residues, resulting in reduced cyclic nucleotide hydrolysis (Londesborough and Lukkari, 1980).

Although transcriptomic responses to acidification and warming may vary between different organisms, there are some common patterns observed, mainly related to ion regulation, metabolic processes, calcification and stress responses (Strader et al., 2020). Also the type of sample used is important, since energetic and metabolic changes are tissue type and life-stage specific. Studies that have used samples from the mantle (i.e. the primary shell forming organ in molluscs) or samples from rigorous developing larvae, indicated that expression of extracellular matrix genes, such as shell matrix proteins, become differentially regulated under acidified conditions (Li et al., 2016). In adults, low pH induced differential expression of genes regulating ion transport and acid-base homeostasis, including calcium binding, likely to maintain calcification (Strader et al., 2020).

5. Conclusions

Transcriptomics are a widely-used method to characterize whole organism responses due to ocean acidification on a molecular level, and also to describe the mechanisms that may explain relevant changes in phenotypes and physiology (Strader et al., 2020). The present study focused on transcriptome changes in the foot tissue of *H. trunculus* under conditions of higher temperature and lower pH. The gastropod foot directs functions related to locomotion, adhesion and attachment, substrate probing, and sensory functions (Denny, 1980; Horváth et al., 2020), which can be disrupted by environmental stressors. The transcriptomic analyses showed responses on calcium activity and ion transport which can be related to shell calcification, as well as to muscle functioning. In addition, aerobic respiration, transmembrane cell transport and cyclic-nucleotide phosphodiesterase were also affected by low pH conditions, revealing holistic responses. Acidification drives dominant shifts in muscle system processes, metabolic demands and ion regulatory responses. These foot-specific molecular signatures highlight the constraints of locomotion and sensory functions in dynamic coastal habitats. Complementary mantle and whole-body RNA sequencing would likely reveal signatures more closely related to shell formation, highlighting tissue partitioning in transcriptomic responses. This study presents the first *de novo* assembly of the foot transcriptome for the marine gastropod *H. trunculus*, as well as experimental treatment-specific transcriptome profiles. Our findings advance the understanding of gastropod resilience to environmental stressors, highlighting the need for multi-tissue profiling to inform Mediterranean conservation and predictive modelling actions.

CRedit authorship contribution statement

Elena Sarropoulou: Writing – review & editing, Writing – original draft, Formal analysis. **Panos Grigoriou:** Writing – review & editing, Methodology, Investigation. **Aglaia Antoniou:** Writing – review & editing, Investigation. **Thekla I. Anastasiou:** Writing – review & editing, Formal analysis. **Eva Chatzinikolaou:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Data statement

All raw sequencing reads have been submitted to the publicly accessible BioProject database NCBI under the accession number PRJNA1378232.

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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.2026.108426>.

Data availability

Research data link provided.

The effects of ocean warming and acidification on the gene expression patterns of *Hexaplex trunculus* using transcriptome analysis (Original data) (NCBI)

References

- Abidli, S., Castro, L.F.C., Lahbib, Y., Reis-Henriques, M.A., Trigui El Menif, N., Santos, M. M., 2013. Imposse development in *Hexaplex trunculus* (Gastropoda: Caenogastropoda) involves changes in the transcription levels of the retinoid X receptor (RXR). *Chemosphere* 93, 1161–1167. <https://doi.org/10.1016/j.chemosphere.2013.06.054>.
- Abidli, S., Lahbib, Y., Trigui El Menif, N., 2009. Effects of TBT on imposex development, reproduction and mortality in *Hexaplex trunculus* (Gastropoda: Muricidae). *J. Mar. Biol. Assoc. U. K.* 89, 139–145. <https://doi.org/10.1017/S0025315408002282>.
- Abidli, S., Santos, M.M., Lahbib, Y., Castro, L.F.C., Reis-Henriques, M.A., Trigui El Menif, N., 2012. Tributyltin effects on *Hexaplex trunculus* and *Bolinus brandaris* (Gastropoda: Muricidae): imposex induction and sex hormone levels insights. *Ecol. Indic.* 13, 13–21. <https://doi.org/10.1016/j.ecolind.2011.05.001>.
- Afgan, E., Baker, D., Batut, B., van den Beek, M., Bouvier, D., Čech, M., Chilton, J., Clements, D., Coraor, N., Grüning, B.A., Guerler, A., Hillman-Jackson, J., Hiltmann, S., Jalili, V., Rasche, H., Soranzo, N., Goecks, J., Taylor, J., Nekrutenko, A., Blankenberg, D., 2018. The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2018 update. *Nucleic Acids Res.* 46, 537–544. <https://doi.org/10.1093/nar/gky379>.
- Aguilera, F., McDougall, C., Degnan, B.M., 2014. Evolution of the tyrosinase gene family in bivalve molluscs: independent expansion of the mantle gene repertoire. *Acta Biomater.* 10, 3855–3865. <https://doi.org/10.1016/j.actbio.2014.03.031>.
- Ahi, E.P., Lindeza, A.S., Miettinen, A., Primmer, C.R., 2025. Transcriptional responses to changing environments: insights from salmonids. *Rev. Fish Biol. Fish.* 35, 681–706. <https://doi.org/10.1007/s11160-025-09928-9>.
- Anastasiou, T.I., Chatzinikolaou, E., Mandalakis, M., Arvanitidis, C., 2016. Imposse and organotin compounds in ports of the Mediterranean and the Atlantic: is the story over? *Sci. Total Environ.* 569–570, 1315–1329. <https://doi.org/10.1016/j.scitotenv.2016.06.209>.

- Bolger, B.M., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30, 2114–2120. <https://doi.org/10.1093/bioinformatics/btu170>.
- Calhoun, R.D., Gillette, R., 1983. Ca^{2+} -activated and pH-sensitive cyclic AMP phosphodiesterase in the nervous system of the mollusc *Pleurobranchaea*. *Brain Res.* 271, 371–374. [https://doi.org/10.1016/0006-8993\(83\)90304-9](https://doi.org/10.1016/0006-8993(83)90304-9).
- Calvin, K., Dasgupta, D., Krinner, G., Mukherji, A., Thorne, P.W., Trisos, C., Romero, J., Aldunce, P., Barrett, K., Blanco, G., Canales, N., Gannon, K., Geden, O., Grady, C., Huang, M., Joos, F., McElwee, P., Mustonen, T., Nkem, J., et al., 2023. Climate Change 2023: Synthesis Report. Contribution of Working Groups I, II and III to the Sixth Assessment Report of the IPCC. IPCC, Geneva. <https://doi.org/10.59327/ipcc/ar6-9789291691647>.
- Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., Madden, T.L., 2009. BLAST+: architecture and applications. *BMC Bioinf.* 10, 421. <https://doi.org/10.1186/1471-2105-10-421>.
- Carducci, F., Biscotti, M.A., Mosca, A., Greco, S., Gerdol, M., Memmola, F., Barucca, M., Canapa, A., 2022. The mantle transcriptome of *Chamelea gallina* (Mollusca: Bivalvia) and shell biomineralization. *Animals* 12, 1196. <https://doi.org/10.3390/ani12091196>.
- Chaar, D.L., Li, Z., Shang, L., Ratliff, S.M., Mosley, T.H., Kardias, S.L.R., Zhao, W., Zhou, X., Smith, J.A., 2025. Multi-ancestry transcriptome-wide association studies of cognitive function, white matter hyperintensity, and Alzheimer's disease. *Int. J. Mol. Sci.* 26, 2443. <https://doi.org/10.3390/ijms26062443>.
- Chatzinikolaou, E., Grigoriou, P., Keklikoglou, K., Faulwetter, S., Papageorgiou, N., 2017. The combined effects of reduced pH and elevated temperature on the shell density of two gastropod species measured using micro-CT imaging. *ICES (Int. Council. Explor. Sea) J. Mar. Sci.* 74, 1135–1149. <https://doi.org/10.1093/icesjms/fsw219>.
- Chatzinikolaou, E., Grigoriou, P., Martini, E., Steriotti, A., 2019. Impact of ocean acidification and warming on the feeding behaviour of two gastropod species. *Mediterr. Mar. Sci.* 20, 669–679. <https://doi.org/10.12681/mms.19187>.
- Chatzinikolaou, E., Keklikoglou, K., Grigoriou, P., 2021a. Morphological properties of gastropod shells in a warmer and more acidic future ocean using 3D micro-computed tomography. *Front. Mar. Sci.* 8, 645660. <https://doi.org/10.3389/fmars.2021.645660>.
- Chatzinikolaou, E., Keklikoglou, K., Grigoriou, P., Arvanitidis, C., 2021b. Micro-CT image gallery visually presenting the effects of ocean warming and acidification on marine gastropod shells. *Biodivers. Data J.* 9, e75358. <https://doi.org/10.3897/BDJ.9.e75358>.
- Chen, Y., Gao, J., Xie, J., Liang, J., Zheng, G., Xie, L., Zhang, R., 2018. Transcriptional regulation of the matrix protein Shematrin-2 during shell formation in pearl oyster. *J. Biol. Chem.* 293, 17803–17816. <https://doi.org/10.1074/jbc.RA118.005281>.
- Conway, J.R., Lex, A., Gehlenborg, N., 2017. UpSetR: an R package for the visualization of intersecting sets and their properties. *Bioinformatics* 33, 2938–2940. <https://doi.org/10.1093/bioinformatics/btx364>.
- De Wit, P., Durland, E., Ventura, A., Langdon, C.J., 2018. Gene expression correlated with delay in shell formation in larval Pacific oysters (*Crassostrea gigas*) exposed to experimental ocean acidification provides insights into shell formation mechanisms. *BMC Genom.* 19, 160. <https://doi.org/10.1186/s12864-018-4519-y>.
- Denny, M., 1980. The role of gastropod pedal mucus in locomotion. *Nature* 285, 160–161. <https://doi.org/10.1038/285160a0>.
- Dickson, A.G., Miller, F.J., 1987. A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. *Deep-Sea Res. Part A* 34, 1733–1743. [https://doi.org/10.1016/0198-0149\(87\)90021-5](https://doi.org/10.1016/0198-0149(87)90021-5).
- Dickson, A.G., Sabine, C.L., Christian, J.R., 2007. Guide to Best Practices for Ocean CO₂ Measurements, 3. PICES Special Publication.
- Doney, S.C., Busch, D.S., Cooley, S.R., Kroeker, K.J., 2020. The impacts of ocean acidification on marine ecosystems and reliant human communities. *Annu. Rev. Environ. Resour.* 45, 83–112. <https://doi.org/10.1146/annurev-environ-012320-083019>.
- Doyle, J.J., Doyle, J.L., 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.* 19, 11–15.
- ENCODE Consortium, 2016. Standards, guidelines and best practices for RNA-Seq. version 2. Available at: <https://www.encodeproject.org>.
- Friedlingstein, P., O'Sullivan, M., Jones, M.W., Andrew, R.M., Bakker, D.C.E., Hauck, J., Le Quéré, C., Peters, G.P., Peters, W., Pongratz, J., Sitch, S., Canadell, J.G., Ciais, P., Jackson, R.B., Alin, S.R., Anthoni, P., Bates, N.R., Becker, M., Bellouin, N., et al., 2022. Global carbon budget 2021. *Earth Syst. Sci. Data* 14, 1917–2005. <https://doi.org/10.5194/essd-14-1917-2022>.
- Gallagher, K.M., Albano, P.G., 2023. Range contractions, fragmentation, species extirpations and extinctions of commercially valuable molluscs in the Mediterranean Sea—a climate warming hotspot. *ICES (Int. Council. Explor. Sea) J. Mar. Sci.* 80, 1382–1398. <https://doi.org/10.1093/icesjms/fsad065>.
- Gingerich, P.D., 2019. Temporal scaling of carbon emission and accumulation rates: modern anthropogenic emissions compared to estimates of PETM onset accumulation. *Paleoceanogr. Paleoclimatol.* 34, 329–335. <https://doi.org/10.1029/2018PA003379>.
- Grabherr, M.G., Haas, B.J., Yassour, M., Levin, J.Z., Thompson, D.A., Amit, I., Adiconis, X., Fan, L., Raychowdhury, R., Zeng, Q., Chen, Z., Mauceli, E., Hacohen, N., Gnirke, A., Rhind, N., di Palma, F., Birren, B.W., Nusbaum, C., Lindblad-Toh, K., Friedman, N., Regev, A., 2011. Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nat. Biotechnol.* 29, 644–652. <https://doi.org/10.1038/nbt1883>.
- Henson, S., Beaulieu, C., Ilyina, T., John, J.G., Long, M., Séférian, R., Tjiputra, J., Sarmiento, J.L., 2017. Rapid emergence of climate change in environmental drivers of marine ecosystems. *Nat. Commun.* 8, 14682. <https://doi.org/10.1038/ncomms14682>.
- Horváth, R., Elek, K., Kristóf, A., Pásztor, I., Kiss, T., Zvara, Á., 2020. Chemical-neuroanatomical organization of peripheral sensory-efferent systems in the pond snail (*Lymnaea stagnalis*). *Brain Struct. Funct.* 225, 2563–2575. <https://doi.org/10.1007/s00429-020-02145-z>.
- Jiang, Y., Sun, Q., Fan, M., He, J., Zhang, X., Xu, H., Liao, Z., 2020. Recombinant transgelin-like protein 1 from *Mytilus* shell induces formation of CaCO₃ polymorphic crystals in vitro. *FEBS Open Bio* 10, 2216–2234. <https://doi.org/10.1002/2211-5463.12972>.
- Jordan-Thaden, I.E., Chanderbali, A.S., Gitzendanner, M.A., Soltis, D.E., 2015. Modified CTAB and TRIzol protocols improve RNA extraction from chemically complex Embryophyta. *Appl. Plant Sci.* 3, 1400105. <https://doi.org/10.3732/apps.1400105>.
- Kendrick-Jones, J., Lehman, W., Szent-Györgyi, A.G., 1970. Regulation in molluscan muscles. *J. Mol. Biol.* 54, 313–326. [https://doi.org/10.1016/0022-2836\(70\)90432-8](https://doi.org/10.1016/0022-2836(70)90432-8).
- Kolberg, L., Raudvere, U., Kuzmin, I., Adler, P., Vilo, J., Peterson, H., 2023. g:Profiler—interoperable web service for functional enrichment analysis and gene identifier mapping (2023 update). *Nucleic Acids Res.* 51, 207–212. <https://doi.org/10.1093/nar/gkad347>.
- Kolde, R., 2025. Pheatmap: pretty heatmaps. R Package Version 1.0.13. <https://CRAN.R-project.org/package=pheatmap>.
- Lahbib, Y., Abidli, S., Trigui El Menif, N., 2010. Laboratory study of the intracapsular development and juvenile growth of the banded murex, *Hexaplex trunculus*. *J. World Aquacult. Soc.* 41, 18–34. <https://doi.org/10.1111/j.1749-7345.2009.00310.x>.
- Lahbib, Y., Abidli, S., Trigui El Menif, N., 2012. TBT pollution in Tunisian coastal lagoons as indicated by imposex in *Hexaplex trunculus* (Gastropoda: Muricidae). *Transit. Waters Bull.* 6, 34–41. <https://doi.org/10.1285/i1825229Xv6n1p34>.
- Li, B., Dewey, C.N., 2011. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC Bioinf.* 12, 323. <https://doi.org/10.1186/1471-2105-12-323>.
- Li, S., Liu, C., Huang, J., Liu, Y., Zhang, S., Zheng, G., Xie, L., Zhang, R., 2016. Interactive effects of seawater acidification and elevated temperature on the transcriptome and biomineralization in the pearl oyster *Pinctada fucata*. *Environ. Sci. Technol.* 50, 1157–1165. <https://doi.org/10.1021/acs.est.5b05107>.
- Liao, Z., Liu, F., Wang, Y., Fan, X., Li, Y., He, J., Buttino, I., Yan, X., Zhang, X., Shi, G., 2023. Transcriptomic response of *Mytilus coruscus* mantle to acute seawater acidification and shell damage. *Front. Physiol.* 14, 1289655. <https://doi.org/10.3389/fphys.2023.1289655>.
- Liu, Y., Zhou, J., White, K.P., 2014. RNA-seq differential expression studies: more sequence or more replication? *Bioinformatics* 30, 301–304. <https://doi.org/10.1093/bioinformatics/btt688>.
- Londesborough, J., Lukkari, T.M., 1980. The pH and temperature dependence of the activity of the high Km cyclic nucleotide phosphodiesterase of bakers' yeast. *J. Biol. Chem.* 255, 9262–9267. [https://doi.org/10.1016/S0021-9258\(19\)70556-0](https://doi.org/10.1016/S0021-9258(19)70556-0).
- Manni, M., Berkeley, M.R., Seppey, M., Zdobnov, E.M., 2021. BUSCO: assessing genomic data quality and beyond. *Curr. Protoc.* 1, e323. <https://doi.org/10.1002/cpz1.323>.
- Marin, F., Le Roy, N., Marie, B., 2012. The formation and mineralization of mollusk shell. *Front. Biosci. (Scholar Edition)* 4, 1099–1125. <https://doi.org/10.2741/S321>.
- Mehrbach, C., Culbertson, C.H., Hawley, J.E., Pytkowicz, R.M., 1973. Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. *Limnol. Oceanogr.* 18, 897–907. <https://doi.org/10.4319/lo.1973.18.6.0897>.
- Natsidis, P., Schiffer, P.H., Salvador-Martinez, I., Telford, M.J., 2019. Computational discovery of hidden breaks in 28S ribosomal RNAs across eukaryotes and consequences for RNA Integrity Numbers. *Sci. Rep.* 9, 19477. <https://doi.org/10.1038/s41598-019-55573-1>.
- Poloczanska, E.S., Burrows, M.T., Brown, C.J., Molinos, J.G., Halpern, B.S., Hoegh-Guldberg, O., Kappel, C.V., Moore, P.J., Richardson, A.J., Schoeman, D.S., Sydeman, W.J., 2016. Responses of marine organisms to climate change across oceans. *Front. Mar. Sci.* 3, 62. <https://doi.org/10.3389/fmars.2016.00062>.
- Sayers, E.W., Beck, J., Bolton, E.E., Brister, J.R., Chan, J., Connor, R., Feldgarden, M., Fine, A.M., Funk, K., Hoffman, J., Kannan, S., Kelly, C., Klimke, W., Kim, S., Lathrop, S., Marchler-Bauer, A., Murphy, T.D., O'Sullivan, C., Schmieder, E., Skripchenko, Y., Stine, A., Thibaud-Nissen, F., Wang, J., Ye, J., Zellers, E., Schneider, V.A., Pruitt, K.D., 2025. Database resources of the National Center for Biotechnology Information in 2025. *Nucleic Acids Res.* 53, 20–29. <https://doi.org/10.1093/nar/gkac979>.
- Schultz, L., Wessely, J., Dullinger, S., Albano, P.G., 2024. The climate crisis affects Mediterranean marine molluscs of conservation concern. *Divers. Distrib.* 30, e13805. <https://doi.org/10.1111/ddi.13805>.
- Seppey, M., Manni, M., Zdobnov, E.M., 2019. BUSCO: assessing genome assembly and annotation completeness. *Methods Mol Biol.* 1962, 227–245. https://doi.org/10.1007/978-1-4939-9173-0_14.
- Simão, F.A., Waterhouse, R.M., Ioannidis, P., Kriventseva, E.V., Zdobnov, E.M., 2015. BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics* 31, 3210–3212. <https://doi.org/10.1093/bioinformatics/btv351>.
- Sleight, V.A., Thorne, M.A., Peck, L.S., Arivalagan, J., Berland, S., Marie, A., Clark, M.S., 2016. Characterisation of the mantle transcriptome and biomineralisation genes in the blunt-gaper clam, *Mya truncata*. *Mar. Genom.* 27, 47–55. <https://doi.org/10.1016/j.margen.2016.01.003>.
- Smeets, P.J., Cho, K.R., Kempen, R.G., Sommerdijk, N.A.J.M., De Yoreo, J.J., 2015. Calcium carbonate nucleation driven by ion binding in a biomimetic matrix revealed by in situ electron microscopy. *Nat. Mater.* 14, 394–399. <https://doi.org/10.1038/nmat4193>.

- Strader, M.E., Wong, J.M., Hofmann, G.E., 2020. Ocean acidification promotes broad transcriptomic responses in marine metazoans: a literature survey. *Front. Zool.* 17, 7. <https://doi.org/10.1186/s12983-020-0350-9>.
- Sun, L., Zhang, Y., Li, R., Li, W., Li, Q., Wang, S., Zhang, L., Xu, F., Zhang, G., Li, Y., 2025. Shallow-water mussels (*Mytilus galloprovincialis*) adapt to deep-sea environment through transcriptomic and metagenomic insights. *Commun. Biol.* 8, 46. <https://doi.org/10.1038/s42003-024-07382-0>.
- Varet, H., Brillet-Guéguen, L., Coppée, J.-Y., Dillies, M.A., 2016. SARTools: a DESeq2- and EdgeR-based R pipeline for comprehensive differential analysis of RNA-Seq data. *PLoS One* 11 (6), e0157022. <https://doi.org/10.1371/journal.pone.0157022>.
- Vargas-Yáñez, M., García, M.J., Salat, J., García-Martínez, M.C., Pascual, J., Moya, F., 2008. Warming trends and decadal variability in the Western Mediterranean shelf. *Global Planet. Change* 63, 177–184. <https://doi.org/10.1016/j.gloplacha.2007.09.001>.
- Vasconcelos, P., Gaspar, M.B., Castro, M., 2006. Imposex in *Hexaplex trunculus* from the Ria Formosa lagoon (Algarve coast—southern Portugal). *Mar. Pollut. Bull.* 52, 332–356. <https://doi.org/10.1016/j.marpolbul.2005.10.023>.
- Venegas, R.M., Acevedo, J., Trembl, E.A., 2023. Three decades of ocean warming impacts on marine ecosystems: a review and perspective. *Deep-Sea Res. Part II Top. Stud. Oceanogr.* 212, 105318. <https://doi.org/10.1016/j.dsr2.2023.105318>.
- Wahl, M., Saderne, V., Sawall, Y., 2016. How good are we at assessing the impact of ocean acidification in coastal systems? Limitations, omissions and strengths of commonly used experimental approaches with special emphasis on the neglected role of fluctuations. *Mar. Freshw. Res.* 67, 25–36. <https://doi.org/10.1071/MF14154>.
- Yarra, T., Ramesh, K., Blaxter, M., Hüning, A., Melzner, F., Clark, M.S., 2021. Transcriptomic analysis of shell repair and biomineralization in the blue mussel, *Mytilus edulis*. *BMC Genom.* 22, 437. <https://doi.org/10.1186/s12864-021-07751-7>.
- Zeebe, R.E., Wolf-Gladrow, D., 2001. *CO₂ in Seawater: Equilibrium, Kinetics, Isotopes*. Elsevier Science, Amsterdam.
- Zhang, G., Xu, M., Zhang, C., Jia, H., Zhang, H., He, M., Liu, W., 2021. Comparative transcriptomic and expression profiles between the foot muscle and mantle tissues in the giant triton snail *Charonia tritonis*. *Front. Physiol.* 12, 632518. <https://doi.org/10.3389/fphys.2021.632518>.