



Transcriptomic Insights into the Adaptive Strategies of *Mytilus galloprovincialis* to Extreme Thermal Stress in Intertidal Zone

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ABSTRACT

Mytilus galloprovincialis is an ecologically and economically essential species in China. Under the background of the global climate change, temperature fluctuations have exerted a significant impact on the growth and survival of *M. galloprovincialis*. To explore the molecular mechanisms underlying its response to extreme thermal stress, we herein performed transcriptome analysis on *M. galloprovincialis* under high-temperature conditions. Specially, *M. galloprovincialis* individuals were exposed to 18°C (control group) and 30°C (heat stress group) for 24 h, simulating the thermal stress encountered during low tide in the intertidal zone. Subsequently, gill and mantle tissues were collected for transcriptomic profiling, which led to the identification of 2673 differentially expressed genes (DEGs) between the 18°C and 30°C treatment groups. Among these DEGs, those associated with immune responses were significantly up-regulated under heat stress, including those involved in the antigen processing and presentation pathway, TNF signaling pathway, and protein processing in the endoplasmic reticulum pathway. Further KEGG enrichment analysis highlighted key biological processes, such as antigen processing and presentation, ubiquitin-mediated proteolysis, endoplasmic reticulum protein processing, and protein export. These results indicate that *M. galloprovincialis* copes with heat stress through post-translational protein modification mechanisms. Our findings provide preliminary insights into the molecular basis of thermal adaptation in *M. galloprovincialis* and establish a theoretical foundation for understanding the environmental adaptability of marine organisms in the context of global climate change.

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Key words

Mytilus galloprovincialis, Thermal stress, Transcriptome analysis, Thermal adaptation

INTRODUCTION

Ocean warming is reshaping the global marine ecosystem at an unprecedented rate. Since the Industrial Revolution, global average temperature has risen by about 1.5 °C compared to pre industrial levels (Hallegatte *et al.*, 2016; Johnson *et al.*, 2020). The IPCC predicts that by the end of this century, seawater temperatures will rise by 1.5 °C to 4 °C (Bongaarts, 2019). Various marine organisms gradually migrate to deeper waters and higher latitudes due to the continuous

rise in seawater temperature and frequent ocean heat waves (Hogg *et al.*, 2011). Additionally, Ocean warming can lead to abnormal metabolic rate, increase energy consumption, and large reproduction failure (Keith *et al.*, 2016). It has been demonstrated that ocean warming has negative impact on marine molluscs (Tate *et al.*, 2017). Pereira *et al.* (2020) also found that ocean warming significantly impact shell growth, lipid synthesis and survival rate of oyster. Thus, ocean warming not only reshapes the biogeographical pattern, but also affects various life activities such as survival, metabolism, reproduction, development, and immune response of marine organisms through cascading reactions of physical and chemical processes, seriously threatening the health and survival of marine organisms. Therefore, it is great significance to explore the adaptability of marine organisms and adaptive limits under global climate change by revealing the physiological, behavioral, and genetic mechanisms.

The temperature of intertidal seawater is highly susceptible to the impact of climate change, resulting in extreme temperature fluctuation, compared with deep sea area (Vafeiadou *et al.*, 2018). Intertidal marine organisms

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has attracted wide attention due to community building function, sensitive response to heat stress caused by sessile lifestyle and conservative evolution (Guo *et al.*, 2024). *Mytilus galloprovincialis* as an economically and ecologically important species, is mainly distributed in the North Atlantic, Mediterranean, Western Pacific, and Eastern Pacific (Oyarzún *et al.*, 2024). By virtue of their distinctive biological traits and considerable nutritional value, *M. galloprovincialis* occupy a significant place in China farmed shellfish industry with its main production area located along the northern coast. *M. galloprovincialis* typically inhabits hard substrates in the intertidal zone, such as rocks and reefs, where it providing habitats for various organisms. *M. galloprovincialis* eating and filtering behavior helps improve energy transfer and water filtration of intertidal ecosystem (Puri *et al.*, 2021). However, with the continuous expansion of aquaculture scale, issues such as increased mortality and decreased larval settlement rates caused by thermal stress have posed a serious threat to the health and sustainable development of China's shellfish farming industry. In recent years, mass mortality of mussels due to extreme heat events has further inflicted significant impact on the global mussel industry (Moyen *et al.*, 2020). Although the *M. galloprovincialis* is widely distributed, the sessile lifestyle limits its ability to avoid thermal stress through migration, which has also driven the evolution of unique environmental adaptation mechanisms. *M. galloprovincialis* lacks robust thermotolerance mechanisms (Feidantsis *et al.*, 2020). Our previous work demonstrated that high temperature could induce oxidative reaction and inflammatory reaction increase and biomolecules damage in mussels (Guo *et al.*, 2024). With the trend of global climate change, the high temperature pressure faced by *M. galloprovincialis* will become more. Wang *et al.* (2025) indicated that *M. coruscus* adapt high temperature through energy conversion, regulation signal transduction pathway and immune response (Wei *et al.*, 2025; Wang *et al.*, 2025). Therefore, exploration of the adaptive mechanism under extreme high temperature helps to comprehensively understand the physiological and ecological adaptation mechanisms of mussels, and provides important scientific basis for protection and management measures.

To decipher the initial molecular response of *M. galloprovincialis* to acute extreme heat, a condition increasingly encountered during summer low tides, we subjected mussels to 30 °C for 24 h. We then integrated physiological profiling with RNA-seq under extreme high temperature (30 °C) to clarify the heat response in *M. galloprovincialis* (Mesas *et al.*, 2015). The expression profiling of temperature-responsive gene, regulatory networks expand the molecular reference base for intertidal

heat-stress studies. These results provide genomics-based indicators for shellfish farmers, which can provide theoretical basis for the selection, breeding or adaptive management strategies of mussels under the increasingly severe marine heatwave.

MATERIALS AND METHODS

Experimental animals and experiment design

Mytilus galloprovincialis were collected from Zhangzi Island, Dalian, Liaoning Province in December. The *M. galloprovincialis* were acclimated in 10L tanks containing artificial seawater (with a salinity of 30 and continuous oxygen supply) at 18 °C for 7 days and fed with *Spirulina platensis* dry algae powder twice daily. After acclimation, 60 individuals (30±3.0 g, 5±1.5 cm) were randomly assigned to either control (18 °C) or heat stress (30 °C) groups in triplicate tanks (n= 30 per group) for 24 h. gill and mantle tissues were sampled from three individuals per group (three biological replicates) and stored at -80 °C for transcriptome analysis.

RNA extraction

Total RNA was extracted using TRIzol (Invitrogen, USA) and assessed with NanoDrop and Agilent 2100. mRNA was enriched using oligo(dT) beads, fragmented, and used for cDNA synthesis. Libraries were constructed using BGI's standard protocol and sequenced on the DNBSEQ-G50 platform (BGI, Shenzhen) with paired-end 150 bp reads. Each sample yielded ~6.4 Gb clean data, with Q20 > 95% and Q30 > 88%, ensuring adequate depth for differential expression analysis. Raw reads have been deposited in NCBI SRA under BioProject ID: PRJNA954971.

Sequence assembly, mRNA identification and functional annotation of differentially expressed genes (DEGs)

The reference genome index was constructed using HISAT2 (v2.2.1). Clean reads were aligned to this indexed reference genome employing HISAT2 (v2.2.1). Subsequently, StringTie (v2.2.1) was utilized to assemble, quantify, and merge the transcripts, incorporating both known and novel coding transcripts.

Differential expression analysis was carried out via DESeq2 (v1.4.5), with a significance threshold of P value < 0.05 and a fold change cutoff of > 1.5. The assembled and merged transcripts were annotated by eggNOG-mapper (v2.1.9). To explore phenotypic changes, KEGG enrichment analysis of the differentially expressed genes was performed using TBtools (v1.1.3.2), with pathway significance levels adjusted by P value to a stringent threshold (P value < 0.05). Throughout these processes,

all parameters were set to their default values, and TBtools was also employed to compute the statistics of the assembled transcriptome.

qRT-PCR

Eight target genes were selected for validation using qRT-PCR. β -actin was used as the internal reference gene, which has been previously validated as stable in *Mytilus* under thermal stress (Wang *et al.*, 2023; Wei *et al.*, 2025). Primers were designed using Primer Premier 5 and synthesized by Qingke Biotech (Table I). Reactions were performed in 20 μ L volumes using SYBR Green on a QuantStudio 3 system (Thermo Fisher). The reaction mixture consisted of 10 μ L of SYBR Green Premix Ex Taq II Fast qPCR (2 $\times$), 2 μ L of cDNA template, 6 μ L RNase-free water, and 1 μ L of each primer. The qRT-PCR program included an initial step at 95 $^{\circ}$ C for 30 s, followed by 40 cycles of 95 $^{\circ}$ C for 5 s and 60 $^{\circ}$ C for 10 s. Relative expression was calculated using the $2^{-\Delta\Delta C_t}$ method.

Statistical analysis

One-way ANOVA in PASW Statistics 18 (SPSS Inc.) was used to analysis statistical significance. All data were presented mean $\pm$ SD, and difference were considered statistically significant at $p < 0.05$. Plots were generated with the R package ggplot2 and polished in TB tools.

RESULT

Transcriptome analysis of *Mytilus galloprovincialis*

As shown in Table II, a total clean reads base for each sample ranging from 6.33Gb to 6.5Gb were obtained. The Q20 for all samples was between 95.26% and 96.23%, and Q30 was between 87.91% and 90.16%. The cleaned

reads have been released to the Sequence Read Archive (BioProject ID: SRR24150749).

Table I. Primers for qRT-PCR.

Gene	Primer 5' $\rightarrow$ 3'
<i>HSPA1s</i>	F: AGTAGACGTGGCTCCACTGTCC R: TGCTTACTGCTGGCTGGTTGTC
<i>HSPB1</i>	F: ACGGAAGAGGTCTGAGGATGGT R: AGCCATTGGTGCCCGGATAGTA
<i>DNAJB5</i>	F: TTGGCAGACCACGGCAATGTT R: GCACGTCTTCGATTGGCACTGA
<i>CRYAB</i>	F: CCAGTGCTTTGGAACCGCTACA R: CGCCTCCTACGCATTGCCATT
<i>SQSTM1</i>	F: GCCTGCCAAGGAGGTTCCAATG R: GGTCGGGTTGTTGTCGCTTTGA
<i>BIRC2_3</i>	F: AGACCAATCACCACGCCAGATG R: CGTCACCTGCTTCCCAGTTTCT
<i>SYNE1</i>	F: ACGAAGAAGGCACAGCAGTCAG R: TGCTGGCTGTTTCCTTCTGATCC
β -actin	F: GTGTGATGTCATATCCGTAAGGA R: GCTTGGAGCAAGTGCTGTGA

DEGs analysis

Figure 1 shows that a total of 2,673 differentially expressed genes (DEGs) were identified in gill tissues, comprising 555 upregulated and 1,008 downregulated genes. In mantle tissues, 1,288 DEGs were identified, comprising 286 upregulated and 402 downregulated genes. The volcano plot visually depicts the overall magnitude and directionality of the transcriptomic response in gill and mantle tissues under thermal stress.

Table II. Transcriptome sequencing project quality control summary.

Tissue	Group	Sample	Total raw reads (m)	Total clean reads (m)	Total clean bases (gb)	Clean reads Q20 (%)	Clean reads Q30 (%)	Clean reads ratio (%)
Gill	Control	C_G_1	48.93	43.19	6.48	95.93	89.42	88.27
		C_G_2	47.19	43.05	6.46	95.66	88.77	91.24
		C_G_3	47.19	42.6	6.39	95.9	89.35	90.28
	Heat	C_H_1	48.93	43.35	6.5	95.84	89.2	88.59
		C_H_2	48.93	42.24	6.34	96.19	90	86.31
		C_H_3	50.68	43.05	6.46	96.11	89.86	84.94
Mantle	Control	C_M_1	48.93	43.26	6.49	96	89.59	88.41
		C_M_2	47.95	42.25	6.34	95.26	87.91	88.12
		C_M_3	48.93	42.21	6.33	95.86	89.28	86.26
	Heat	H_M_1	50.68	43.25	6.49	95.96	89.5	85.33
		H_M_2	50.68	42.91	6.44	96.23	90.16	84.67
		H_M_3	52.43	43.31	6.5	95.73	88.97	82.6

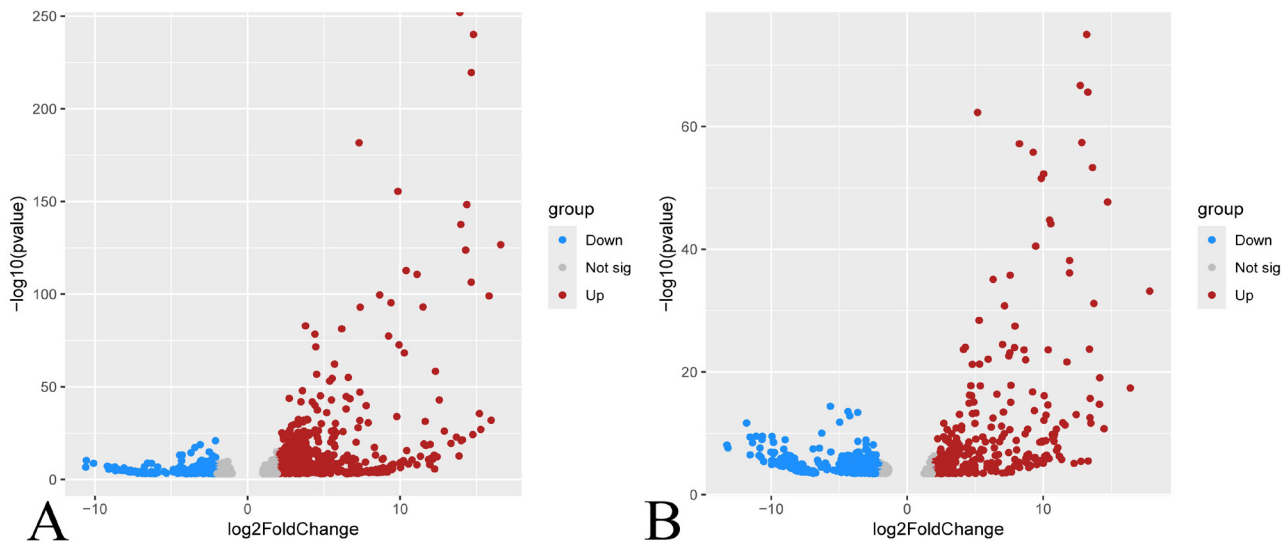


Fig. 1. The volcano plot of DEGs in gill (A) and mantle (B).

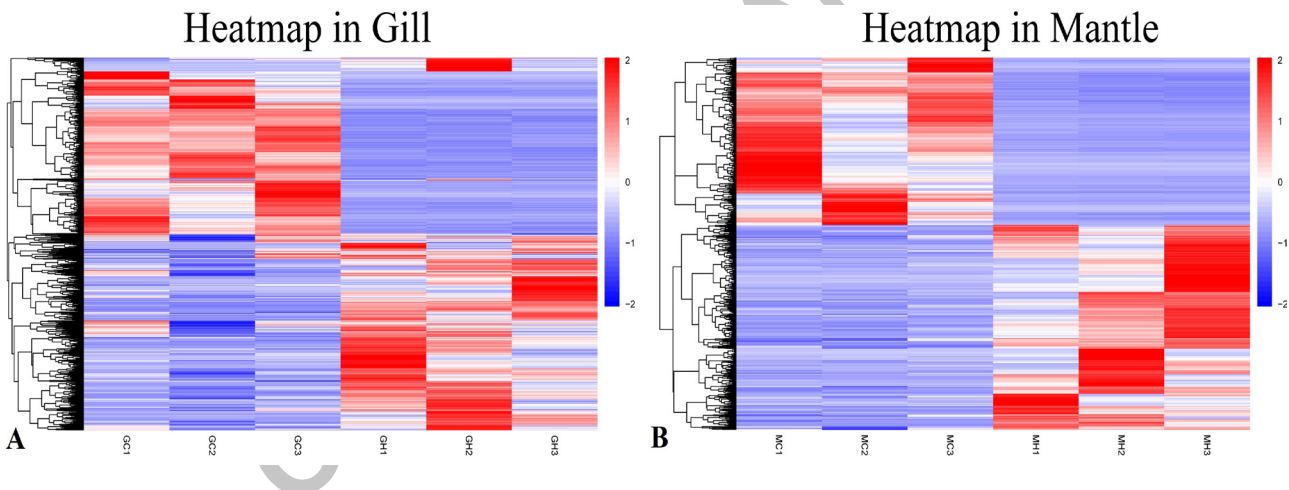


Fig. 2. DEGs heatmap of gill (A) and mantle (B).

As shown in Figure 2, further heatmap clustering analysis of differentially expressed genes revealed clear separation of gene expression patterns between control and heat treated groups in both gill and mantle tissues. This distinct group clustering demonstrates that 24 h treatment at 30 °C is sufficient to induce significant, tissue specific transcriptomic remodeling in both tissues.

DEGs enrichment

In the gill, 1563 out of 2673 differentially expressed genes (58.47 %) were successfully assigned KEGG orthology terms, whereas the mantle library yielded 688 annotated DEGs, representing 53.42 % of the 1 288 candidates (Table III).

Table III. KEGG annotation summary of differentially expressed genes (DEGs) in gill and mantle tissues.

Tissue	Total DEGs	Annotated DEGs (KEGG)	Percentage (%)
Gill	2673	1563	58.47
Mantle	1288	688	53.42

KEGG enrichment analysis (Fig. 3) revealed tissue specific stress response pathways. DEGs in mantle tissues were significantly enriched in pathways including apoptosis, protein processing in the endoplasmic reticulum, MAPK signaling, and IL-17 signaling (Fig. 3A), suggesting that the mantle may prioritize regulation of cell fate and

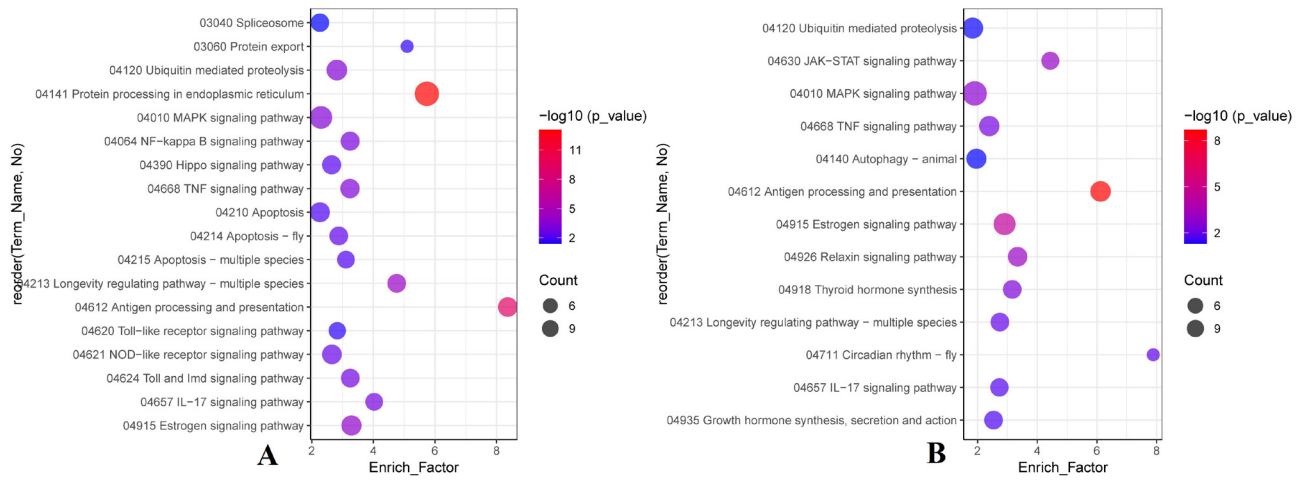


Fig. 3. KEGG enrichment analysis of DEGs. A, KEGG enrichment analysis results of DEGs in mantle. B, KEGG enrichment analysis results of DEGs in gill.

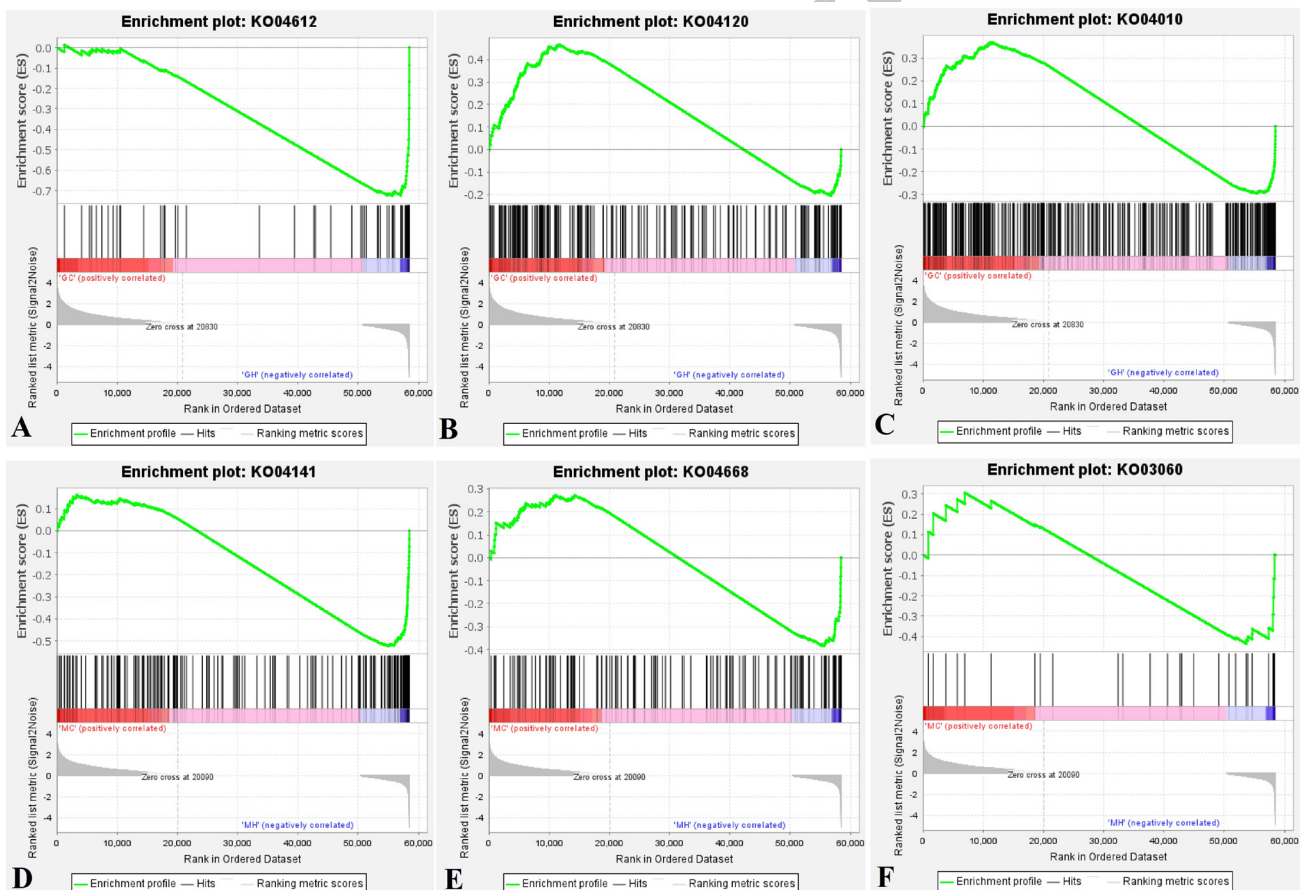


Fig. 4. The results of gene set enrichment analysis (GSEA) for different pathways. A, GSEA of genesets for antigen processing and presentation. B, GSEA of genesets for ubiquitin mediated proteolysis. C, GSEA of genesets for MAPK signaling pathway. D, GSEA of genesets for protein processing in endoplasmic reticulum. E, GSEA of genesets for TNF signaling pathway. F, GSEA of genesets for protein export.

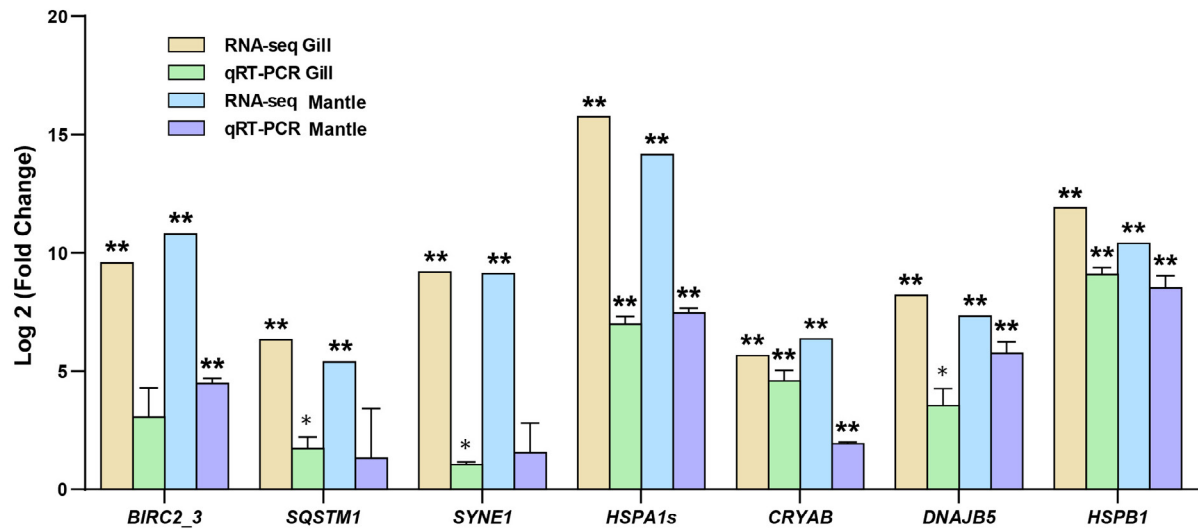


Fig. 5. qPCR results of DEGs.

inflammatory responses under thermal stress. In contrast, DEGs in gill tissues were significantly enriched in the TNF signaling pathway, JAK-STAT signaling pathway, ubiquitin-mediated proteolysis, and autophagy (Fig. 3B), indicating that immune activation and maintenance of protein homeostasis may be more central in gill tissues during heat stress responses.

Gene set enrichment analysis (GSEA) of DEGs

To comprehensively investigate the underlying mechanisms of the heat stress response, we performed GSEA. The results (Fig. 4) revealed that multiple key biological processes were significantly enriched after heat stress, including antigen processing and presentation, ubiquitin-mediated proteolysis, protein processing in the endoplasmic reticulum, MAPK signaling pathway, TNF signaling pathway, and protein export. GSEA further corroborated the findings of the KEGG enrichment analysis, indicating that the heat stress response in *M. galloprovincialis* involves a coordinated network of immune activation, protein quality control, and cellular signal transduction.

Validation of DEGs analysis

To validate our RNA seq results, we selected seven representative DEGs (BIRC2_3, SQSTM1, SYNE1, HSPA1, CRYAB, DNAJB5, HSPB1) for qRT-PCR analysis. As shown in Figure 5, the expression patterns of these genes were highly consistent with the RNA seq data. These results confirm the accuracy of our transcriptomic data and underscore the important roles of these genes in the heat stress response of *M. galloprovincialis*.

DISCUSSION

An analysis of the transcriptomic data can help identify the genes differentially expressed to significant levels under conditions of heat-induced stress, thus enabling a deeper understanding of the functions of the expanded genes related to heat-stress resistance. It is well-known that the gills and the mantle are crucial in providing bivalve molluscs with environmental adaptability (Luckenbach and Epel, 2008; Malachowicz and Wenne, 2019). Gills are in direct contact with the external aquatic environment and are considered an important tissue in contact with the external environment, participating in processes such as gas exchange, ion regulation, osmoregulation, acid-base balance, ammonia excretion, immune defense, and feeding (Zhou *et al.*, 2024; Cannuel *et al.*, 2009; de Oliveira David *et al.*, 2008). The periostracum the outermost, water-resistant coating of bivalve shells first appears at the base of the periostracal groove that separates the outer and middle mantle folds. It fulfils two pivotal roles: (a) provision of an organic framework on which calcium-carbonate crystals nucleate during shell biomineralisation, and (b) protection of the underlying shell against environmental insults. Nevertheless, the precise cellular origin and differentiation pathways that give rise to this secretion are still only fragmentarily understood (Salas *et al.*, 2022). Thus, maintaining the proper function of both organs is vital for resistance to heat-induced stress in *M. galloprovincialis*.

Transcriptomic analysis reveals that the expression of the expanded genes related to heat-induced stress resistance was generally up-regulated. For example, the genes related to protein folding mentioned earlier are significantly up-

regulated in at least one type of tissue, with none being notably down-regulated. These results indicated that the genes associated with protein folding played a substantial role in the response to heat-induced stress. Furthermore, various genes in the six pathways of the two organizations mentioned earlier also played a role. When temperatures rise, *M. coruscus* mobilizes an integrated network that encompasses ribosomal, endocytic, ER-stress, proteolytic and lysosomal modules to defend proteostasis. Transcriptional up-regulation of central protectors such as Hsp70, Hsp20, Hsp110, the lysosomal marker LAMP, the ER chaperone BiP and the anti-apoptotic factor BIRC7 equips the mussel to buffer thermal insult and prolong survival (Wang *et al.*, 2023b). HSPs enhance protein stability by helping other proteins to not unfold during heat stress (Xu *et al.*, 2020). The cells synthesis misfolded proteins under heat-induced stress, affecting their normal function (Droll *et al.*, 2013). Under heat stress, Heat shock heat 70kda protein family (HSP70) members prioritize the renaturation of mis-folded polypeptides and, in concert with degradation systems, eliminate irreversibly damaged proteins to preserve cellular proteostasis (Mathangasinghe *et al.*, 2021). While most transcripts plateau or decline during metabolic depression, loci linked to the heat-shock and oxidative-stress responses, exemplified by HSP70 and CYP450, continue to be actively transcribed and maintain elevated mRNA levels. This persistent up-regulation is believed to equip intertidal mussels with the cytoprotective capacity needed to endure the sharply fluctuating and often extreme thermal regimes characteristic of rocky-shore habitats (Wang *et al.*, 2023a). The up-regulation of HSP70 expression in the results indicates its crucial role in responding to heat stress. Heat shock protein family A (Hsp70) member 5 (HSPA5) modulates cellular metabolism with a noted impact on lipid pathways (Rehati *et al.*, 2023). Moreover, the protein expressed by the HSPA5 gene is BiP, which is typically located in the endoplasmic reticulum (ER). In the Gill group and Mantle group discussed earlier, the HSPA5 gene is present and up-regulated in both the endoplasmic reticulum pathway for protein processing and the MAPK signaling pathway, thereby enabling the expression of more BiP, thus indicating its role in the response to heat stress.

In addition, our results also revealed that inflammatory pathways in both gill and mantle tissues was significantly activated. The significant enrichment of the TNF and JAK-STAT signaling pathways in gills, and the IL-17 signaling pathway in the mantle, suggests that thermal stress is perceived not only as a proteotoxic insult but also as an event triggering sterile inflammation (Fig. 3). The up-regulation of genes within these pathways may serve to alert the immune system, orchestrate the clearance of

damaged cells, and prime tissue for repair, highlighting a previously underappreciated axis of thermotolerance in bivalves (Rosani *et al.*, 2015; Sun *et al.*, 2025).

Our results indicate that the BIRC7 gene also plays an important role in responding to thermal stress. the BIRC7 gene is a key gene and is upregulated in both the Gill group and the Mantle group. Concomitantly, the significant enrichment of the apoptosis pathway in the mantle and the up-regulation of anti-apoptotic genes like BIRC7 (Fig. 5) point to a critical cellular fate decision process under heat stress. The co-activation of pro-inflammatory and anti-apoptotic signals suggests a coordinated strategy to limit tissue damage by suppressing excessive programmed cell death while initiating inflammatory signals necessary for homeostasis restoration. This delicate balance between survival and death pathways could be a key determinant of individual resilience (Wang *et al.*, 2023).

Comparative perspective with a congeneric species. When contrasted with transcriptomic studies on the heat response of *Mytilus coruscus* (Wang *et al.*, 2025; Wei *et al.*, 2025), intriguing differences emerge. While both species activate stress-responsive chaperones, *M. coruscus* under similar acute stress shows a more prominent enrichment in pathways related to energy metabolism and general signal transduction. In contrast, our data on *M. galloprovincialis* reveal a more striking and immediate upregulation of specific immune (TNF, JAK-STAT, IL-17) and apoptotic pathways. This divergence may suggest species-specific strategic emphases: *M. galloprovincialis* might rely more heavily on rapid immune activation and precise apoptosis control as a first line of defense against acute thermal injury, possibly reflecting adaptations to its specific intertidal thermal history. A dedicated comparative transcriptomics study across multiple mussel species would be invaluable to unravel the evolutionary landscape of thermal adaptation strategies in this ecologically vital group.

Our study provides the first transcriptomic profile of 24 h of heat stress response at 30°C in *M. galloprovincialis*. This focused design allows us to define the core early-response network to an extreme temperature. Future studies incorporating thermal gradients (e.g., 26, 32, 34°C) and time-series sampling (e.g., 6, 48, 96 h, including recovery) will be essential to delineate the dynamics of these pathways, identify potential tipping points, and elucidate the progression from acute response to chronic adaptation or injury.

To summarize, comparing gene families and transcriptomic analyses suggested that a few of the expanded genes mentioned previously were up-regulated and played an adaptive role under heat-induced stress. In addition, this may be one of the reasons for the appreciable

heat adaptability of *M. galloprovincialis*.

CONCLUSIONS

This study clarifies the transcriptomic responses of *M. galloprovincialis* to heat stress, uncovering a complex gene-pathway network supporting thermal adaptability. It demonstrates the mussel modulates immune, protein degradation and signal transduction genes to tolerate high temperatures. The identified key genes and pathways guide aquaculture practice, which aiding in the cultivation of heat-tolerant varieties and optimizing culture strategies under global warming. This research deepens understanding of thermal adaptability of intertidal mussels, offering insights to enhancing the climate resilience of marine species and ensuring the sustainability of aquatic resources in the context of global climate change.

DECLARATIONS

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IRB approval

The animal study protocol was approved by the Institutional Animals Care and Use Committee of Zhejiang Ocean University (protocol code 2026185 and date of approval: September 1, 2025).

Data availability

The complete sequencing datasets are publicly accessible through the NCBI SRA under accession numbers PRJNA922052 (genomic) and PRJNA954971 (transcriptomic). The datasets supporting the conclusions of this article will be publicly available upon article acceptance.

Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Statement of conflict of 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.

REFERENCES

- Bongaarts, J., 2019. Intergovernmental panel on climate Changespecial report on global warming of 1.5°C Switzerland: IPCC, 2018. *Popul. Dev. Rev.*, **45**: 251-252. <https://doi.org/10.1111/padr.12234>
- Cannuel, R., Beninger, P.G., McCombie, H. and Boudry, P., 2009. Gill development and its functional and evolutionary implications in the blue mussel *Mytilus edulis* (Bivalvia: Mytilidae). *Biol. Bull.*, **217**: 173-188. <https://doi.org/10.1086/BBLv217n2p173>
- De-Oliveira, D.J.A., Salaroli, R.B. and Fontanetti, C.S., 2008. Fine structure of *Mytella falcata* (Bivalvia) gill filaments. *Micron*, **39**: 329-336. <https://doi.org/10.1016/j.micron.2007.06.002>
- Droll, D., Minia, I., Fadda, A., Singh, A., Stewart, M., Queiroz, R. and Clayton, C., 2013. Post-transcriptional regulation of the trypanosome heat shock response by a zinc finger protein. *PLoS Pathog.*, **9**: e1003286. <https://doi.org/10.1371/journal.ppat.1003286>
- Feidantsis, K., Giantsis, I.A., Vratsistas, A., Makri, S., Pappa, A.Z., Drosopoulou, E., Anestis, A., Mavridou, E., Exadactylos, A., Vafidis, D. and Michaelidis, B., 2020. Correlation between intermediary metabolism, Hsp gene expression, and oxidative stress-related proteins in long-term thermal-stressed *Mytilus galloprovincialis*. *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, **319**: R264-R281. <https://doi.org/10.1152/ajpregu.00066.2020>
- Guo, S., Li, J., Yang, X., Qin, Y., Zhao, Y., Wei, J., Ma, H., Yu, Z., Zhao, L. and Zhang, Y., 2024. Resistance of an intertidal oyster (*Saccostrea mordax*) to marine heatwaves and the implication for reef building. *Sci. Total Environ.*, **928**: 172474. <https://doi.org/10.1016/j.scitotenv.2024.172474>
- Hallegatte, S., Rogelj, J., Allen, M., Clarke, L., Edenhofer, O., Field, C.B., Friedlingstein, P., Van Kesteren, L., Knutti, R., Mach, K.J., Mastrandrea, M., Michel, A., Minx, J., Oppenheimer, M., Plattner, G.K., Riahi, K., Schaeffer, M., Stocker, T.F. and Van Vuuren, D.P., 2016. Mapping the climate change challenge. *Nat. Climate Change*, **6**: 663-668. <https://doi.org/10.1038/nclimate3057>
- Hogg, O.T., Barnes, D.K. and Griffiths, H.J., 2011.

- Highly diverse, poorly studied and uniquely threatened by climate change: An assessment of marine biodiversity on South Georgia's continental shelf. *PLoS One*, **6**: e19795. <https://doi.org/10.1371/journal.pone.0019795>
- Johnson, G.C. and Lyman, J.M., 2020. Warming trends increasingly dominate global ocean. *Nat. Climate Change*, **10**: 757. <https://doi.org/10.1038/s41558-020-0822-0>
- Keith, S.A., Maynard, J.A., Edwards, A.J., Guest, J.R., Bauman, A.G., Van Hooidonk, R., Heron, S.F., Berumen, M.L., Bouwmeester, J., Piromvaragorn, S., Rahbek, C. and Baird, A.H., 2016. Coral mass spawning predicted by rapid seasonal rise in ocean temperature. *Proc. Biol. Sci.*, **283**. <https://doi.org/10.1098/rspb.2016.0011>
- Luckenbach, T. and Epel, D., 2008. ABCB- and ABCC-type transporters confer multixenobiotic resistance and form an environment-tissue barrier in bivalve gills. *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, **294**: R1919-1929. <https://doi.org/10.1152/ajpregu.00563.2007>
- Malachowicz, M. and Wenne, R., 2019. Mantle transcriptome sequencing of *Mytilus* spp. and identification of putative biomineralization genes. *PeerJ*, **6**: e6245. <https://doi.org/10.7717/peerj.6245>
- Mathangasinghe, Y., Fauvet, B., Jane, S.M., Goloubinoff, P. and Nillegoda, N.B., 2021. The Hsp70 chaperone system: Distinct roles in erythrocyte formation and maintenance. *Haematologica*, **106**: 1519-1534. <https://doi.org/10.3324/haematol.2019.233056>
- Mesas, A. and Tarifeño, E., 2015. Temperaturas letales superiores para el mejillón, *Mytilus galloprovincialis* (Lamarck, 1819), en la costa de Chile central. *Latin Am. J. Aquat. Res.*, **43**: 473-483.
- Moyen, N.E., Somero, G.N. and Denny, M.W., 2020. Mussel acclimatization to high, variable temperatures is lost slowly upon transfer to benign conditions. *J. exp. Biol.*, **223**. <https://doi.org/10.1242/jeb.222893>
- Oyarzún, P.A., Toro, J.E., Nuñez, J.J., Ruiz-Tagle, G. and Gardner, J.P.A., 2024. The Mediterranean mussel *Mytilus galloprovincialis* (Mollusca: Bivalvia) in Chile: Distribution and genetic structure of a recently introduced invasive marine species. *Animals* (Basel), **14**. <https://doi.org/10.3390/ani14060823>
- Pereira, R.R.C., Scanes, E., Gibbs, M., Byrne, M. and Ross, P.M., 2020. Can prior exposure to stress enhance resilience to ocean warming in two oyster species? *PLoS One*, **15**: e0228527. <https://doi.org/10.1371/journal.pone.0228527>
- Puri, V., Juan, M., Catarina, R.O., Leandro, S. and Rubal, M., 2021. Public perception of ecosystem services provided by the Mediterranean mussel *Mytilus galloprovincialis* related to anthropogenic activities. *PeerJ*, **9**: e11975. <https://doi.org/10.7717/peerj.11975>
- Rehati, A., Abuduaini, B., Liang, Z., Chen, D. and He, F.P., 2023. Identification of heat shock protein family A member 5 (HSPA5) targets involved in nonalcoholic fatty liver disease. *Genes Immun.*, **24**: 124-129. <https://doi.org/10.1038/s41435-023-00205-y>
- Rosani, U., Varotto, L., Gerdol, M., Pallavicini, A. and Venier, P., 2015. IL-17 signaling components in bivalves: Comparative sequence analysis and involvement in the immune responses. *Dev. Comp. Immunol.*, **52**: 255-268. <https://doi.org/10.1016/j.dci.2015.05.001>
- Salas, C., Bueno-Pérez, J.D., López-Téllez, J.F. and Checa, A.G., 2022. Form and function of the mantle edge in Protobranchia (Mollusca: Bivalvia). *Zoology (Jena)*, **153**: 126027. <https://doi.org/10.1016/j.zool.2022.126027>
- Sun, B., Lan, X., Bock, C., Shang, Y., Hu, M. and Wang, Y., 2025. Effects of ocean acidification and warming on apoptosis and immune response in the mussel *Mytilus coruscus*. *Fish Shellfish Immunol.*, **158**: 110134. <https://doi.org/10.1016/j.fsi.2025.110134>
- Tate, R.D., Benkendorff, K., Ab Lah, R. and Kelaher, B.P., 2017. Ocean acidification and warming impacts the nutritional properties of the predatory whelk, *Dicathais orbita*. *J. exp. Marine Biol. Ecol.*, **493**: 7-13. <https://doi.org/10.1016/j.jembe.2017.03.006>
- Vafeiadou, A.M., Bretaña, B.L.P., Van Colen, C., Dos Santos, G.A.P. and Moens, T., 2018. Global warming-induced temperature effects to intertidal tropical and temperate meiobenthic communities. *Mar. Environ. Res.*, **142**: 163-177. <https://doi.org/10.1016/j.marenvres.2018.10.005>
- Wang, H., Wei, B., Dong, Z., Xin, Z., Lin, H., Zhang, W., Ye, Y., Liao, Z., Wang, W., Yan, X., Guo, B. and Song, M., 2025. Whole transcriptomic analysis reveals the lncRNA-miRNA-mRNA regulatory mechanism underlying the heat-hardening formation in *Mytilus coruscus*. *Comp. Biochem. Physiol. Part D: Genom. Proteom.*, **55**: 101468. <https://doi.org/10.1016/j.cbd.2025.101468>
- Wang, J., Ma, L.X. and Dong, Y.W., 2023. Coping with harsh heat environments: Molecular adaptation of metabolic depression in the intertidal snail

- Echinolittorina radiata*. *Cell Stress Chaper.*, **28**: 477-491. <https://doi.org/10.1007/s12192-022-01295-9>
- Wang, Y.X., Lin, S.R., Xu, L.Z., Ye, Y.Y., Qi, P.Z., Wang, W.F., Buttino, I., Li, H.F. and Guo, B.Y., 2023. Comparative transcriptomic analysis revealed changes in multiple signaling pathways involved in protein degradation in the digestive gland of *Mytilus coruscus* during high-temperatures. *Compar. Biochem. Physiol. D: Genom. Proteom.*, **46**. <https://doi.org/10.1016/j.cbd.2023.101060>
- Wei, B., Wang, H., Dong, Z., Xin, Z., Zhang, W., Lin, H., Wang, W., Song, M. and Guo, B., 2025. Transcriptomic analysis reveals the survival strategies of *Mytilus coruscus* under short-term rising seawater temperatures. *Comp. Biochem. Physiol. D: Genom. Proteom.*, **55**: 101481. <https://doi.org/10.1016/j.cbd.2025.101481>
- Xu, J., Fang, M., Li, Z., Zhang, M., Liu, X., Peng, Y., Wan, Y. and Chen, J., 2020. Third-generation sequencing reveals LncRNA-regulated HSP genes in the *Populus × Canadensis* Moench heat stress response. *Front. Genet.*, **11**: 249. <https://doi.org/10.3389/fgene.2020.00249>
- Zhou, T., Meng, Q., Sum, R., Xu, D., Zhu, F., Jia, C., Zhou, S., Chen, S. and Yang, Y., 2024. Structure and gene expression changes of the gill and liver in juvenile black porgy (*Acanthopagrus schlegelii*) under different salinities. *Comp. Biochem. Physiol. D: Genom. Proteom.*, **50**: 101228. <https://doi.org/10.1016/j.cbd.2024.101228>