Integrated physiological, transcriptomics and metabolomics analyses provide insights into thermal resistance of razor clam, *Sinonovacula constricta*

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1. Introduction

- Extreme high temperature is believed as a major inducer to summer mortality syndrome in molluscs, which frequently occurs and has caused serious economic losses.
- *S. constricta* is a typical mudflat bivalve that lived in estuarine and intertidal zone with limited range of movement. This lifestyle makes razor clam vulnerable to water temperature and climate change.
- The abundance of pathogenic microorganisms is increased in coastal areas in response to warming, which inevitably challenge the growth and reproduction of razor clams.
- Gill, as the primary respiration and metabolism organ, plays a critical role in mollusk physiological homeostasis under environmental adaptation.
- In this study, we investigated the response mechanism of the clams at 32℃ for 24 h and 15 d, with a combined physiological, transcriptomic and untargeted metabolomic analysis.

2. Methods

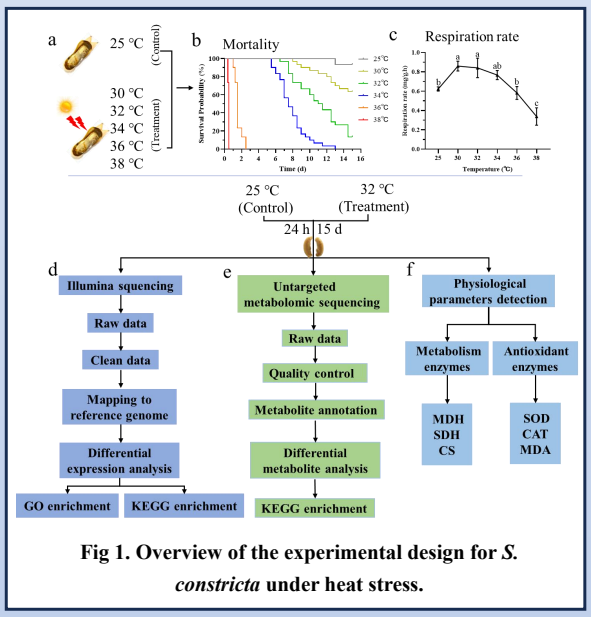


Fig 1. Overview of the experimental design for *S. constricta* under heat stress.

- Firstly, the mortality and respiration rate of razor clams were measured under different temperatures (Fig 1a, b, c).
- According to the results of Kaplan-Meier cumulative survival curves and respiration rate, 32°C was chosen as heat stress.
- To validate the RNA-seq and metabolome data, gene expression and the activities of metabolic enzymes were detected (Fig 1d, e, f).

3. Results

◆ Transcriptomics analysis under heat stress

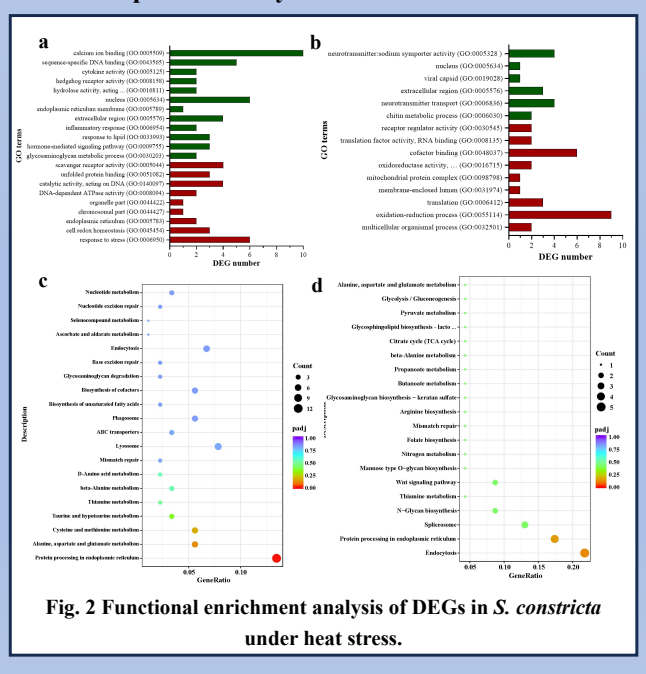


Fig. 2 Functional enrichment analysis of DEGs in *S. constricta* under heat stress.

- ER-related signal pathways were significantly enriched in both KEGG pathway and GO enrichment after heat stress 24 h.
- GO terms showed that cell homeostasis process, energy metabolism and ion transport processes were enriched at 24 h. Oxidation-reduction process, translation and transport process were significantly enriched at 15d (Fig. 2a, b).

- KEGG enrichment showed that amino acid metabolism, folding, sorting and degradation and transport and catabolism pathways were significantly enriched with DEGs under heat stress 24 h. DEGs were mainly enriched in pathways of carbohydrate metabolism, amino acid metabolism and glycan biosynthesis and metabolism at 15 d (Fig. 2c, d).

◆ ER quality control of clams to against short term heat stress

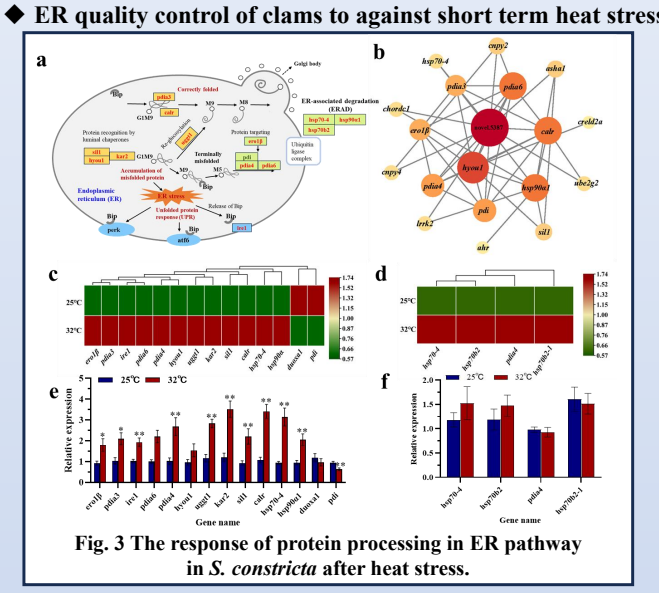


Fig. 3 The response of protein processing in ER pathway in *S. constricta* after heat stress.

- Protein processing in ER was the most significantly affected pathway at 24 h, including 11 upregulated and 1 downregulated DEGs (Fig 3a).
- PPI analysis showed *hyou1*, *hsp90a1*, *calr and pdis* were identified as the hub genes at 24 h (Fig 3b).
- The DEGs exhibited consistent expression patterns between RNA-seq (Fig 3c, d) and qRT-PCR (Fig 3e, f).

◆ Metabolomics analysis under heat stress

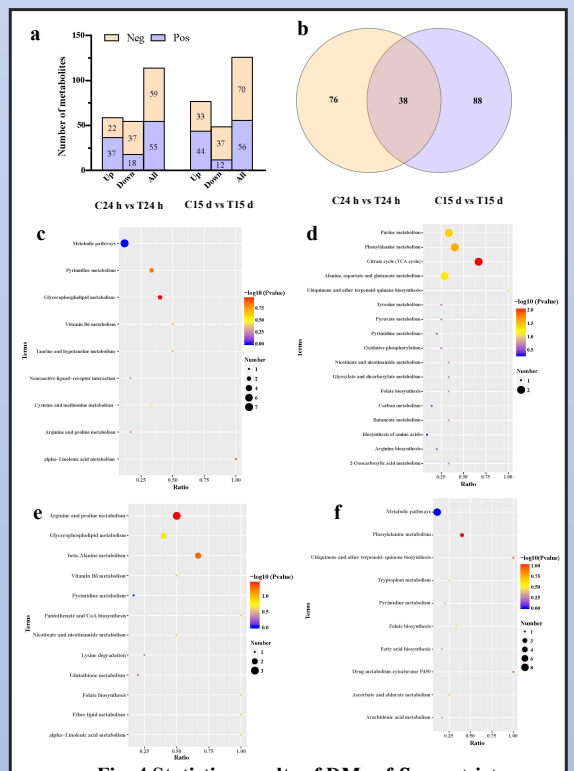


Fig. 4 Statistics results of DMs of *S. constricta* under heat stress.

- Glycerophospholipid metabolism and TCA cycle pathways were remarkable enriched at 24 h (Fig 4 c, d) .
- Arginine and proline metabolism pathway, glycerophospholipid metabolism phenylalanine metabolism pathway and beta-alanine metabolism pathway were significantly enriched at 15 d (Fig 4 e, f) .

◆ Clustering analysis of changes for DMs after heat stress

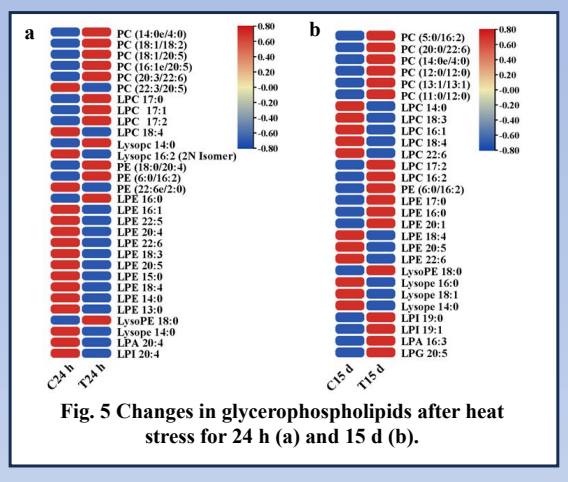


Fig. 5 Changes in glycerophospholipids after heat stress for 24 h (a) and 15 d (b).

◆ Metabolomics analysis under heat stress

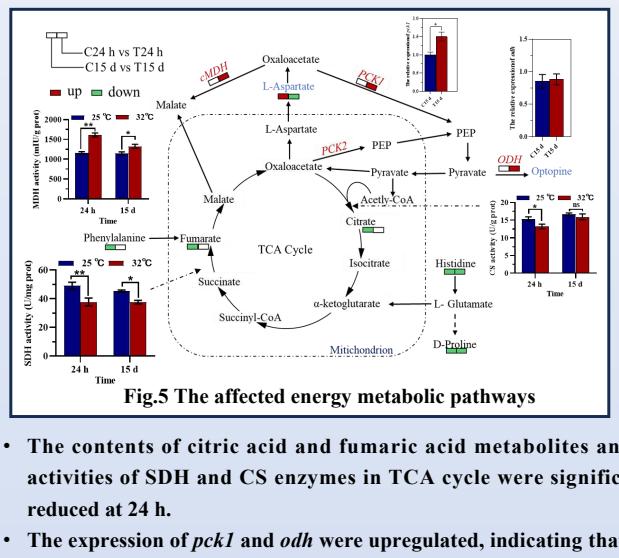


Fig.5 The affected energy metabolic pathways

- The contents of citric acid and fumaric acid metabolites and the activities of SDH and CS enzymes in TCA cycle were significantly reduced at 24 h.
- The expression of *pck1* and *odh* were upregulated, indicating that heat stress may limit the aerobic capacities of the razor clams at 15 d.

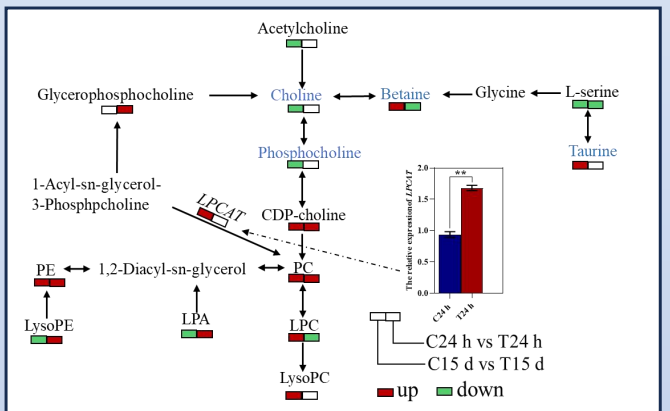


Fig.6 The regulated membrane lipid pathways.

- The metabolite content of PCs and PEs were elevated after heat stress for 24 h and 15 d.
- Content of CDP-choline accumulated with increasing water temperature, which may be due to that high temperature stress decreased the cell membrane fluidity.
- Serine can function as a modifying group in the synthesis of phosphatidylserine and indirect regulates the cellular metabolism and maintains the cell membrane structure stability.

◆ Effects of heat stress on physiological parameters

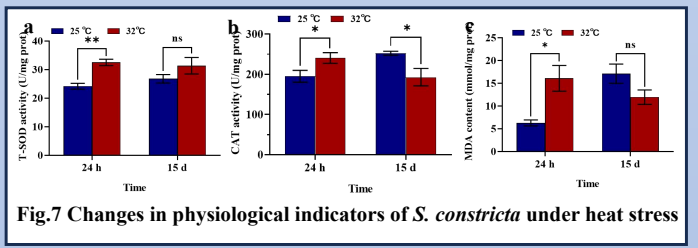
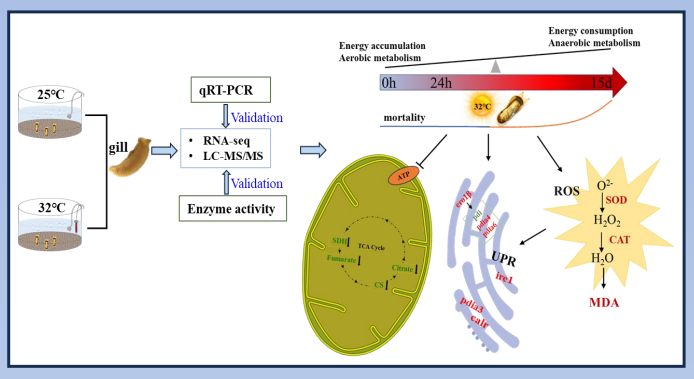


Fig.7 Changes in physiological indicators of *S. constricta* under heat stress

4. Conclusions

- Heat stress interfered clams ER protein homeostasis and cell membrane fluidity.
- TCA cycle and fatty acid synthesis was suppressed of clams exposed to 32℃ for 24 h.
- Heat stress inhibited lipid metabolism, enhanced antioxidant capacity of clams.
- Clam switched from aerobic to anaerobic metabolism with the extension of stress time.



■ Bibliography

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