The Influencing Mechanism of Heat Stress on Parent *Eriocheir sinensis* under Histology, Physiology and Molecular Biology

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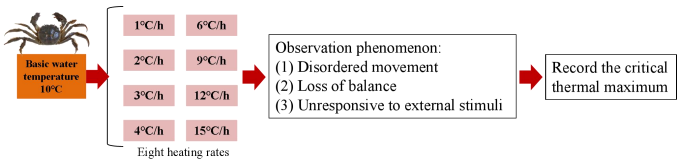
Introduction

The extreme heat weather occurs frequently and water temperature increases obviously in the influence of global warming. Heat stress can damage tissue structure and destroy the antioxidant defence in aquatic animals. Moreover, the response of animals to heat stress also includes the rapid changes in gene expression and the synthesis of proteins involved in environmental adaptation. At present, the integrative analysis of the transcriptomics and proteomics has become a research hotspot in the response of complex molecules to heat stress. *Eriocheir sinensis*, a representative crab in crustaceans, belongs to an aquatic ectotherm which is sensitive to the change of water temperature, especially parent crabs in reproduction period. Meanwhile, the studies of acute heat stress have shown that high temperature can affect antioxidant activity, immune defense and metabolic ability, leading to the disorder of free radical metabolism. However, most studies on heat stress of *E. sinensis* focused on the juvenile crabs, which can't well reveal the response mechanism of high temperature on *E. sinensis*.

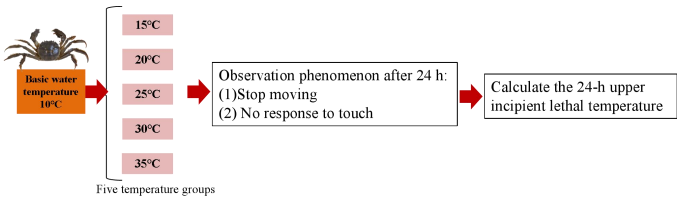
Materials and Methods

Determination of thermal tolerance

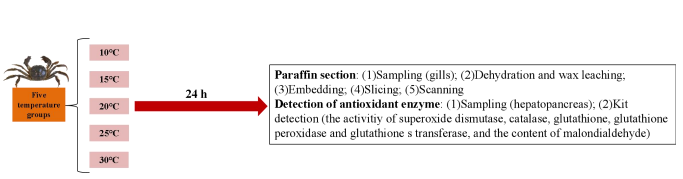
- The critical temperature method



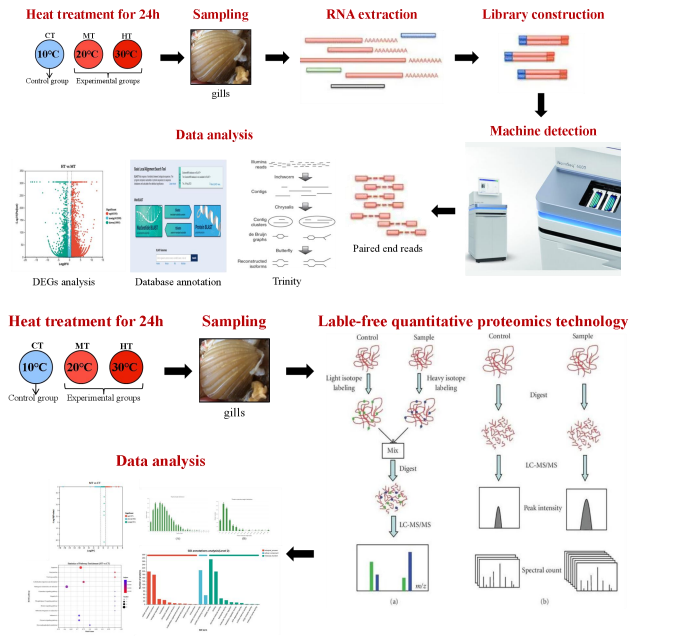
- The lethal temperature method



Paraffin section and detection of antioxidant enzyme



Transcriptomic and proteomic profiling

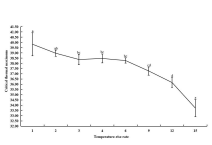


Quantitative real-time polymerase chain reaction

Gene Name	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')	Gene Name	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')
β-actin	GGCATCCACGAGACCACTT	CTCCTGCTTGCTGATCCACAT			
PIK3CA	GCTCAGCATAGGCATAAT	AGGAGATACGCCAAATAG	*EIF4*	ATCCACGACACCCTACAAG	AGCCCAGTCATTCACTAACA
CASP7	GATGCTGGAGGACAGGGAT	TAGCGATGGTTGAAGATG	*ATPase*	CGCCCATCCTCAACTCCT	CTCCACCAAACAGACCAATC
HSP70	CCAGCGTCAAGCAACCAA	TCAACTTCACGGCGGAGC	*V-ATPase*	CCAGAACCAGCGTTACCA	CTCCTCTACCCTCAGCATC
HSP90	AGGTCGTCCGAGTCCACA	GTCGCTGCTCTTCATTCC	*ALF*	TAGTAGTTCCTGGCTGTTCCC	CCTCTATCGGCTGGTTCTG

Results

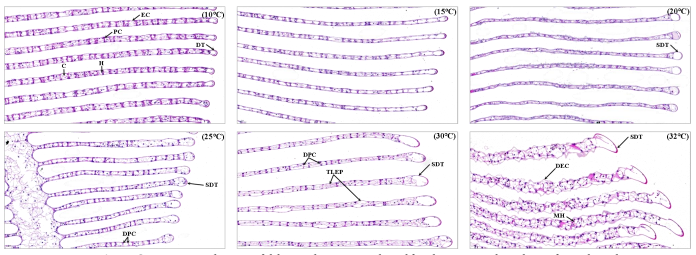
Determination of thermal tolerance



	10°C	15°C	20°C	25°C	30°C	32°C	35°C
Actual water temperature/°C	10.40 ± 0.32	14.99 ± 0.23	19.87 ± 0.27	25.14 ± 0.20	30.02 ± 0.15	31.96 ± 0.24	35.17 ± 0.12
Dissolved oxygen/mg·L^{-1}	8.10 ± 0.72^{a}	6.99 ± 0.17^{a}	6.88 ± 0.20^{a}	6.18 ± 0.24^{a}	4.49 ± 0.37^{a}	4.58 ± 0.44^{a}	2.57 ± 0.46^{b}
Mortality rate/%	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	5.56 ± 9.62^{c}	11.11 ± 9.62^{c}	44.45 ± 19.24^{b}	100.00 ± 0.00^{a}

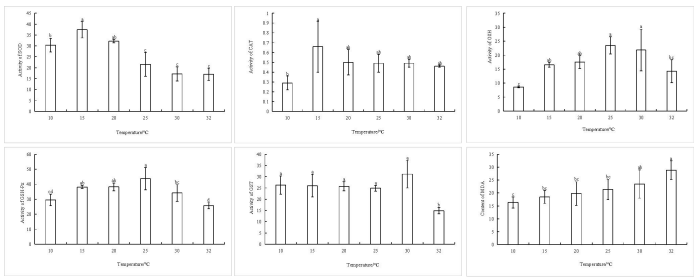
The critical thermal maximum of parent crabs increased with the decrease of the heating rates, and the maximum CTM reached 39.83 ± 1.35℃ at the heating rate of 1 ℃·h^{-1}. The 24-h upper incipient lethal temperature was 31.26℃.

Paraffin section



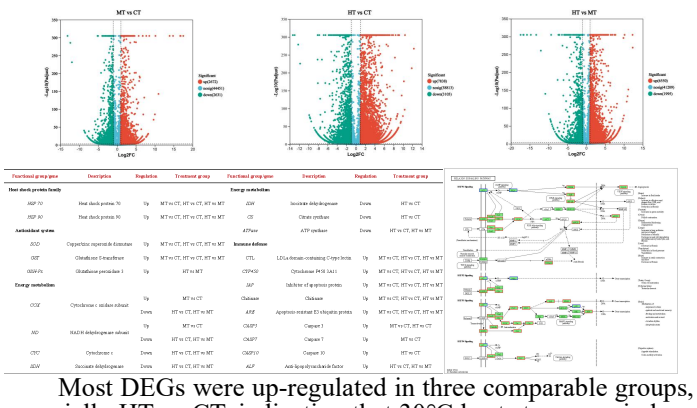
At 15-25℃, the gills showed slight pathological changes. At 30-32℃, the gills showed obvious pathological changes, and the structure was seriously damaged.

Antioxidant system



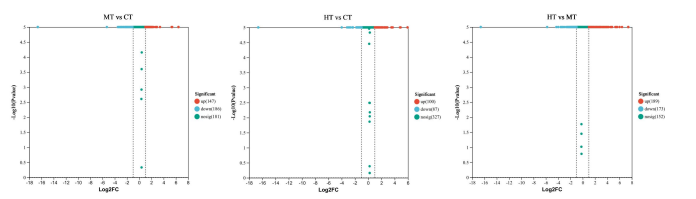
The activity of SOD, CAT, GSH and GSH-Px increased at 15℃-25℃. The activities of SOD, GST and GSH-Px decreased significantly, increasing the content of MDA sharply at 30℃-32℃.

Comparison and enrichment analysis of DEGs



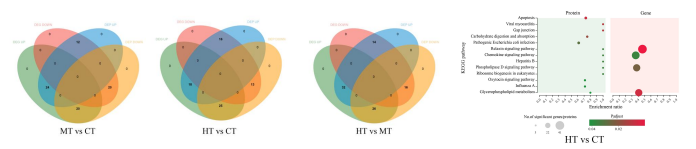
Most DEGs were up-regulated in three comparable groups, especially HT vs CT, indicating that 30℃ heat stress can induce more genes in the gills of *E. sinensis*. DEGs related to heat shock protein family, antioxidant system, energy metabolism and immune defense were significantly differentially expressed. Relaxin signaling pathway was significantly enriched with abundant up-regulated DEGs under 30℃ stress.

Comparison and enrichment analysis of DEPs



The number of up-regulated DEPs in MT vs CT was slightly higher than that in HT vs CT, indicating that 20℃ stress can induce more proteins in the gills of *E. sinensis*. DEPs related to heat shock protein family, antioxidant system and energy metabolism were significantly differentially expressed.

Integrative analysis of transcriptome and proteome



76 associated differentially expressed genes and proteins were associated. Notably, 24 co-expressed genes and proteins were up-regulated, such as HSP70 and GST. In HT vs CT, relaxin signaling pathway were significantly enriched at gene and protein levels.

Conclusions

The thermal tolerance of parent crab

The thermal tolerance of parent crabs was better at **a lower heating rate (1-2 ℃·h^{-1})**, which can provide sufficient time for parent crabs to adapt to changes in external water temperature.

Regulation mechanism at physiological level

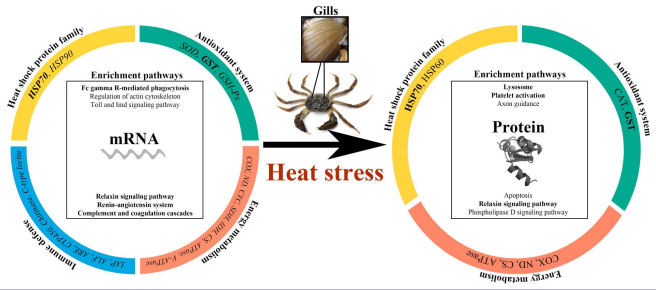
In the face of heat stress (15-25℃), parent crabs can alleviate the degree of tissue damage by increasing the activity of antioxidant enzymes and decreasing the increased content of MDA, so as to reduce the mortality of parent crabs through internal adjustment to the water environment. However, **high temperature stress over 30℃ can lead to the decrease of antioxidant enzyme activity, the sudden increase of MDA content, serious tissue damage**, and body can not adapt to the high temperature in a short time, which will lead to a large increase in the death rate of parent crabs.

Regulation mechanism at molecular level

HSP70 plays an important role in combating heat stress, and the highly expressed HSP70 gene can inhibit the generation of oxygen radical and the apoptosis rate induced by heat stress. HSP70 was up-regulated at both the gene and protein levels under heat stress, and the expression of HSP70 was significantly higher at 30℃ than 20℃, indicating that **the highly expressed HSP70 is involved in the regulatory mechanism of heat stress, especially higher temperature.**

The antioxidant system is also an important component of resistance to oxidative damage caused by heat stress. GST was co-up-regulated at two levels in this study. GST can effectively remove reactive oxygen species in time, thus **the significant up-regulation of GST implied that the antioxidant system is activated in response to heat stress.**

Relaxin (RLN) can reduce the accumulation of peroxide products, inhibit oxidative damage, and improve the antioxidant capacity of endothelial cells. *RXFP1*, *PI3K*, *AC*, *PKC* and *CREB* were up-regulated in relaxin signaling pathway, promoting the generation of cAMP and thus activating gene transcription such as NOS1. **The activation of relaxin signaling pathway at the gene level implied that RLN may play an important role in the response of *E. sinensis* gills to heat stress.**



Acknowledgments

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