华中农业大学 HUAZHONG AGRICULTURAL UNIVERSITY

2024中国水产学会范蠡学术大会

水产学院 COLLEGE OF FISHERIES

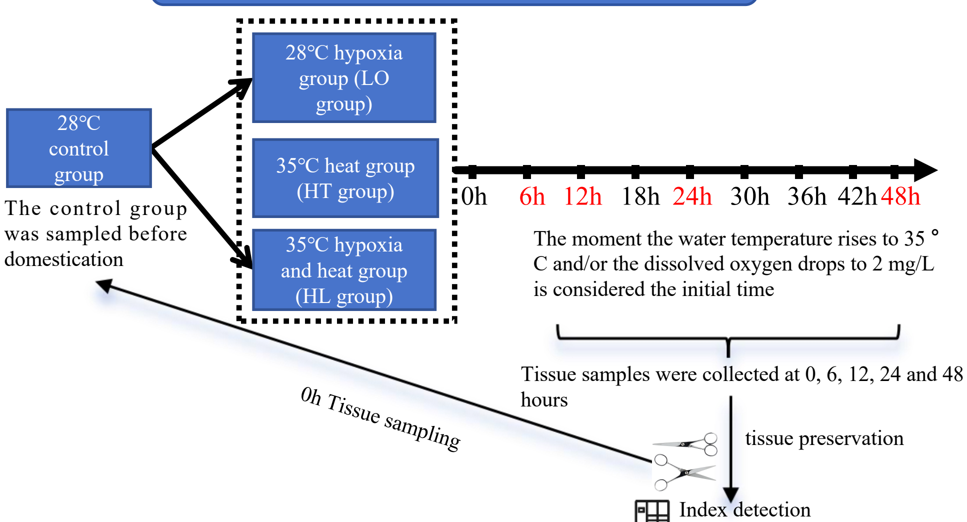
Impact of heat and hypoxia stresses on the antioxidant status, immunity, apoptosis and metabolism of *Megalobrama amblycephala* under global climate changes: defense against heat and hypoxia stresses

Kang Chen, Yihui Jia, Zheng He, Peiyu Xie, Hong Liu, Zexia Gao, Huanling Wang

Abstract

Megalobrama amblycephala, a main herbivorous fish with notable economic benefits in China, faces serious challenges to its survival and growth due to hypoxia and heat caused by factors such as global warming and intensive aquaculture. To evaluate the combined effects of these stressors, we performed a two-factor crossover test to assess the impacts of simultaneous exposure to hypoxia (2 mg/L) and heat (35 °C) on oxidative stress, immunity, apoptosis and metabolism in M. amblycephala. These results showed that hypoxia and heat exposure significantly enhanced the expression of oxygen-sensing and heat shock protein (HSP) genes, hypoxia inducible factor 1α (Hif-1α), HIF-prolyl hydroxylase-2 (phd2) and factor inhibiting Hif-1 (fih-1), as well as hsp70 and hsp90α. Furthermore, M. amblycephala suffering from hypoxia and heat exposure exhibited several changes in liver tissues, with the most severe lesions observed in those subjected to simultaneous exposure. Moreover, the combined hypoxia and heat exposure initially triggered an increase in the activities of total antioxidant capacity (T-AOC), superoxide dismutase (SOD) and catalase (CAT), and glutathione (GSH) contents, followed by a reduction, and the accumulation of malondialdehyde (MDA), which induced oxidative stress. This was accompanied by an increase and subsequent reduction in the contents of alkaline phosphatase (AKP), acid phosphatase (ACP), complement component 3 (C3) and C4, leading to immunosuppression. Additionally, hypoxia and heat exposure up-regulated the expression of antioxidant enzyme genes (nrf2, cu/zn-sod, mn-sod cat, ho-1, pi3k and gpx-1a), inflammatory genes (interleukin il-1β, il-8 and tnf-α), immunity effectors (igm and lyz), as well as apoptosis genes (casp3, casp8, casp9 and p53) and activated p-Akt/Akt, suggesting apoptosis may be linked with oxidative stress and inflammation and mediated through the PI3K/Akt signaling pathway. Based on GC-MS analysis, 100, 108 and 108 differential metabolites were found in the LO, HT and HL groups, respectively. There were 58 common differential metabolites in the three groups, of which 16 were up-regulated and 10 were down-regulated. Meanwhile, abnormal metabolites such as amino acids, nucleotides and fatty acids in the liver were found. In addition, accumulation of anaerobic metabolic biomarkers marks the onset of anaerobic metabolism, and accumulation of glycerophospholipids may maintain cell membrane stability. Overall, both hypoxia and heat exposure affected homeostasis, and simultaneous exposure led to more deleterious effects on M. amblycephala than exposure to the individual stressor. Candidate metabolites were also identified from the metabolome for new feed development.These results will help to understand the mechanism of hypoxia and heat-induced stress in fish and provide a theoretical basis for aquaculture management.

Materials and methods



biochemical indicators

Immune indicators
C3, C4 and ACP and AKP
Antioxidant indicators
SOD, CAT, MDA
pathological examination

Gene and protein expression

Hif - 1 alpha pathway
PI3K/Akt pathway
apoptotic pathways

GC-MS

Difference analysis of metabolites

Result

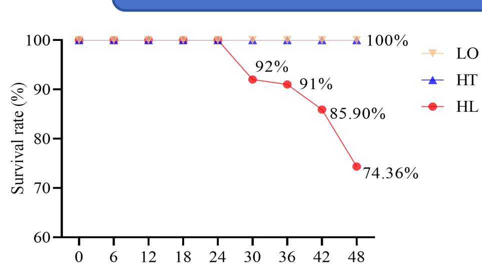


Fig. 1. The survival rate of *M. amblycephala* after hypoxia and/or heat exposure.

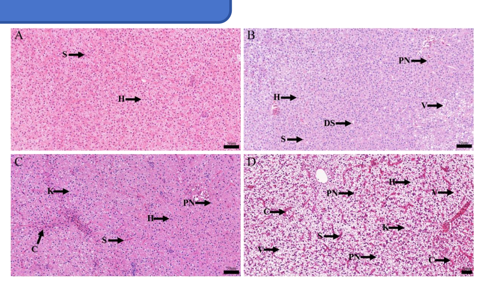


Fig. 2. Histopathological changes of the liver after hypoxia and/or heat exposure.

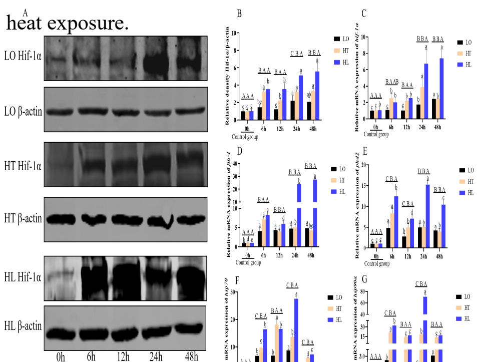


Fig.3. The impact of hypoxia and/or heat exposure on oxygen-sensing genes and heat shock protein genes in M. amblycephala liver.

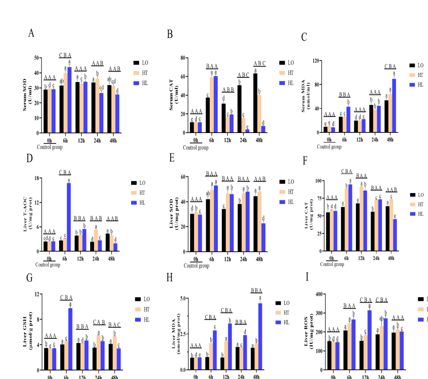


Fig.4. The changes of antioxidant indexes, lipid peroxidative products and ROS level in M. amblycephala liver and serum after hypoxia and/or heat exposure.

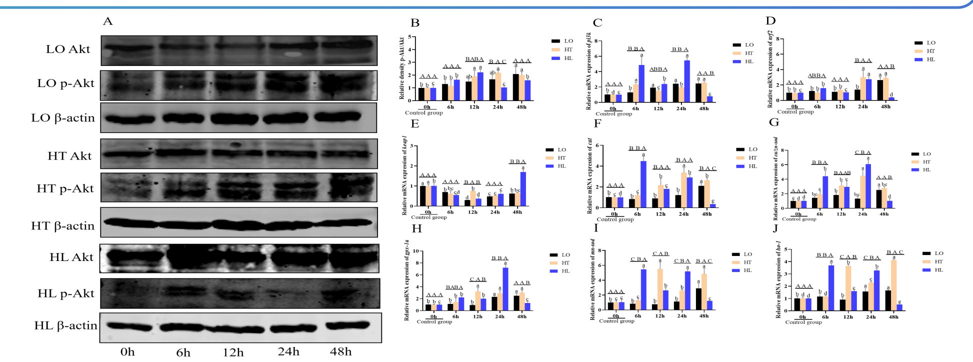


Fig.5. The relative expression of key genes in PI3K/Akt/Nrf2 pathway in *M. amblycephala* liver after hypoxia and/or heat exposure.

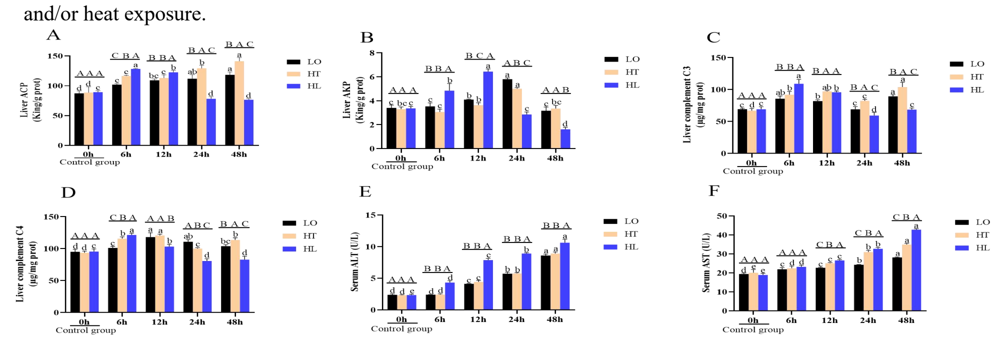


Fig.6. The effects of hypoxia and/or heat exposure on immunological parameters of *M. amblycephala* liver and serum.

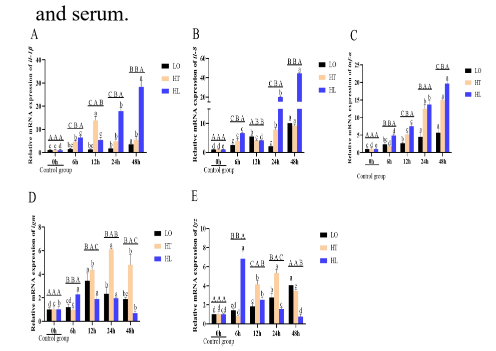


Fig.7. Hypoxia and/or heat exposure induced expression of inflammatory genes and immunity effector genes in *M. amblycephala* liver.

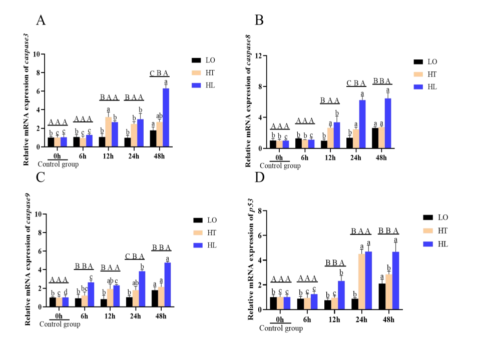


Fig.8 Hypoxia and/or heat exposure induced expression of apoptosis genes in *M. amblycephala* liver.

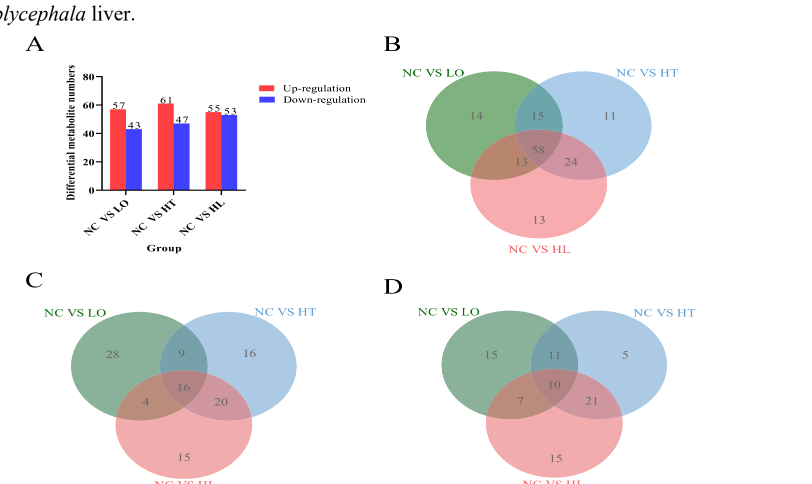


Fig.9. Venn diagram of differential metabolite numbers and unique and shared differential metabolites under hypoxia and/or heat exposure

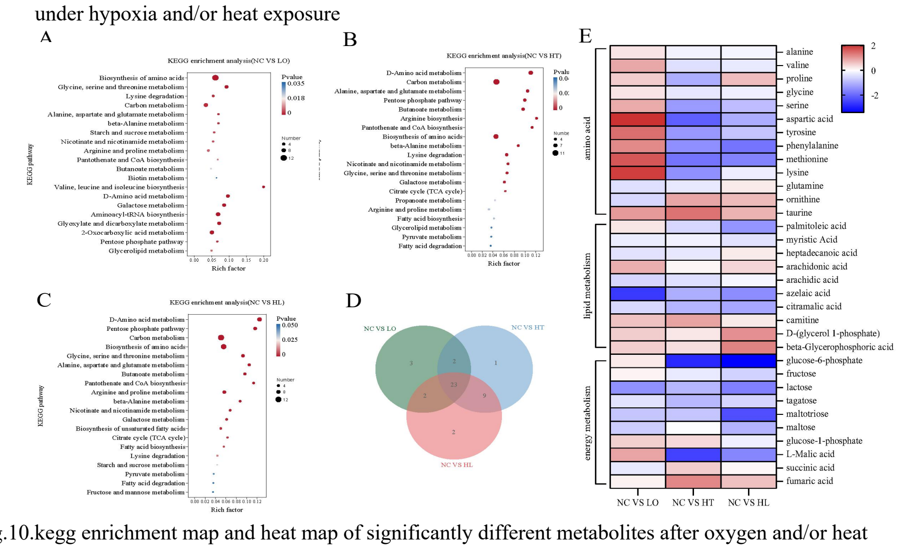


Fig.10.kegg enrichment map and heat map of significantly different metabolites after oxygen and/or heat exposure

Conclusion

1.Combined effect of hypoxia and heat exposure may mediate oxidative stress, immune response and apoptosis in *M. amblycephala* through the PI3K/Akt pathway.

2.Combined exposure to stress caused greater damage than stress alone.

3.Accumulation of glycerophospholipid may maintain cell membrane stability.