Effects of acute low-temperature stress on respiratory metabolism, antioxidants, and metabolomics of red swamp crayfish, Procambarus clarkii

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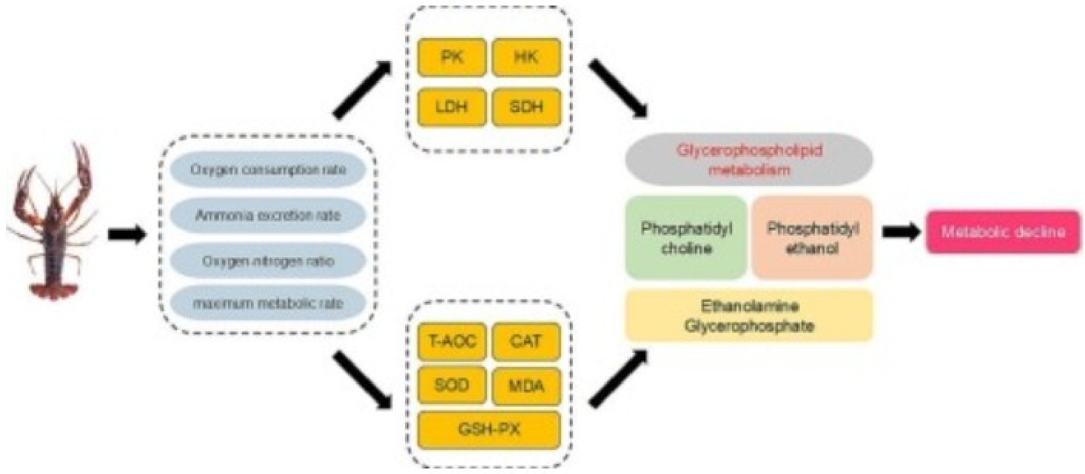
Abstract

Crayfish (Procambarus clarkii) aquaculture is threatened by abrupt temperature decreases caused by climatic phenomena, such as cold waves and seasonal fluctuations. In this study, crayfish were exposed to an abrupt temperature change from 17 °C to 7 °C for 24 h to investigate the effects of acute low-temperatures on respiratory metabolism, antioxidants, and metabolomics. The results showed that acute low-temperatures significantly reduced the activities of pyruvate kinase, lactate dehydrogenase, and succinate dehydrogenase in the gills and hemolymph, associated with decreases in anaerobic and aerobic respiratory capacities, and significant decreases in oxygen consumption, ammonia excretion, and maximum metabolic rates. Antioxidant enzymes in the hepatopancreas and hemolymph initially increased then decreased within 24 h. Metabolomics revealed that glycerophospholipid metabolism and glycosylphosphatidylinositol anchor biosynthesis pathways responded to acute low-temperatures, with glycerophospholipids being the most significantly differentially expressed metabolites. These results supported the hypothesis that crayfish exhibit lower metabolic activity at low temperatures. Our data provide mechanistic insight into the biological changes induced by acute low-temperature and may provide insight into culture of P. clarkii in cold waters.

Graphical abstract



Introduction

Production of the freshwater crayfish Procambarus clarkii in China reached 2.89 million tons in 2022 (Yu et al., 2023). Crayfish farming areas are mainly concentrated in the middle and lower reaches of the Yangtze River and Huaihe River Basin (Yue et al., 2021), with Jiangsu Province, a major crayfish farming area, producing 115,400 tons of crayfish. However, crayfish production in the cold northern regions, such as the Xinjiang and Heilongjiang provinces, is as high as 979 and 701 tons, respectively (Yu et al., 2023). These northern regions are highly susceptible to strong cold air activity from the Arctic and Siberia, resulting in frequent and sudden acute temperature drops from October to April (Hu et al., 2023). The China Meteorological Administration defines the climatic phenomenon in which the temperature drops by more than 10 °C within 24 h during this period as a cold wave. Moreover, crayfish aquaculture in China is mainly based on integrated rice-crayfish aquaculture, and the water temperature of smaller aquaculture water bodies in this system is highly susceptible to the impact of climate change, such as cold waves; therefore, crayfish frequently experience acute low-temperature stress resulting in frostbite and/or permanent damage (Chen et al., 2011; Wang et al., 2012; Li and Chen, 2021; Liu et al., 2023). Current research has focused on the effects of high temperatures on crayfish, with insufficient attention paid to the damage caused by low temperatures (Zhou et al., 2024). Therefore, addressing the research gap concerning the metabolic and physiological responses of crayfish to low-temperature conditions is necessary.

Decapod crustaceans have limited thermoregulatory capacity, which leads to lower physiological tolerance in cold environments (Ren et al., 2021). Indeed, low-temperature stress directly affects the respiratory metabolism and physiology of crustaceans (Hennig and Andreatta, 1998; Mubiana and Blust, 2007), leading to, for example, reduced oxygen consumption and ammonia excretion rates in Macrobrachium rosenbergii (Chen and Kou, 1996). Under low temperature, NADH dehydrogenase and mitochondrial ATP synthase of Cherax quadricarinatus are downregulated, and the ATP producing processes such as the citric acid cycle and electron transfer chain, are inhibited, resulting in energy shortage (Wu et al., 2019). As gills are the main site of respiration, exploring changes in respiratory metabolism at this tissue can be a sensitive indicator of the impact of environmental factors in crayfish (Wood and Soivio, 1991).

Low temperatures lead to the accumulation of reactive oxygen species (ROS) and oxidative stress in crustaceans, and crustaceans regulate enzyme activities through the antioxidant system to alleviate the prooxidant effects of ROS (Qiu et al., 2011; Zhu and Yao, 2015; Jiang et al., 2019; Xu et al., 2018). The hepatopancreas is an integrated tissue for metabolism and immunity, and it is responsive to environmental factors such as temperature, salinity, and dissolved oxygen. Moreover, it is a commonly used tissue for assessing oxidative damage in crustaceans (Jiang et al., 2009; Gao et al., 2012; Rőszer, 2014; Fan et al., 2016; Zhang et al., 2019). For example, low temperatures lead to elevated hepatopancreatic catalase activity in Litopenaeus vannamei (Qiu et al., 2011; Zhu and Yao, 2015). In addition, the hemolymph, as a conduit between the uptake of gases at the gill, and the metabolic activity at the hepatopancreas, can also be a tissue in which changes in respiratory metabolism and antioxidant status are insightful into organism responses to environmental stress (Gao et al., 2021).

With the development of modern bioinformatics technology, high throughput approaches are advantageous in revealing mechanisms of biological change (Bayona et al., 2022). For example, metabolomic studies have investigated the low-temperature response mechanisms of L. vannamei showing that the involvement of metabolites such as amino acids in energy metabolism pathways enhances its low-temperature tolerance (Zhu et al., 2024a). Meanwhile, the up-regulation of phospholipids such as phosphatidylcholine and phosphatidylserine increased the antioxidant capacity and cell membrane fluidity of L. vannamei, and this self-protection mechanism mitigated the stressful effects of low temperature (Zhu et al., 2024b). However, it is not clear whether low-temperature responses such as differential metabolite expression are consistent between crustaceans, especially those that differ in tolerance to low temperature.

The aims of this study were therefore to (i) investigate physiological changes in respiratory metabolism and antioxidant capacity of crayfish under low temperature, (ii) analyze the mechanism of low-temperature tolerance of crayfish via the combination of physiological response and metabolomics, and to (iii) verify whether the low-temperature response of crayfish was consistent with low-temperature intolerant crustaceans such as L. vannamei.

Section snippets

Source and acclimation of experimental crayfish

Procambarus clarkii was obtained from Suqian, Jiangsu Province, China. A total of 150 healthy, intermolt crayfish (body length, 71 ± 4 mm and weight 14 ± 2 g), were held in three tanks with a volume of 300 L (119 × 89 × 32 cm, water depth 16 cm). Crayfish were reared indoors in a recirculating water culture system at a temperature of 17 ± 0.5 °C, a pH of 7.5 ± 0.5, and under a 12 h light/dark cycle for 14 d. Commercial feed was provided twice daily at 8:00 am and 8:00 pm, at a daily feeding

Respiratory metabolism

At 4 h after the initiation of the temperature decrease, the oxygen consumption rate was significantly lower than the 17 °C control, and although oxygen consumption increased from the 4-h mark at 8 h, it dropped again at 12 h and remained low for the remainder of the 24-h duration of the exposure ($P < 0.001$; Fig. 2A). The ammonia excretion rate of crayfish gradually decreased over time, becoming significantly lower at 8 h, and being maintained at values around half of those of the 17 °C control

Effects of acute low-temperature on the respiratory metabolic capacity

As an ecotherm, the oxygen consumption rate of Procambarus clarkii would be expected to decline with a reduction in environmental temperature. Indeed, at all measurements conducted in 7 °C water, the oxygen consumption rate of crayfish was lower than that at 17 °C. This is a pattern commonly reported in other crayfish species (e.g., Villarreal, 1990; Whitledge and Rabeni, 2002; Simčič et al., 2014). The test temperature in this study (7 °C) represents the critical temperature for dormancy in P.

Conclusions

Acute low-temperature stress decreased the activity of respiratory metabolism enzymes in crayfish. Moreover, a transient stress response was induced, where antioxidant enzyme activity briefly increased and then decreased or returned to normal levels with gradual acclimatization to low-temperature environments. In addition, a significant decline in the glycerophospholipid levels implied a reduction in energy metabolism in crayfish hemolymph, which is contrary to the mechanism of low-temperature

Author statement

Yu Ding: Experimental design, Manuscript writing, Experiment conducting, Data analysis. **Wenbin Sha**: Manuscript writing, Experiment conducting. **Yunfei Sun**: Methodology, Experimental design, Writing – review and editing. **Yongxu Cheng**: Project administration, Methodology, Funding acquisition.

Declaration of competing interest

The authors declare no competing financial interests or personal relationships that could appear to have influenced the work reported in this paper.

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