



Health & Welfare

# Pacific white shrimp responses to temperature fluctuations at low salinity

Monday, 30 November 2020

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# Results showed that species can effectively adapt to some temperature variations

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Results of this study assessing physiological responses in L. vannamei juveniles subjected to temperature fluctuations in low salinity water showed that the shrimp can effectively adapt to some temperature variation. Photo by Fernando Huerta.

The Pacific white shrimp (*Litopenaeus vannamel*) – with its wide range of salinity tolerance, rapid growth and several other characteristics appropriate for intensive aquaculture – has become the most important cultured shrimp species globally. However, a variety of environmental factors can affect the growth of shrimp, such as changes in pH, salinity, dissolved oxygen (DO), temperature, and also chemical compounds like nitrite, ammonia and sulfide.

The annual cold wave that affects the shrimp farming industry in southern China during the winter months (November to January) causes significant economic losses to the *L. vannamei* aquaculture industry. However, little information is available regarding the physiological responses of the shrimp during the process of gradual temperature cooling and warming.

In shrimp, histology [the study of the microscopic anatomy of tissues and cells of animals and plants] of their hepatopancreas has been reported by researchers as a tool to monitor the impact of environmental stressors that can cause ultrastructural alterations at the onset of stress. For example, environmental stress like pH changes can cause change or damage of hepatopancreas cells. However, for temperature fluctuations, so far there is no definite information on any changes in hepatopancreas.

This article – adapted and summarized from the <u>original publication (https://doi.org/10.3389/fphys.2019.01025)</u> (Wang, Z. et al. 2019. Physiological Responses of Pacific White Shrimp *Litopenaeus vannamei* to Temperature Fluctuation in Low-Salinity Water. *Front. Physiol.*, 13 August 2019) – reports on a study that investigated several physiological responses in *L. vannamei* juveniles subjected to temperature fluctuations (28 to 13 to 28 degrees-C) in low salinity water.

# Study setup

*L. vannamei* juveniles (average weight  $5.4 \pm 0.7$  grams) from a commercial farm in Panyu (Guangdong, China) were transported to the lab and acclimated in filtered and aerated seawater tanks for several days before the experiment. During the acclimation stage, the water salinity and temperature in tanks were consistent with those of the farm

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culture ponds (salinity 5 ppt, pH  $8.3 \pm 0.1$  and temperature  $28 \pm 1$  degrees-C) where the shrimp were collected. The shrimp were fed commercial feed two times daily at 5 percent of their body weight.

Of these shrimp, selected, healthy individuals were randomly divided into three replicate tanks, and placed in an artificial climate incubator. The water temperature was decreased from acclimation temperature (AT, 28 degrees-C) to 13 degrees-C with a cooling rate of 7.5 degrees-C daily (2.5 degrees-C per 8 hours). After 13 degrees-C for 24 hours, the water temperature was increased back to 28 degrees-C at the same rate.

At various temperature points – 28, 23, 18, 13 and 13 degrees-C for 24 hours during the cooling process and at 18 and 28 degrees-C during the warming-back process – whole hepatopancreas from experimental animals were dissected and preserved for various analyses.

For detailed information on the experimental design and animal husbandry; collection and preservation of tissue samples; histology, RNA and DNA extractions, real-time polymerase chain reaction (qPCR) and other tests; and statistical analyses, refer to the original publication.

## **Results and discussion**

In this study, we investigated various physiological responses – including hepatopancreas histological changes, plasma metabolites concentrations, the expression of various genes, and other processes – in *L. vannamei* juveniles exposed to water temperature fluctuations (28 to 13 to 28 degrees-C). All these responses and processes were affected as temperatures decreased, but generally recovered during the re-warming stage, and evidenced that *L. vannamei* shrimp can adapt to a certain level of temperature fluctuations.

The crustacean hepatopancreas is a vital organ involved in excretion, molting, diverse metabolic activities and storage of energy reserves. Results of our study showed that the number and volume of certain cells (B-cells) in the hepatopancreas tubules were significantly increased after the shrimp underwent cold stress. This may be related to the fact that B-cells are the main site of absorption and digestion of nutrients. It is possible that the high rate of synthesis and release of digestive enzymes in B-cells accelerated the mobilization of nutrients in hepatopancreas tubules, which would help the shrimp better adapt to the temperature stress.

In shrimp, the hepatopancreas is known to have a high self-repairing ability. For example, researchers have reported that *L. vannamei* can repair its hepatopancreas injuries after long-term exposure to low zinc levels and low pH. And that the weight of the hepatopancreas of *L. vannamei* significantly declined after fasting, but then increased immediately after animals began feeding again. In our study, histological damage of the hepatopancreas was reversed after the animals were returned to higher water temperatures, confirming this reported ability to self-repair.

Regarding changes in the shrimp plasma [liquid portion in shrimp blood, the hemolymph] during temperature fluctuations, our results showed that lipids and protein in *L. vannamei* plasma responded more rapidly to temperature fluctuation, while glucose levels remained stable before experimental water temperature reached 13 degrees-C, and recovered to acclimation levels after temperature increased back to 28 degrees-C.

The hepatopancreas is typically high in lipids and appears to be the main site for gluconeogenesis [a metabolic pathway that generates glucose from certain non-carbohydrate carbon substrates] in decapod crustaceans, those with five pairs of walking legs, like shrimp. Thus, combined with our observed hepatopancreas histology and plasma results, we conclude that the increase of B-cells in the hepatopancreas facilitates the gluconeogenesis to synthesize glucose from protein and lipid, through which shrimps supplied glucose demand under experimental cold stress. However, after the water temperature dropped to 13 degrees-C, the rupture of hepatopancreas tubules causes lipids and proteins to enter the hemolymph, resulting in an increase of lipid and protein content in plasma, and the glucose content decreased at the same time due to the damage to the hepatopancreas.

Nonspecific immunity plays an important role in the immune defense of aquatic animals. Shrimp like *L. vannamei* depend entirely on cellular and humoral immunity to prevent external injury. The enzyme Alkaline Phosphatase (ALT) is directly involved in several metabolic pathways and plays a significant role in the shrimp immune system against various pathogens, probably because it can help protect the hepatopancreas and hemolymph from cold-stress damage

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The plasma metabolites concentration analysis also showed that the activity of the enzyme ALT reached its highest level at 13 degrees-C – the activity of ALT in plasma is inversely proportional to hepatopancreas health. This finding is consistent with previous studies and confirms the self-repair ability of *L. vannamei* shrimp. In addition, the expressions of many genes we assessed in our study, as well as hemocyte [type of cell involved in invertebrate immune system] numbers, reached their highest level in the hepatopancreas at 13 degrees-C.

### Perspectives

Results of our study showed that proteins and lipids were the main energy source of *L. vannamei* during temperature fluctuations. During the re-warming stage, all histopathological symptoms assessed generally reversed and all plasma metabolite concentrations and gene expressions returned to acclimation temperature levels. Overall, results suggest that *L. vannamei* can adapt to a certain level of temperature fluctuation, but the detailed adaptation mechanism in this species of shrimp still needs further study.

References available from the original publication.

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