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Original Research Articles

GLUT2 gene from *Penaeus monodon*: Molecular characterization, expression and association with tolerance to low salinity stress

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The full-length cDNA sequence of *Penaeus monodon* glucose transporter-2 (*PmGLUT2*) was cloned in this study using the RACE method. Quantitative real-time PCR was used to analyze the differential expression of *PmGLUT2* during the development of *P. monodon* larvae in different tissues and under low salinity stress. The *PmGLUT2* cDNA exhibited a total length of 2018 base pairs, with 94 base pairs located in the 5' untranslated region (UTR) and 352 base pairs in the 3' UTR. Additionally, the sequence contained 29 base poly (A) tails and 1572 base pairs within the open reading frame (ORF), capable of encoding 523 amino acids. Through a comparative analysis of the amino acid sequences of *PmGLUT2* and *LvGLUT2*, it was determined that *PmGLUT2* had 94.77% homology with *Tret1* gene of *Litopenaeus vannamei,* and 60.54% homology with *GLUT2* gene of *L. vannamei*. The results indicated that there was a fluctuation in *PmGLUT2* expression levels from zygote to postlarva development, with initial reduction followed by an increase, although the difference was not statistically significant. According to the molting analysis, the highest expression level of *PmGLUT2* was observed in the hepatopancreas during the premolt period, while the gill and gut exhibited peak expression levels during the intermolt period. *PmGLUT2* was found to be most abundant in lymph tissue, followed by gill tissue, and least abundant in ootheca, according to the tissue expression analyses. Following 96 hours of acute salt stress, there was a notable inhibition in the expression of *PmGLUT2* in the hepatopancreas and gills. Additionally, the expression level in the gills at 96 hours was significantly lower compared to the baseline level at 0 hours.

INTRODUCTION

The glucose transporter 2 (*GLUT2*), a member of the glucose transporter family (*GLUTs*), was initially discovered in the human liver and kidney, with specific expression in the liver, kidney, and small gut. Subsequent research revealed a significant presence in pancreatic islet β cells as well.[1] *GLUT2*, characterized by its low affinity and high capacity for glucose transport, is primarily localized in the plasma membrane of epithelial cells in the aforementioned tissues, where it facilitates glucose transport.[2] Currently, most studies on *GLUT2* are related to mammalian diseases together with sodium-glucose cotransporter, and few studies have been conducted in aquatic animals. Terova et al.[3] conducted a study on the role of *GLUT2* in the European wolf bass, demonstrating that *GLUT2* may be involved in facilitating glucose transport from liver glycogen degradation in liver cells during hypoxic conditions. Hall et al.[4] conducted a study on the expression of *GLUT2* in the liver of Atlantic cod and observed a significant correlation between liver *GLUT2* expression and blood glucose levels when values for *GLUT* expression from all individuals were plotted

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against blood glucose levels. *GLUT2* was also found in the kidney, gut, and liver of rainbow trout, according to some researchers.[5] Martinez-Quintana et al.[6] studied the expression of *GLUT2* in the hepatopancreas of *Litopenaeus vannamei*, the results showed that the expression of *GLUT2* was up-regulated in the hepatopancreas under hypoxia. These studies suggest that the function and regulatory mechanisms of *GLUT2* in aquatic animals deserve further in-depth study, especially the mechanism of action under different environmental stresses.

Salinity is an important environmental factor affecting the physiological metabolism of aquatic animals. The change in salinity would affect the growth, survival, reproduction, molting, physiological metabolism, and osmotic regulation of aquatic animals.[7,8] Salinity exhibits noticeable diurnal fluctuations and seasonal variations under natural conditions. Additionally, factors such as severe weather events like rainstorms and typhoons, as well as water changes in aquaculture practices, can also alter salinity levels in the water environment. This dramatic fluctuation of salinity stress can easily cause shrimp's stress response.

In this study, we utilized RACE technology to obtain the full-length cDNA sequence of *GLUT2* from *P. monodon* and analyzed its expression pattern during larval development, in different tissues, during molting stages, and under low-salinity stress. This study aims to offer fundamental data for investigating the regulatory mechanism underlying the response to low salt stress in *P. monodon*. Additionally, it contributes valuable insights for breeding for salinity tolerance of *P. monodon*.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

Specimens of *P. monodon* were obtained from the Shenzhen Experimental Base of the South China Sea Fisheries Research Institute. These shrimps, measuring 8 cm in length and weighing approximately 10 g, are currently being temporarily cultivated in cement ponds filled with natural seawater, maintained at a temperature of 27°C.Three male and three female shrimps were randomly selected for the study. Tissue samples were collected from the hepatopancreas, gills, heart, gut, stomach, lymph, epidermis, muscle, eyestalk ganglion, ootheca, and testis of each shrimp as appropriate. The samples from the three shrimp individuals were then pooled into a single tube, preserved in RNAlater at 4°C overnight, and subsequently stored at -80°C for future use.

During the summer breeding of *P. monodon* in the Shenzhen base of the South China Sea Fisheries Research Institute, samples (3 groups of parallel samples) of its larval development stages were collected, including zygote (Z), nauplius (N), zoea I (Z1), zoea II (Z2), zoea III (Z3), mysis I (M1), mysis II (M2), mysis III (M3) and postlarva stage (P). The larval development stages of *P. monodon* refer to the Breeding Technology of *P. monodon*.[9] The samples were placed in RNA*later* solution at 4 °C overnight and then at -80 °C for standby.

P. monodon with a body mass of 20 ± 2g and a body length of 12 ± 1cm was selected for sampling in each stage of molting, including stage A (postmolt), C (intermolt), D (premolt), E (ecdysis).[10] At each molting stage, 6 ± 1 shrimp's hepatopancreas, gills and gut were stored in RNA*later* Solution at 4 °C overnight and then stored at - 80 °C.

Healthy shrimps were randomly selected from the temporary breeding pool as experimental materials. The experiment was divided into two groups, with salinity of 30 ‰ (control group) and salinity of 3 ‰ (experimental group). Each group was provided with 3 parallel tanks, in which 300 L seawater with different salinity was added, 60 shrimps were put in each tank, and the culture temperature was 28 ± 2 °C. Throughout the duration of the experiment, deceased shrimp were retrieved from individual plastic buckets at 3-hour intervals and documented.

The salinity concentration in the experiment was achieved by mixing seawater and freshwater for aquaculture and was adjusted to the target salinity using a salinity tester (AZ8371, Hengxin, Taiwan). In the control group, three individuals with better vitality were selected, and their gill tissues, gut tissues, and hepatopancreas tissues were collected. These tissues were then mixed and uniformly preserved in RNA*later* solution. At the 3rd, 6th, 12th, 24th, 48th, 72nd, and 96th hours, respectively, three individuals with better vitality during the molting interval were selected from the plastic bucket with a salinity of 3‰. The same types of tissues as those from the control group were collected, mixed, and uniformly preserved in RNA*later* solution at 4°C overnight, followed by storage at -80°C.

EXPERIMENTAL METHODS

EXTRACTION OF TOTAL RNA AND CONSTRUCTION OF CDNA LIBRARY

According to the protocol outlined in the HiPure Fibrous RNA Plus Kit, total RNA extraction was performed on each sample. A 1:5 ratio of ten loading buffers and RNA was prepared. The purity of the total RNA was assessed through 1.5% agarose gel electrophoresis conducted at 150V for 15 minutes using a nucleic acid electrophoresis apparatus. Additionally, the integrity of the RNA was evaluated using a NanoDrop 2000 nucleic acid quantitative assay instrument. When the ratio of 260 nm/280 nm measured by the NanoDrop 2000 falls within the range of 1.8 to 2.0, and the 28s and 18s bands of the RNA samples are clearly visible without any observable drag, it suggests that the extracted total RNA is of high quality and suitable for the construction of a cDNA library and the subsequent generation of 3' and 5' RACE templates. Prime ScriptTM II Reverse Transcriptase Kit kit's method was utilized to create the cDNA library from the total RNA of the aforementioned tissues. Following the experiment, the cDNA isolated from various tissues must be suitably diluted and kept at -80 °C for standby.

CDNA CLONING OF PMGLUT2

The sequence was obtained from the transcriptome library of *P. monodon* in the laboratory, and was preliminarily

Table 1. Primer sequences and purpose.

Primer	Sequence (5'-3')	Purpose
PmGLUT2-F	GCCGTATTATTACTGGCTTCTGTG	Sequence validation
PmGLUT2-R	GGAGTTTTCTCCCTGCTTTGTC	Sequence validation
M13-F	CAGGAAACAGCTATGACC	DNA sequencing
M13-R	TGTAAAACGACGGCCAGT	DNA sequencing
PmGLUT2-5'GSP1-1	GATCCTGAGTCTTCAAAGATGGTGGTG	5'RACE
PmGLUT2-5'GSP2-1	GAAGATCCCTAAATGAAGCCTTGTTGCG	5'RACE
PmGLUT2-5'GSP1-2	GATGGCTCTCCATGCTGCGGTTTAC	5'RACE
PmGLUT2-5'GSP2-2	GACCAGGGACTTGTTCTGATGGGATG	5'RACE
PmGLUT2-3'GSP1-1	TTGGGGGTGTCTGCCTCAATAAGC	3'RACE
PmGLUT2-3'GSP2-1	GTGGCTGGCTTTTTATTGCTTTTGCTC	3'RACE
PmGLUT2-3'GSP1-2	ATGGAGGATGAGGTATGGGCAGTGG	3'RACE
PmGLUT2-3'GSP2-2	TGACTTTGGTGTCTACTGGCTGTTTGC	3'RACE
PmGLUT2-qPCR-F	CTCCTGCTGATTGTTTCTGCTTC	qPCR
PmGLUT2-qPCR-R	GCCTAAGGTTTCCACTGCCC	qPCR
EF-1α-F	AAGCCAGGTATGGTTGTCAACTTT	qPCR
EF-1α-R	CGTGGTGCATCTCCACAGACT	qPCR

named as the glucose transporter 2 (*PmGLUT2*) after NCBI comparison.[11-14] *PmGLUT2* was sent to Beijing Ruibo Xingke Guangzhou Branch for synthesis, and the sequence obtained was validated. The primers required for the experiment were designed using Primer Premier 5.0. According to SMARTer ® RACE 5 '/3' Kit instructions were used to extract the complete length of the *PmGLUT2* gene. The primers required in the experiment were shown in Table 1.

BIOINFORMATICS ANALYSIS

The resultant sequence from the 3'/5'-RACE PCR clone was merged using DNAMAN 8 software to achieve the complete length of *PmGLUT2*. Find the open reading box with ORF Finder (https://www.ncbi.nlm.nih.gov/orffinder/). Use EMBOSS to predict amino acid sequence (http://www.bioinformatics.nl/emboss-explorer/). Use NCBI's BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) tool to compares the predicted amino acid sequence with the protein database for similarity analysis. The Clustal X program was employed to compare the *GLUT2* sequence of *PmGLUT2* with that of the closely related *L. vannamei*. Utilizing ExPASy ProtParam (https://web.expasy.org/protparam/), calculate the concentrations of different amino acids, as well as their theoretical molecular weight and isoelectric point. Protein domain was analysis by using SMART4.0 (http://smart.embl-heidelberg.de/smart/set_mode.cgi?GENOMIC=1). Utilize the expected glycosylation sites from the Net NGlyc 1.0 Server (http://www.cbs.dtu.dk/services/Net NGlyc/). Utilize the predicted phosphorylation sites from NetPhos 3.1 Server (http://www.cbs.dtu.dk/services/Net Phos/). Third level structure prediction uses SWISS MODEL (https://swissmodel.expasy.org/). In order to build the system evolution tree, Cluster X and MEGA 6.0 software were both used.

ANALYSIS OF TISSUE DIFFERENTIAL EXPRESSION AND EXPRESSION ANALYSIS UNDER SALINITY STRESS

Fluorescent quantitative primer *PmGLUT2*-qPCR-F/R for *PmGLUT2* gene expression analysis designed with Beacon Designer 7.0 software (Table 1) and used EF-1α-F/R for internal reference gene (elongation factor 1α, Gen Bank number was DQ021452).[15-18] The column with a vertical axis of value 1 was used as a reference. Using the cDNA of the larval development stages, tissues and molting stages of *P. monodon* as the template was amplified by Quantitative real-time PCR use Roche LightCycler480II. The reaction volume was 12.5 μL, including 5.25 μL TB GreenTM Premix Ex TaqTM (Tli RNase H Plus), 0.5 μL upstream and downstream primers, 1 μL template cDNA (about 40 ng), ddH_2O added to 12.5 μL. Three replicates and negative controls were set for each sample, and no template was added to the negative controls The reaction conditions included an initial denaturation step at 95℃ for 30 seconds, followed by 40 cycles of denaturation at 95℃ for 5 seconds, annealing at 60℃ for 30 seconds, and extension at 95℃ for 5 seconds. Subsequently, the temperature was raised to 95℃ at a rate of 60℃ per minute, followed by a final extension step at 50℃ for 8 minutes. *PmGLUT2*-qPCR-F/R and EF-1α-F/R were used to analyze the expression of *PmGLUT2* under salinity stress (Table 1).

STATISTICAL ANALYSIS

Quantitative real-time PCR data were analyzed using the relative Ct method ($2^{-\Delta\Delta Ct}$), and statistical analysis was conducted using One-Way ANOVA with the Tukey post-hoc test in SPSS 24.0 software. A significance level of $P<0.05$ was used to determine statistical significance.

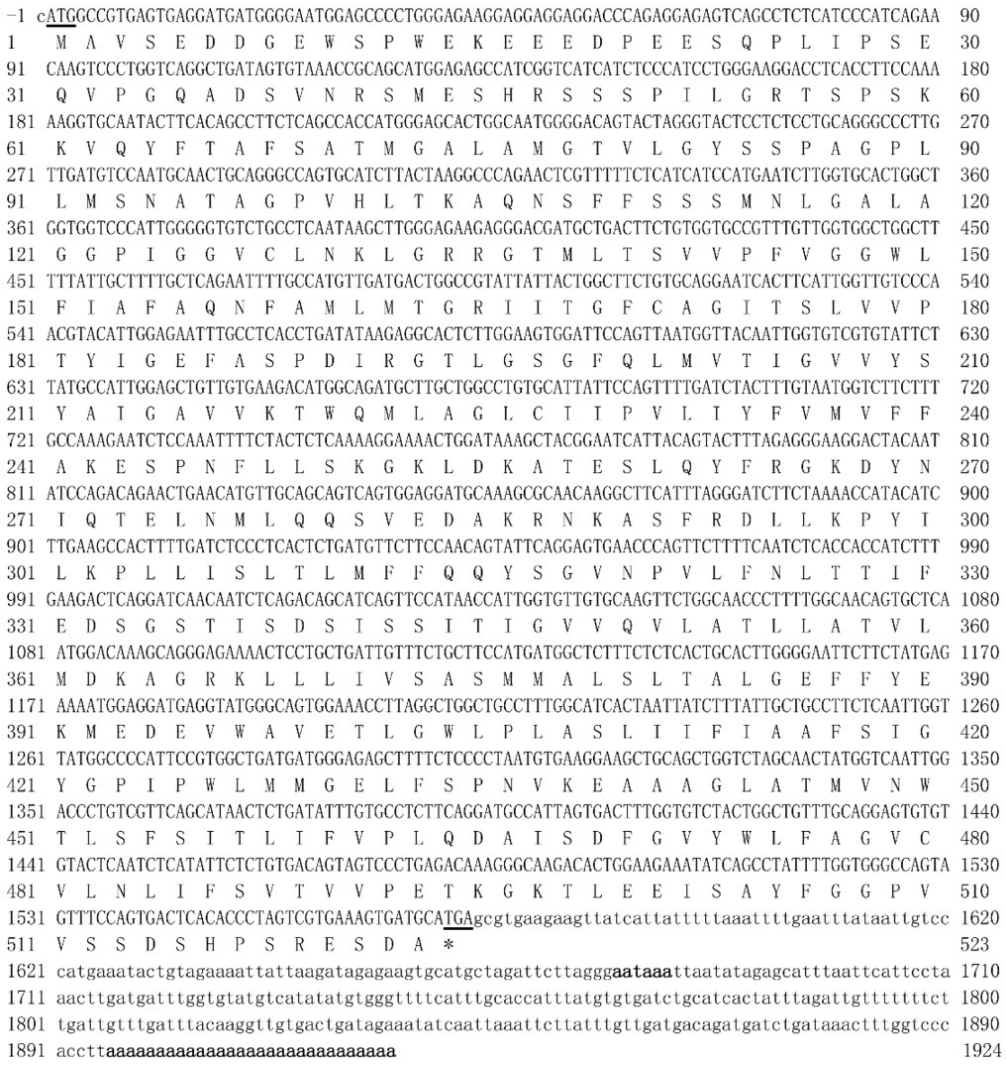

Figure 1. Nucleotide and deduced amino acid sequence of *PmGLUT2*.

RESULTS

PMGLUT2 SEQUENCE ANALYSIS

The full length of *PmGLUT2* cDNA was obtained by cloning, and the GenBank login number was MK094070. *PmGLUT2* had a total length of 2018 bp, a 5' UTR of 94 bp, a 3' UTR of 352 bp, including 29 base poly (A) tails, an open reading frame (ORF) of 1572 bp, and can encode 523 amino acids (Figure 1). ExPASy ProtParam predicted that its molecular weight was 56.568 kD and its theoretical isoelectric point was 4.96. NetPhos 3.1 Server predicted that it contains 55 phosphorylation sites (including 37 serine sites, 15 threonine sites and 3 tyrosine sites), NetNGlyc 1.0 Server predicted that it contains 4 N-glycosylation sites, and SignalP-5.0 predicted that this sequence does not contain signal peptide. SMART 4.0 predicts that *PmGLUT2* had 12 transmembrane domains (Figure 1), including a glucose (and other small molecule carbohydrates) transport functional domain located at 54~506 aa.

HOMOLOGY ANALYSIS

The amino acid sequence of *PmGLUT2* was analyzed for homology with *GLUT2* in NCBI BLAST, revealing a high degree of similarity with that of *P. vannamei*. The three-dimensional structure of the protein was constructed using SWISS-MODEL, which was similar to the three-dimensional structure of *L. vannamei GLUT2* (Figure 2). Using Cluster X software, protein sequence comparisons were made between the *GLUT2* amino acid sequence of *L. vannamei* retrieved from NCBI and *PmGLUT2* amino acid sequence. The results showed that the *GLUT2* of *P. monodon* and *L. vannamei* (AIT97017.1) were relatively conservative, with a homology of 60.54% (Figure 3). Utilizing MEGA 6.06 software and employing the NJ (Neighbor Joining) method, a phylogenetic tree was constructed for *PmGLUT2* and *L. vannamei* through 1000 iterations with the Bootstrap method (Figure 4). Within the group, the glucose transporter *GLUT2* of *P. monodon* and the trehalose-promoting transporter *Tret1* of *L. vannamei* were grouped together in a distinct branch, which subsequently clustered with the *GLUT2* of *L. vannamei*.

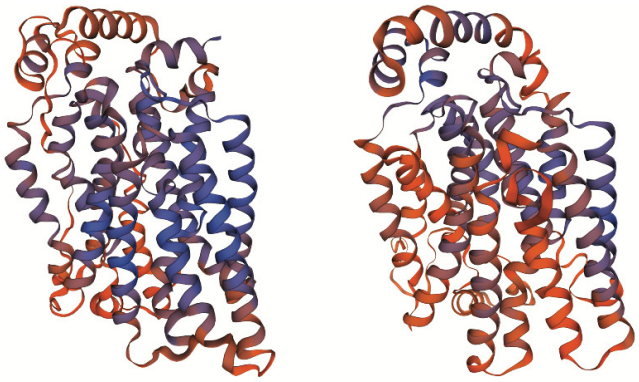

Figure 2. Three-dimensional ribbon structure of *L. vannamei GLUT2* and *PmGLUT2*.

The left image shows the three-dimensional structure of *GLUT2* in *L.vannamei*, and the right image shows the three-dimensional structure of *GLUT2* in *P. monodon*.

ANALYSIS OF PMGLUT2 EXPRESSION DURING LARVAL DEVELOPMENT

The expression of *PmGLUT2* in each stage of the larval development of *P. monodon* was detected. From the zygote to the postlarvae stage, the expression of *PmGLUT2* decreased first and then increased, but there was no significant difference (Figure 5).

PMGLUT2 TISSUE EXPRESSION ANALYSIS

The expression of *PmGLUT2* in various tissues of *P. monodon* was detected. *PmGLUT2* was expressed in all tissues of *P. monodon* (Figure 5), with the highest expression level in lymph tissue, followed by a higher expression level in gill tissue, about 12 times that in muscle, and a lower expression level in muscle and ootheca.

ANALYSIS OF PMGLUT2 EXPRESSION IN DIFFERENT STAGES OF MOLTING

The expression of *PmGLUT2* in each stage of the molting of *P. monodon* was detected. The expression of *PmGLUT2* in the hepatopancreas was the highest in the premolt and the lowest in the intermolt. The expression of *PmGLUT2* in gills was the highest in intermolt, which was significantly different from that in premolt, ecdysis and postmolt ($P<0.05$). The expression of *PmGLUT2* in the gut was the highest in the intermolt and the lowest in the premolt and then increased gradually (Figure 6).

EXPRESSION ANALYSIS OF PMGLUT2 UNDER SALINITY STRESS

The relative expression of *PmGLUT2* in hepatopancreas, gill, and gut tissues of *P. monodon* under salinity stress was studied by qRT-PCR. In the experiment of the salinity acute stress group, the expression of *PmGLUT2* in hepatopancreas, gills, and gut tissues is shown in Figure 7. The expression of *PmGLUT2* in hepatopancreas decreased significantly at the 3rd hour, which was significantly different from the control group ($P<0.05$). The expression remained low at the 72nd hour and then increased at the 96th hour, which was no significant difference from the control group. The expression of *PmGLUT2* in gills decreased significantly at the 3rd hour, which was significantly different from that in the control group ($P<0.05$). Subsequently, the expression level fluctuated at a low level, and there was no significant difference. The expression of *PmGLUT2* in the gut did not change significantly in the first 24 hours but increased significantly at the 48th and 72nd hours ($P<0.05$) and then decreased at the 96th hour, showing no significant difference compared with the control group.

DISCUSSION

The expression of *PmGLUT2* decreased while *P. monodon* larvae developed, then slightly increased up to the larval stage. However, *PmGLUT2* expression was typically low at all phases, it could be due to the fact that the nauplius stage's nourishment came entirely from the yolk, the larva's digestive and absorption systems were imperfect, preventing it from feeding, and there was less glucose absorbed and transported.[2] At the larval stage, the expression level begins to rise as the digestive organs gradually mature, begin to feed on benthos, and begin to get small molecule carbohydrates like glucose from food.

The investigation of *PmGLUT2* expression across various tissues revealed its presence in a diverse range of tissue types. Notably, *PmGLUT2* was highly expressed in the lymph, gills, stomach, and gut, suggesting that these tissues are particularly active in glucose transport or absorption. Shrimp lymph is their immunological system, the gill is the tissue responsible for excretion into the environment, gas exchange, and ion transport. The organs responsible for digestion and absorption include the stomach and gut, while the lymph and gill play a crucial role in the transportation of glucose for energy production. Additionally, the stomach and gut serve as the main locations for glucose absorption. The differential expression of *GLUT2* across various tissues suggested a potential regulatory function in the immune response, digestion, and nutrient absorption processes of *P. monodon*.

The hepatopancreas, gills, and gut were selected for a molting expression study to substantiate the connection between *PmGLUT2* and the molting process in *P. monodon*. The findings demonstrated that *PmGLUT2* expression was highest in the hepatopancreas at the premolt stage and highest in the gills and gut at the intermolt. Intermolt is a period of rapid development and eating for shrimp, characterized by high activity, robust respiration and excretion, robust digesting and absorption, and elevated *PmGLUT2* expression in the gills and guts. The expression of the gut tends to increase in the ecdysis stage, which may be connected to the peritrophic membrane's repair and energy supply.[19]

Based on the results of the experiment conducted under acute low salinity stress conditions, it was observed that the expression of *PmGLUT2* was downregulated in the hepatopancreas and gills, while showing an increasing trend

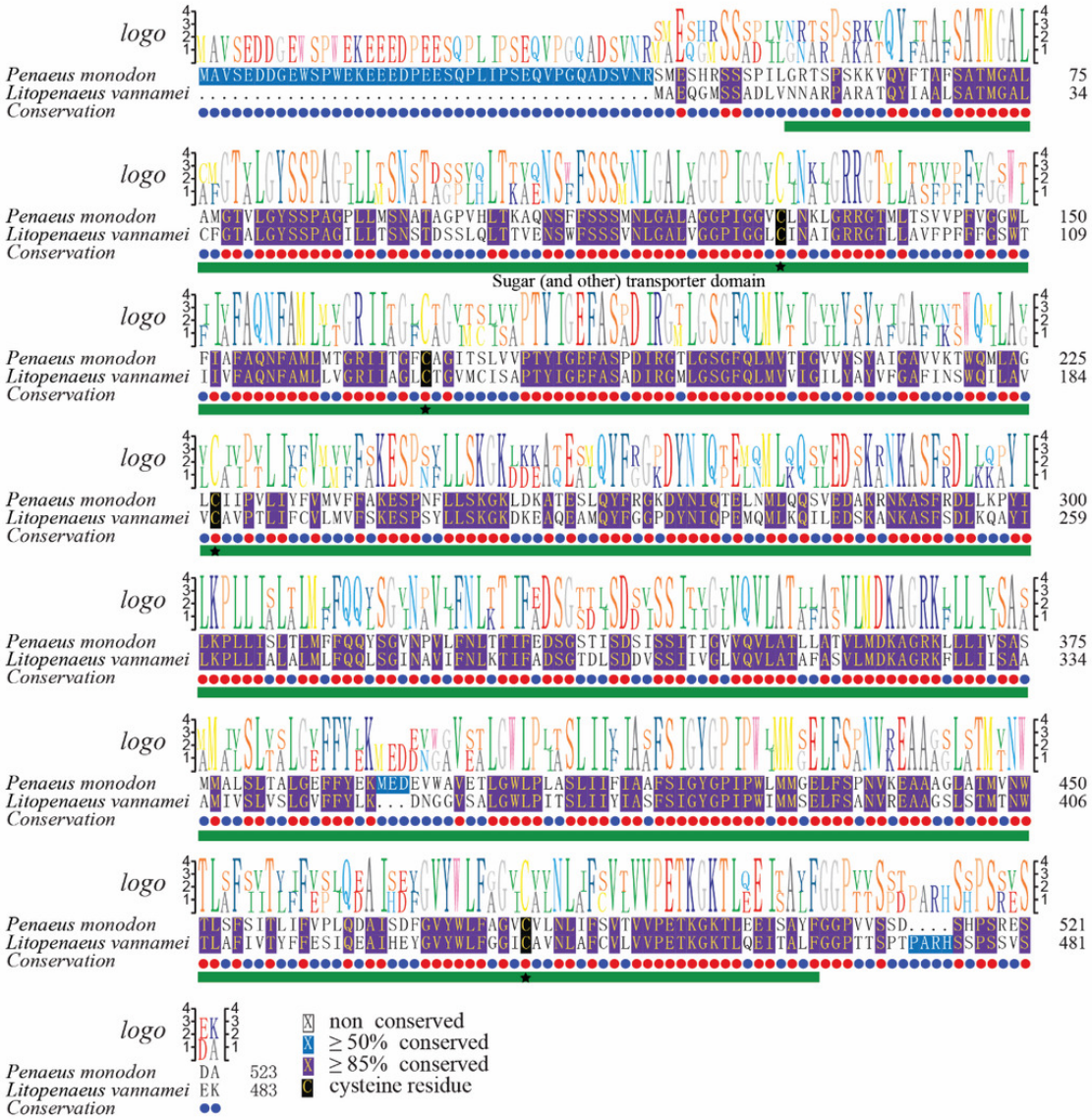

Figure 3. Comparison of multiple alignments of the predicted amino acid sequence of *PmGLUT*2 with other species.

Sequences used for alignment include PmGLUT2, and *LvGLUT*2 (ALG65274.1). The sequence logo representing the similarity is shown at the top of the alignments, and the numbers of amino acids are listed on the right side of the alignments. Green horizontal lines indicate sugar (and other small carbohydrates) transporter domain. Cysteine residues in the mature peptides were framed "★" below and the residences of different similarity were shaded in different colors according to the rule in the end. The "logo" The "logo" in the figure represents the conserved position of each amino acid in the sequence.

in the gut over time. The primary function of *GLUT2* is to facilitate the absorption and transportation of glucose, suggesting that the inhibition of *PmGLUT2* expression in response to acute low salinity stress may be linked to a potential decrease in feeding behavior in *P. monodon*.[2] Fluctuations in salinity levels have been shown to impact the feeding behavior of shrimp, potentially leading to reduced energy intake and subsequent limitations in energy supply.[20] To sustain essential life functions, shrimp may enhance glucose absorption efficiency by modulating the expression of glucose transporters, such as *GLUT2*, in the gut to meet energy demands. Further experimental investigations and thorough analysis are necessary to accurately elucidate the underlying link between reduced shrimp feeding behavior and heightened expression of *GLUT2* in the gut. *L.vannamei*'s immune system was examined by Liu et al[21] in relation to salinity changes. The findings indicated a significant decrease in the blood cell count of *L. vannamei* within 24 hours of a salinity change, with subsequent stabilization after one day. A positive correlation with salinity was observed. Additionally, cytophagy rate, hemolymph phenoloxidase, lysobacteriolytic activity, and α2-macroglobulin activity exhibited peak changes within three days. Salinity changes demonstrated both positive and negative correlations. By day three, the immunity of *L. vannamei* had returned to baseline levels. The study demonstrated a significant decrease in the immunity of *L. vannamei* in response to rapid salinity changes, with a notable dose-dependent effect. Subsequently, a gradual immune recovery process was observed, suggesting that the prawn exhibits a capacity for immunomodulatory adaptation to fluctuations in salinity. Furthermore, this immune adaptation dis-

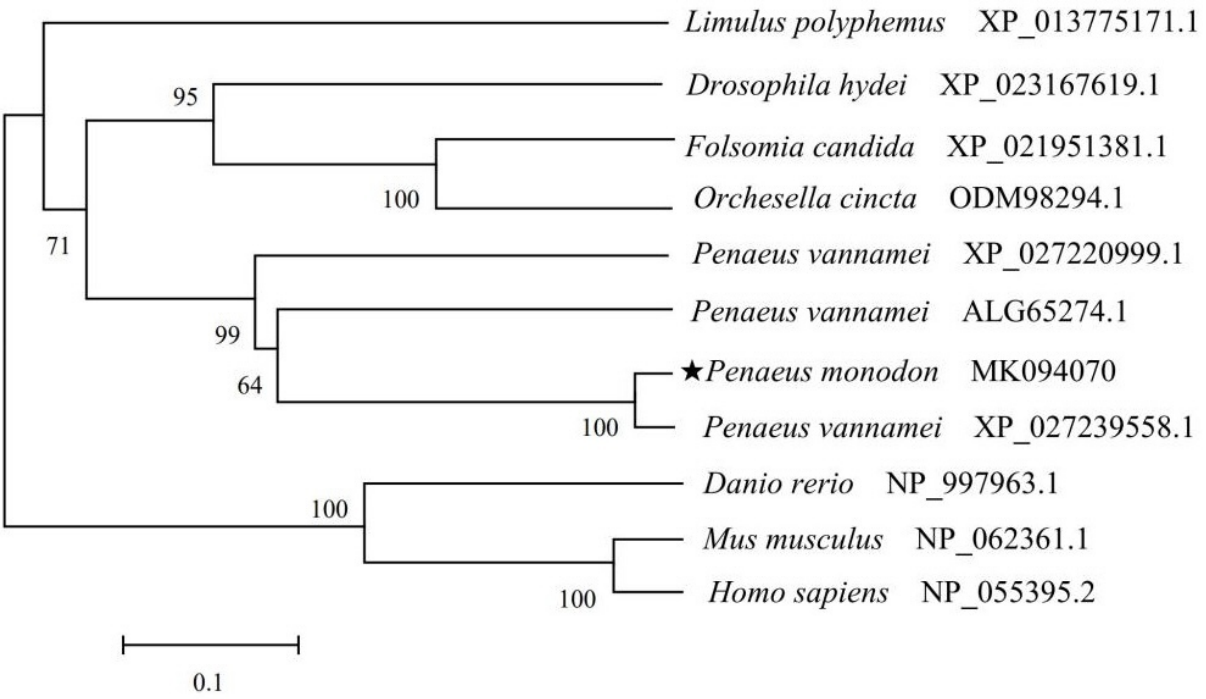

Figure 4. Phylogenetic tree of *PmGLUT*2 by MEGA 6.06.

Sequence accession numbers and species are included in each branch. The percentage of replicate trees of the bootstrap test is shown on the branches.

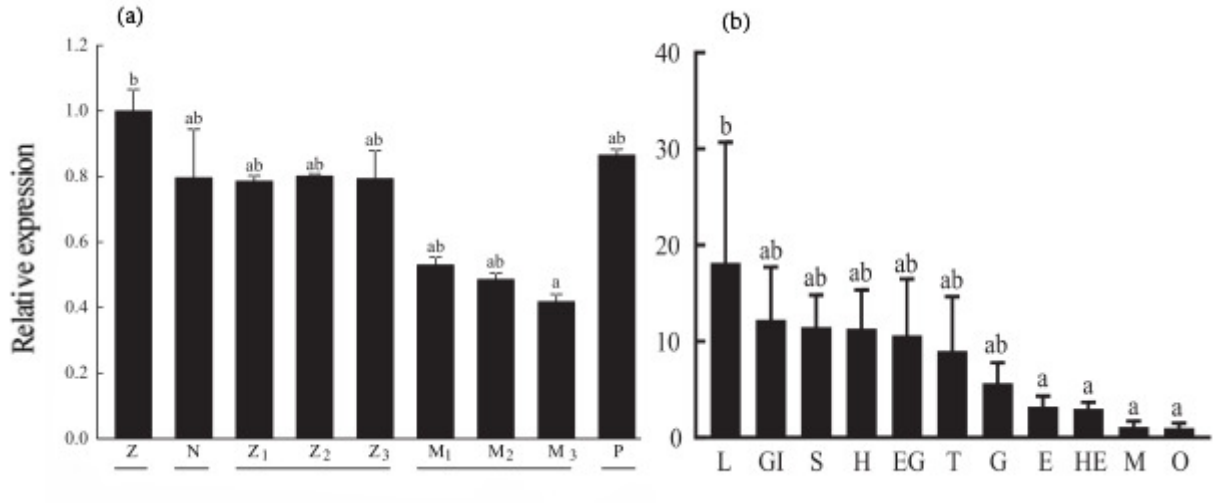

Figure 5. Expression levels of *PmGLUT2* at different larval developmental stages and tissues of *P. monodon* (n=3).

The letters indicate significant difference ($p<0.05$). Z: zygote, N: nauplius, Z1, Z2, Z3: zoea, M1, M2, M3: mysis, P: postlarva stage. L: lymph, GI: gill, S: stomach, H: heart, EG: eyestalk ganglion, T: testis, G: gut, E: epidermis, HE: hepatopancreas, M: muscle, O: ootheca.

played a distinct temporal regularity, with peak recovery occurring after a period of three days.[21] At the 72nd and 48th hours, respectively, the expression of *GLUT2* in the hepatopancreas and gut increased, which may be connected to its adaptation to low salinity immunoregulation. Our study revealed that the expression of *GLUT2* was altered under salinity stress, offering valuable insights for future research. Further, more rigorous experiments were needed to confirm the specific mechanisms and patterns of *GLUT2* modulation in response to salinity stress. The observed alterations suggested a potential association between *GLUT2* and salinity stress, highlighting its significance as a key gene in the response to such environmental factors.

The intricate physiological and metabolic mechanisms of aquatic organisms may involve the combined influence of various genes and pathways under salt stress conditions. Furthermore, factors beyond salinity, such as temperature and dissolved oxygen levels, could also impact the physiological responses and gene regulation in *P. monodon*. These studies would help reveal the molecular mechanisms of low salt tolerance in *P. monodon* and provide theoretical support for the sustainable development of aquaculture.[22-24]

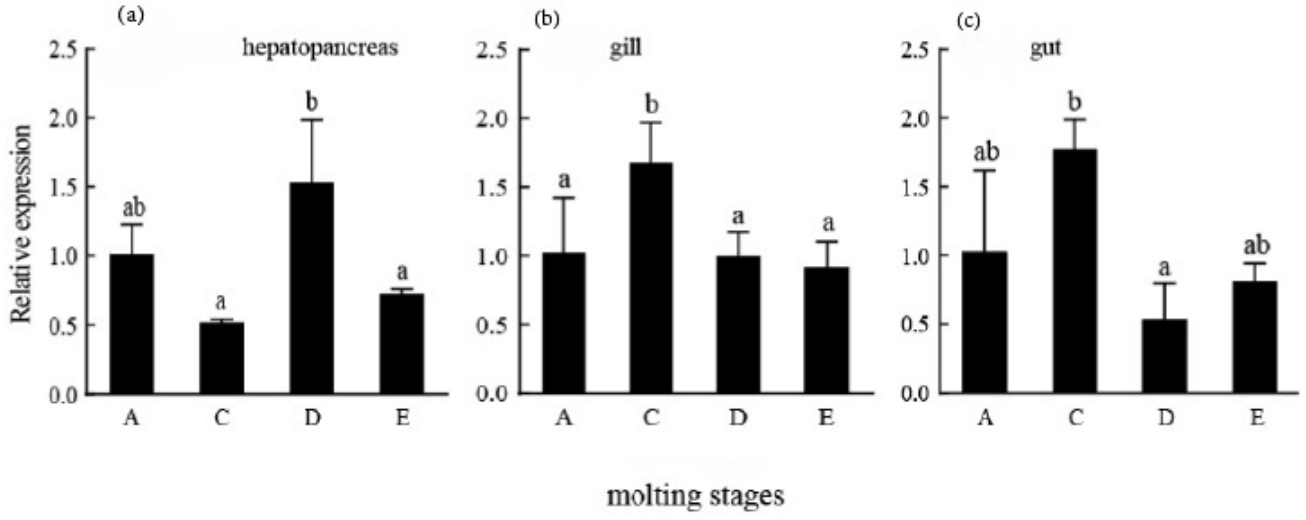

Figure 6. Expression levels of *PmGLUT2* in different tissues during molting stages of *P. monodon* (n=3).

The letters indicate significant difference (p<0.05). A: postmolot, C: intermolt, D: premolt, E: ecdysis.

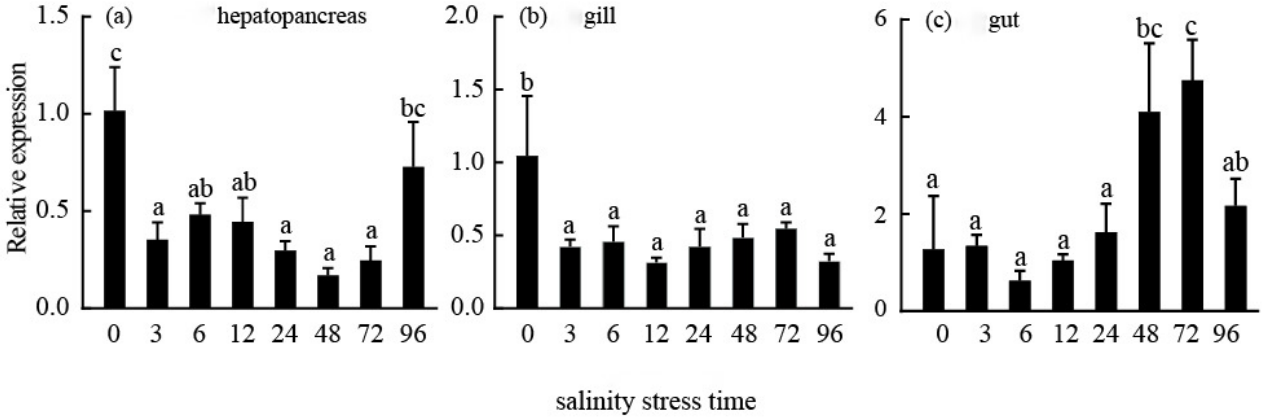

Figure 7. Expression levels of *PmGLUT2* in hepatopancreas, gill, and gut under acute low salinity stress (n=3).

The letters indicate a significant difference (p<0.05).

CONCLUSIONS

The amino acid sequence analysis revealed that *PmGLUT2* lacks a signal peptide and has a transmembrane transport functional domain. The *PmGLUT2* gene is widely expressed in tissues of *P. monodon* and is highly expressed in lymphocytes and gills. *PmGLUT2* is related to the molting and low salinity stress response of *P. monodon*. Investigating the response of *P. monodon* to low salt stress is crucial for elucidating the mechanisms underlying low salt tolerance. This study also aims to identify and develop new *P. monodon* varieties with improved salt tolerance, thereby informing and enhancing production practices.

AUTHOR CONTRIBUTIONS

Data curation: Yundong Li (Equal), Song Jiang (Equal), Qibin Yang (Equal), Lishi Yang (Equal). Software: Yundong Li (Lead). Writing – original draft: Yundong Li (Lead). Writing – review & editing: Yundong Li (Equal), Wenwen Zhang (Equal), Peng He (Equal), Qibin Yang (Equal), Lishi Yang (Equal), Jianhua Huang (Equal), Falin Zhou (Equal). Investigation: Wenwen Zhang (Equal), Peng He (Equal), Jianhua Huang (Equal). Methodology: Song Jiang (Equal), Qibin Yang (Equal), Lishi Yang (Equal). Conceptualization: Falin Zhou (Lead).

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DATA AVAILABILITY

Not applicable.

ETHICS APPROVAL

The Animal Care and Use Committee approved the use of all the shrimps in these experiments at the Chinese Academy of Fishery Sciences (CAFS), and we also applied the national and institutional guidelines for the care and use of laboratory animals at the CAFS.

COMPETING INTERESTS

The authors report no conflict of interest.

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REFERENCES

1. Augustin R. The protein family of glucose transport facilitators: It's not only about glucose after all. *IUBMB Life*. 2010;62(5):315-333. doi:10.1002/iub.315

2. Chen Y. *Study on the Expression and Regulation of Glucose Transporter Proteins in Rat Testis*. Xiamen University; 2004. http://dspace.xmu.edu.cn:8080/dspace/handle/2288/47233

3. Terova G, Rimoldi S, Brambilla F, Gornati R, Bernardini G, Saroglia M. In vivo regulation of GLUT2 mRNA in sea bass (*Dicentrarchus labrax*) in response to acute and chronic hypoxia. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*. 2009;152(4):306-316. doi:10.1016/j.cbpb.2008.12.011

4. Hall JR, Short CE, Driedzic WR. Sequence of Atlantic cod (*Gadus morhua*) GLUT4, GLUT2 and GPDH: developmental stage expression, tissue expression and relationship to starvation-induced changes in blood glucose. *J Exp Biol*. 2006;209(22):4490-4502. doi:10.1242/jeb.02532

5. Krasnov A, Teerijoki H, Mölsä H. Rainbow trout (*Onchorhynchus mykiss*) hepatic glucose transporter. *Biochim Biophys Acta*. 2001;1520(2):174-178. doi:10.1016/S0167-4781(01)00258-5

6. Martínez-Quintana JA, Kikuta S, Felix-Portillo M, Peregrino-Uriarte AB, Yepiz-Plascencia G. A novel functional glucose transporter in the white shrimp *Litopenaeus vannamei-LvGLUT2-is* up-regulated during hypoxia in hepatopancreas. *Mar Environ Res*. 2015;112:61-67. doi:10.1016/j.marenvres.2015.09.007

7. Wang P, Liu JY, Yao ZL, et al. Advances in saline-alkali adaptation physiology of aquatic animals. *Journal of Yangtze University (Natural Science Edition)*. 2015;12(15):44-47.

8. Li YD, Zhou FL, Huang JH, et al. Transcriptome and miRNA Profiles of Black Tiger Shrimp, *Penaeus monodon*, Under Different Salinity Conditions. *Frontiers in Marine Science*. 2020;7. doi:10.3389/fmars.2020.579381

9. Jiang SG, Yang CH, Zhou F. *Ban Jie Dui Xia Zhong Xia Fan Yu Ji Shu*. Ocean Press; 2013.

10. Owens L. A simple technique to stage the moult of *Penaeus monodon*. *Asian Fish Sci*. 2011;24:1-11. doi:10.33997/j.afs.2011.24.1.001

11. Li YD, Zhou FL, Huang JH, et al. Transcriptome reveals involvement of immune defense, oxidative imbalance, and apoptosis in ammonia-stress response of the black tiger shrimp (*Penaeus monodon*). *Fish and Shellfish Immunology*. 2018;83:162-170. doi:10.1016/j.fsi.2018.09.026

12. He P, Jiang SG, Li YD, et al. Cloning and Expression Analysis of GLUT1 Gene cDNA of *Penaeus monodon*. *Southern Fisheries Science*. 2019;15(02):72-82.

13. Fan HD, Li YD, Yang QB, et al. Characterization and expression analysis of MKK7 in black tiger shrimp (*Penaeus monodon*) under different stressors. *Journal of Fishery Sciences of China*. 2020;27(7):748-758. doi:10.3724/SP.J.1118.2020.19289

14. Fan HD, Li YD, Yang QB, et al. Isolation and characterization of a MAPKK gene from *Penaeus monodon* in response to bacterial infection and low-salinity challenge. *Aquaculture Reports*. 2021;20. doi:10.1016/j.aqrep.2021.100671

15. Yang LS, Li XL, Jiang S, et al. Characterization of Argonaute2 gene from black tiger shrimp (*Penaeus monodon*) and its responses to immune challenges. *Fish Shellfish Immunol*. 2014;36(1):261-269. doi:10.1016/j.fsi.2013.11.010

16. Soponpong S, Amparyup P, Tassanakajon A. A cytosolic sensor, *PmDDX41*, mediates antiviral immune response in black tiger shrimp *Penaeus monodon*. *Developmental & Comparative Immunology*. 2018;81:291-302. doi:10.1016/j.dci.2017.12.013

17. Soo TCC, Devadas S, Mohamed Din MS, Bhassu S. Differential transcriptome analysis of the disease tolerant Madagascar-Malaysia crossbred black tiger shrimp, *Penaeus monodon* hepatopancreas in response to acute hepatopancreatic necrosis disease (AHPND) infection: inference on immune gene response and interaction. *Gut Pathogens*. 2019;11:1-13. doi:10.1186/s13099-019-0319-4

18. Phan HNT, Nguyen HLT, Dinh TH, Le NT, Vu HG, Phan TN. Phenoloxidases from black tiger shrimp (*Penaeus monodon*): gene expression and activity distribution in different tissues. *Aquaculture International*. 2023;31(3):1423-1437. doi:10.1007/S10499-022-01033-Z

19. Zhou KM. *Cloning, Characterization and Expression Analysis of Chitinase Genes from* Penaeus Monodon. Shanghai Ocean University; 2017.

20. He ZL, Wang RY, Hector V, et al. Effect of Low Salinity Stress on the Behavior of *Macrobrachium rosenbergii*. *Journal of Zhejiang Ocean University (Natural Science)*. 2018;37(01):20-24.

21. Liu HJ, Pan LQ, Hu FW. The appraisement of immune ability of *Litopenaeus vannamei* under the change of salinity. *Transactions of Oceanology and Limnology*. 2008;26(2):159-166. doi:10.3969/j.issn.1003-6482.2008.02.022

22. Gao Y, Hu CQ, Ren CH, et al. Molecular cloning of aquaporin-4 (AQP4) gene in the Pacific white shrimp (*Litopenaeus vannamei*) and the effect of salinity stress on its expression in hepatopancreas. *Marine Sciences*. 2017;41(02):61-70.

23. Geng ZX, Shan HW, Ma S, Zuo XW, Wang T. Effect of Formula Diet with Freeze-Dried Powder of *Amphithoe* sp. on Accommodation of *Litopenaeus vannamei* to Low Salinity Stress. *Periodical of Ocean University of China*. 2020;50(08):65-74. doi:10.16441/j.cnki.hdxb.20190041

24. Liu SB, Xu C, Jia YY, Gu ZM, Li EC. Analysis of genes and metabolic pathways in response to long-term salinity stress in red crayfish (*Cherax quadricarinatus*). *Journal of Fisheries of China*. 2022;46(06):917-930.