RNA-seq provides novel insights into response to acute salinity stress in oriental river prawn Macrobrachium nipponense

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Research Article

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Abstract

The oriental river prawn *Macrobrachium nipponense* is an important aquaculture species in China, Vietnam, and Japan. This species could survive in the salinity ranging from 7 to 20 ppt and accelerate growth in the salinity of 7 ppt. To identified the genes and pathways in response to acute high salinity stress, *M. nipponense* were exposed to the acute high salinity of 25 ppt. Total RNA from hepatopancreas, gills, and muscle tissues was isolated, and then sequenced using high throughput sequencing method. Differentially expressed genes (DGEs) were identified, and total of 632, 836, and 1246 DEGs with a cutoff of significant two-fold change were differentially expressed in hepatopancreas, gills, and muscle tissues, respectively. Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genome pathway enrichment analysis were conducted. These DEGs were involved in the GO terms of cellular process, metabolic process, membrane, organelle, binding, and catalytic activity. The DEGs of hepatopancreas and gill tissues were mainly enriched in PPAR signaling pathway, longevity regulating pathway, protein digestion and absorption, and the DEGs of muscle tissue in arginine biosynthesis, adrenergic signaling in cardiomyocytes, cardiac muscle contraction, and cGMP-PKG signaling pathway. Real-time PCR conducted with fifteen selected DEGs indicated high reliability of digital analysis using RNA-Seq. This work provides a comprehensive insight into the molecular responses to high salinity stress in *M. nipponense*, which provide a novel contribution to understanding of the molecular mechanisms of adaptation to salinity stress in euryhaline crustaceans.

Introduction

Salinity is one of the most fundamental environmental factors affecting the distribution and physiological activities of aquatic organisms (Deane and Woo 2004; Aguilar et al. 2019). For the crustaceans, salinity could influence metabolism, growth and osmoregulation (Wang et al. 2018), which make profound impacts on survival, molting, oogenesis, embryogenesis and larval quality (Giménez and Anger 2001; Tantulo and Fotedar 2006; Pan et al. 2007; Huang et al. 2019). Under non-isotonic salinity conditions, aquatic organisms can maintain the homeostasis through osmoregulation (Chourasia et al. 2018). Despite some crustaceans could tolerant an extensive salinity range (Bertucci et al. 2017), drastic fluctuation in salinity can induce damage even cause death. In recent years, the utilization of freshwater species for seawater acclimation has already been a new trend in the aquaculture industry (Nikapitiya et al. 2014).

Studies have shown that salinity stress can influence ion channel activity in aquatic animals (Wheatly et al. 2002; Romano and Zeng 2011), in particular, the Na^+/K^+-ATpase (Khodabandeh et al. 2005), the $Na^+/K^+/2CL^-$ cotransporter (NKCC) (Carmosino et al. 2013), and the Sodium calcium exchanger (NCX) (Hiroi et al. 2008). Additionally, the energy used for osmoregulation can occupy 20–50% of the total energy consumption (Chen et al. 2015). A numerous metabolic changes allow organisms adapt to salinity variations, and different salinity stresses can cause various physiological responses (Aranguren Caro et al. 2021). Under intensive culture, variations in salinity may break homeostasis and lead to significant stress (Lindqvist 2004).

The oriental river prawn *Macrobrachium nipponense* (subphylum Crustacea, order Decapoda, family Palaemonidae, genus *Macrobrachium*) is an important aquaculture species in China, Japan and Vietnam (Yu et al. 2019). *M. nipponense* is commonly found in the fresh and brackish waters in most Asian countries (Sun et al. 2015). Few studies have reported the growth performance related with salinity. *M. nipponense* can survive in the salinity range from 7 to 20 ppt (Wang et al. 2002), and the salinity 7 ppt could promote the growth (Huang et al. 2019). Hence, the tolerance to salinity made this species have more potential to be cultured in brackish water or saline-alkali waters (Sun et al. 2015). Furthermore, the nutrition regulation (Ding et al. 2017), germplasm resource (Ma et al. 2012), and immune performance (Tang et al. 2017) of *M. nipponense* were also mostly deliberated. Although considerable progresses have been achieved in the research on *M. nipponense* (Sun et al. 2015; Zhang et al. 2021), relatively fewer studies on salinity adjustment of *M. nipponense*, particularly at the transcriptomic level of this species exposed to acute high salinity stress. In the present study, we characterized the genes, pathways, and transcriptome profile of *M. nipponense* in response to acute high salinity stress by using transcriptome analysis (Liu et al. 2013). The results reveal numbers of differentially expressed genes (DEGs) modified by acute high salinity stress and several important pathways, which will provide valuable insights for discovering the molecular basis of salinity stress adaptation of *M. nipponense*.

Materials And Methods

Ethics Approval

During this study, all experimental procedures involving prawns were conducted in accordance with the approval of the care and utilization of animals for scientific purposes set up by the Institutional Animal Care and the Use Committee (IACUS) of Shanghai Ocean University, Shanghai, China. The prawns were anesthetized in ice prior to removing the tissue samples, which was in accordance with ARRIVE guidance.

Sample Collection

M. nipponense of similar size (average body length of 2.51 ± 0.24 cm, average body weight of 0.34 ± 0.09 g) used in this study were obtained from a freshwater farm in Wuyi (Zhejiang, China). The prawns were transported to Hangzhou Fishery Research Institution (Zhejiang, China) in July 2021, and randomly separated into five rearing tanks with recirculating aerated pond water for two weeks in advance. The culture condition was maintained at 27.0 ± 1.0 °C, pH 7.6 ± 0.5, 6.2 ± 0.6 mg/L of dissolved oxygen, < 0.1 mg/L of total ammonia-nitrogen and natural photoperiod. Before the treatment began, *M. nipponense* was fed on commercial prawn feed twice per day (7:00 and 18:00). After adaptation to the cultivated environment, the total of 120 prawns were randomly selected and equally divided into three separate tanks (25 ppt). The first point of time (0 h) was considered as the control group, and the others (12, 24, 36, and 48 h) were regulated as the salinity stress treatment groups. During the treatment, six prawns from each tank were randomly sampled every 12 hours (0, 12, 24, 36, and 48 h). The gills, hepatopancreas and muscle from the acute high salinity stress treatment group and control group were sampled and immediately frozen in liquid nitrogen for subsequent experiments.

Total RNA Extraction and Library Preparation

Total RNA was extracted from each sample using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. RNA purity and quantification were evaluated using the NanoDrop 2000 spectrophotometer (Thermo Scientific, USA). RNA integrity was assessed using the Agilent 2100 Bioanalyzer (Agilent Technologies, USA). Then the libraries were constructed using TruSeq Stranded mRNA LT Sample Prep Kit (Illumina, USA) according to the manufacturer's instructions.

RNA Sequencing and Differentially Expressed Genes Analysis

The 150 bp paired-end Illumina sequencing reads were generated on an Illumina HiSeq X Ten platform of the OE Biotech Co., Ltd. (Shanghai, China). Raw reads containing poly-N and the low-quality reads were trimmed to obtain the clean data using Trimmomatic version 0.39 (Bolger et al. 2014). Clean data for each sample were retained for subsequent analyses. The clean data were mapped to the *M. nipponense* genome (cngb_100843) (Jin et al. 2021) using HISAT2 (Kim et al. 2015). FPKM (Roberts et al. 2011) of each gene was calculated using Cufflinks (Trapnell et al. 2010), and the read counts of each gene were obtained by HTSeq- count (Anders et al. 2015). Differential expression analysis was performed using the DESeq (2012) R package (Anders and Huber 2012). The *p-values* (*P*) < 0.05 and absolute $\log_2$ fold change (FC) > 2 or FC < 0.5 was considered cut-off criteria for DEGs. Hierarchical cluster analysis of DEGs was performed to demonstrate the expression pattern of genes in three sample tissues of five time points. Gene ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genome (KEGG) (Kanehisa et al. 2008) pathway enrichment analysis of DEGs were performed using R based on the hypergeometric distribution, respectively. GO terms and KEGG pathway annotation were achieved using the Blast2GO program and Kaas (KEGG Automatic Annotation Server) online program (http://www.genome.jp/kaas-bin/kass-man), respectively.

Verification Using Real-time qRT-PCR

Fifteen DEGs identified from the gills, hepatopancreas, and muscle, were selected to verify the reliability of the RNA-seq analysis using quantitative real-time PCR (qRT-PCR) methods. The Primers of the fifteen DEGs were listed in Table S2. The cDNA synthesis was performed using the PrimeScript^{TM} RT reagent Kit (TaKaRa, Dalian, China) according to the manufacturer's instructions. The qRT-PCR was performed on the CFX96^{TM} Real-time PCR Detection System (BioRad, Hercules, CA, USA) with SYBR Green Master Mix (TaKaRa, Dalian China) following the manufacturer's instructions. The expression level of each gene was normalized towards the reference gene (EIF). Relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method (Ma et al. 2012). The data were recorded as the mean ± standard deviation of three replicates. The gene expression level was analysed using one-way analysis of variance. Differences were considered statistically significant at $p < 0.05$.

Results

Preliminary Analysis of the Transcriptomic Sequencing Data

To detect the time-dependent transcription expression of *M. nipponense* exposed to acute high salinity stress, whole transcriptome was sequenced from hepatopancreas, gills and muscle of five time point under acute high salinity stress (0, 12, 24, 36 and 48 h). After removing the adaptor and trimming the low-quality reads, a total of 726.26 Gb clean reads was obtained from hepatopancreas, gills and muscle tissues. The minimum of base score of Q30 was over 93.31% (Table S1). Subsequently, the clean data were mapped to the *M. nipponense* reference genome.

Different Expressed Genes in Response to Salinity Stress

To elucidate the gene expression pattern under salinity stress, the numbers of DEGs of the four time point treatments (12, 24, 36 and 48 h) was compared with the control group (0h) respectively. In the hepatopancreas, 6477 DEGs (2813 up- and 3665 down-regulated) at 12 h, 7807 DEGs (2696 up- and 5111 down-regulated) at 24 h, 5886 DEGs (2208 up- and 3678 down-regulated) at 36 h and 6948 (2261up- and 4687 down-regulated) DEGs at 48 h were identified to be significantly differentially expressed respectively (Figure 1.C). Total of 632 DEGs were found to be differentially expressed with the fold change (FC) of in all the four time points (FC $\geq$ 2, $P < 0.05$). In addition, stricter limitations (FC $\geq$ 4, $P < 0.01$) were set to determine the significant DEGs. The results showed that there were 83 DEGs differentially expressed (Figure 1.F). In the gill tissue, 6015 DEGs (2751up- and 3264 down-regulated) at 12 h, 8783 DEGs (3587 up- and 5196 down-regulated) at 24 h, 12164 DEGs (3904 up- and 8260 down-regulated) at 36 h and 8575 DEGs (3785 up- and 4790 down-regulated) at 48 h were identified to be significantly differentially expressed with the fold change (FC $\geq$ 2, $P < 0.05$), respectively (Figure 1.B). Totally, 836 DEGs were found were found to be differentially expressed in four treatment time points. In addition, we set stricter limitations (FC $\geq$ 4, $P<0.01$) and there were 146 DEGs were differentially expressed (Figure 1.E). In the muscle, 9198 (5561up- and 3637 down-regulated), 9435 (3764 up- and 5671 down- regulated), 9207(4324 up- and 4883down-regulated) and 8524 (3119 up- and 5405down-regulated) DEGs were obtained at 12, 24, 36, 48 h, respectively (Figure 1.A). Generally, a total of 1246 DEGs were found to be differentially expressed in four treatment time points (FC $\geq$ 2, $P<0.05$). In addition, there were 99 DEGs were significantly differentially expressed (Figure 1.D). Generally, a total of 16735, 23013 and 21241 DEGs were detected in hepatopancreas, gills and Muscle, respectively.

To understand how acute high salinity stress impacts the osmoregulation of *M. nipponense*, the DEGs were further conducted to GO analysis for potential functions. The results illustrated that the primary significant biological process of DEGs were similar in the three tissues (hepatopancreas, gills and muscle) under acute high salinity stress. These GO terms were involved in cellular process (GO: 0009987; level 2, biological process; level 1), metabolic process (GO: 0008152; level 2, biological process; level 1), membrane (GO: 0016020; level 2, cellular component; level 1), organelle (GO: 0043226; level 2, cellular component; level 1), binding (GO: 0005488; level 2, molecular function; level 1), and catalytic activity (GO: 0003824; level 2, molecular function; level 1) (Figure 2).

The networks of molecular interactions were identified by mapping the DEGs to the KEGG pathway (Figure. 3) of the top 20 enriched pathways. For further analysis, the common and differentially enriched

pathways were also recorded from the top 20 pathways (Table 1). Compared with the control group of hepatopancreas, seven KEGG pathways were significantly enriched in the treatment group, including PPAR signaling pathway (ko03320), longevity regulating pathway-worm (ko04212), antigen processing and presentation (ko04261), carbohydrate digestion and absorption (ko04973), protein digestion and absorption (ko04974), biosynthesis of unsaturated fatty acids (ko01040), and lysine degradation (ko00310). In addition, six KEGG pathways were enriched in gills, including longevity regulating pathway-worm (ko04212), adrenergic signaling in cardiomyocytes (ko04261), cardiac muscle contraction (ko04260), protein digestion and absorption (ko04974), lysosome (ko04142), and oxidative phosphorylation (ko00190). In the muscle, six pathways were enriched, including arginine biosynthesis (ko00220), adrenergic signaling in cardiomyocytes (ko0426), cardiac muscle contraction (ko04260), arginine biosynthesis (ko00220, down), cGMP-PKG signaling pathway (ko04022), and D-Glutamine and D-glutamate metabolism (ko00471).

Validation of DEGs with qRT-PCR

To validate the transcriptome data, qRT-PCR experiments on five cDNA templates of each tissue sample were performed respectively. The fifteen DEGs were randomly selected to meet the strict requirement, which were expressed in the whole treatment (Table S2). Compared with the control group, the relative expression levels of the five candidate DEGs in the four treatment groups are performed (Figure. 4). The results exhibited that the expression trend of the fifteen candidate DEGs were consistent between the qRT-PCR and transcriptome analysis, indicating the high credibility of the transcriptome data. Therefore, the qRT-PCR results confirmed the reliability and accuracy of the RNA-Seq data.

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Discussion

Salinity is a critical environmental factor influencing the osmotic pressure of aquatic animals. Osmoregulation of crustaceans is complex due to their diverse ranges of salinity (Chen et al. 2015). Most crustaceans have certain salinity tolerance and can live within a certain salinity range by osmoregulation. When aquatic animals are subjected to acute salinity, the mechanism will activate the antioxidant system to eliminate the oxidative stress caused by salinity mutation (Lou et al. 2019). However, when exceeding the controllable range, it will cause oxidative damage. Therefore, crustaceans may not exhibit a rapid osmotic regulation capacity when they experience salinity stress (Chen et al. 2015). Hence, comprehending the osmotic regulation mechanism of crustaceans has contributed to increasing attention recently. Although numerous studies of molecular mechanism underlying salinity adaptation have already been carried out, including ion transport in gill (Pan et al. 2014), osmoregulation in hemolymph (Do et al. 2001), and biological process in muscle (Lou et al. 2019), limited evidence is available to explain how acute salinity stress influences the whole tissues of the crustaceans. Through RNA-Seq analysis, several differently expressed genes and pathways were determined.

M. nipponense is an euryhaline crustacean species, which could tolerate a wide range of salinity fluctuation from 0 to 22 ppt, and the semi-lethal salinity for 96 h are above 25 ppt (Huang et al. 2019). Therefore, the salinity of 25 ppt were selected as an acute high salinity stress treatment in the present study. To understand the dynamic changes in gene expression in acute high salinity tolerance, 5-time points during the stress treatment (0, 12, 24, 36, and 48 h) were chosen to conduct transcriptomic analysis in three tissues. To identify the function of DEGs potentially associated with the osmoregulation of *M. nipponense*, a GO term enrichment analysis was conducted in hepatopancreas, gills, and muscle, respectively. The results showed that although the GO terms were not entirely similar, the mainly enrichment terms show higher similarity in chronological order (Figure. 4). These DEGs were significantly enriched in cellular process, metabolic process, membrane, organelle, binding, and catalytic activity. The enrichment of binding and cellular process might represent the immune response (Lou et al. 2019). The fatty acid composition of membrane and metabolic process can influence permeability to water and ions (Palacios and Racotta 2007; Chen et al. 2015). The catalytic function of enzymes regulates on ion exchanges and osmoregulation. Therefore, the salinity stress might contribute to cellular stress response and activate several functional genes of *M. nipponense* to prevent apoptosis through the significant enrichments in binding, cellular process, and metabolic process.

For crustaceans, osmotic balance in high salinity is achieved by distinct biochemical mechanisms that regulate the exchange of ions with the environment. Sodium (Na^+) and chlorine (Cl^-) ions account for a relatively significant proportion of the composition of hemolymph in most crustaceans (Wang et al. 2018). In acute salinity stress, *M. nipponense* should extrude superfluous ions gained from the hyperosmotic environment. The relative genes can facilitate salt extrusion, including NKCC, Na^+/K^+-ATPase, and NCX. Recent research suggests that NCX can remove calcium (Ca^{2+}) from cells and then place 2 Na^+ or Na^+ and K^+ into cells (Hiroi et al. 2008). The expression of NCX was up-regulated at 12h, 24h, 36h, and down-regulated at 48h (Figure S1). Hence, we assumed that the osmoregulation of *M. nipponense* was activated. However, the ability to exchange ions was restrained at acute high salinity. Besides, the Na^+/K^+-ATPase was kept down-regulation in the whole treatment experiments (Figure S1 and Figure S2). This finding corresponded to the previous result (Chung and Lin 2006) that the Na^+/K^+-ATPase might not be activited in response to high salinity stress. In addition, the NKCC was down-expressed in *M. nipponense* exposed to acute high salinity from 12 to 48h (Figure S2). The NKCC may transport Na^+, K^+, and Cl^- into cells from the previous studies (Velotta et al. 2014). However, the expression of NKCC in this study did not match the expectation. Hence, the acute salinity might damage the osmoregulation of *M. nipponense* and restrain the activity of NKCC.

Crustaceans rely on innate immune system to recognize and react to environmental antigens (Zheng et al. 2017). The innate immune system of prawn relies on humoral and cellular immunity (Chen et al. 2020). The proPO can generate the active enzyme phenoloxidase (Po) by producing the melanin and toxic reactive intermediates to regulate the whole proPO system (Chan et al. 2009). In this study, the proPO was found to be down-regulated at the 12h salinity stress treatment (Figure S4), which may suggest acute salinity causes the damage to organism by the accumulation of an excessive cytotoxic substance in *M. nipponense*. Furthermore, antioxidant enzymes, including SOD, can provide a post-phagocytosis self-protection of oxygen-respiring organisms. In our study, the expression of SOD3 was kept down-regulated, except for 36h treatment (Figure S3). From a previous study of SOD, those antioxidant indicators could recover rapidly and reduce the damage antioxidant system (Zheng et al. 2017). We speculated that the acute salinity was beyond the ability from self-recover and caused antioxidant damage, which contributed to the inhabitation of the regulation of SOD3. Lysozyme worked as an antibacterial protein and a component of the innate immune system based on its small molecular weight and bacteriolytic effects (Li and Xiang 2013). However, the expression of Lyz also significantly down-regulated in the whole 48h treatment (Figure S2). Apart from the osmoregulation, we also found the immune-related pathways were enriched in this treatment, including antigen processing pathway and longevity regulating pathway-worm. Heat shock proteins (HSPs), referred to as molecular chaperones or stress proteins, comprise a group of highly conserved proteins that are ubiquitous in both prokaryotic and eukaryotic organisms (Kregel 2002).HSPs also play a pivotal role in maintaining of normal cellular homeostasis by acting as molecular chaperones for other proteins. Hence, the up-regulated of HSP90 (Figure S6) in the 12 and 48 treatments also indicated that the HSPs might be a better indicator gens indicating environmental stress of aquatic organisms. Consequently, we hypothesized that the acute high salinity stress affected the immune system of *M. nipponense*. However, the high salinity may exceed the

ability of self-regulation and cause damage to the innate immune system. Previous studies have reported the changes in antioxidant enzymes and proteins in response to various environmental stressors (Wang et al. 2015), which was consistent with our findings.

Due to the complexity of the physiological response to acute salinity stress in *M. nipponense*. We focused on two significantly enriched metabolism pathways regulated the whole experimental treatment (Table. 1), including biosynthesis of unsaturated fatty acids and oxidative phosphorylation pathway. Besides, the polyunsaturated fatty acid and saturated fatty acid ensure the extra energy for osmoregulation and ion exchange (Chen et al. 2014). However, most expression of DEGs was down-regulated in this study, with the exception of HSD17B12 (Figure S7). The HSD17B12, a typical estrogen 17b-HSD (Luu-The et al. 2006),was firstly identified as a ketoacyl CoA reductase involved in fatty acid chain lengthening (Hiltunen et al. 2019), and was also found during the process of the fish oocyte maturation (Aranyakanont et al. 2020). In the current study, the expression of HSD17B12 was upregulated at 12h and then down-regulated in the following salinity (Figure. S7). Hence, the acute salinity might partly stimulate the sexual determination, establishment, and maintenance of secondary sexual characteristics of *M. nipponense*. Besides, the biosynthesis of PUFAs, including ALA, EPA, and DHA, were not significantly enriched in the current study (Figure S7). The PUFAs are mainly incorporated in cell membranes and can increase membrane permeability and fluidity (Sui et al. 2007). Additionally, the PUFAs can improve the resistance to osmotic shock in aquatic animals (Chen et al. 2015). Therefore, we inferred that the *M. nipponense* might not have dynamic lipid metabolism under the acute high salinity stress. In the oxidative phosphorylation pathway, most DEGs were up-regulated in 48h treatment (Figure S5), compared with other experimental treatments. In this study, genes related to nicotinamide adenine dinucleotide (NADH) dehydrogenase, cytochrome C reductase, cytochrome C oxidase, and F-type ATPase (Eukaryotes) were up-regulated (Figure S5). These enzymes are located on the mitochondrial membrane and constitute electron transfer chains that synthesize ATP through biological oxidation (Dahout-Gonzalez et al. 2006). NADH is an important coenzyme in cells involved in most oxidation-reduction reactions of sugars, fats, and proteins (Ying 2007). Our current findings correspond with the finding that the osmoregulation accompanies with increasing in energy demand (Li et al. 2014). Besides, the large amount of up-regulated DEGs also illustrated the 48h timepoint required more energy in osmoregulation when *M. nipponense* was exposed to acute salinity stress.

Conclusions

The osmoregulation of crustaceans is a complex physiological, biochemical, and molecular process when exposed to acute salinity stress. This study performed transcriptome analysis of *M. nipponense* in response to acute salinity stress using RNA-Seq method. RNA-seq data revealed major changes in the expression of metabolic, immune, and ion exchange related DEGs in the gill, hepatopancreas and muscle tissues of *M.nipponense* under acute high salinity stress. In conclusion, transcriptome analyses provide novel insights into the osmoregulation and molecular mechanisms of *M. nipponense* under acute salinity high stress.

Declarations

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Competing Interests: The authors have no competing interests to disclose.

Authors' Contributions

Jianbin Feng and Jiale Li conceptualized the study. Yaoran Fan, Feiyue Ling, Zefei Wang and Xie Nan collected the specimens and experimented. Yaoran Fan performed the qRT-PCR and bioinformatics work. Yaoran Fan and Jianbin Feng participated in the formal analysis of the results. Yaoran Fan drafted the manuscript. Jianbin Feng, Xueming Hua, and Keyi Ma critically evaluated and approved the article.

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Tables

Table 1: Common and differentially enriched KEGG pathways classification in whole 48h treatment

Term	Classification Level 2	Tissue
Longevity regulating pathway - worm	Aging	hepatopancreas /gill
Lysine degradation	Amino acid metabolism	hepatopancreas
Adrenergic signaling in cardiomyocytes	Circulatory system	muscle/gill
Cardiac muscle contraction	Circulatory system	muscle/gill
Carbohydrate digestion and absorption	Digestive system	hepatopancreas
Protein digestion and absorption	Digestive system	hepatopancreas /gill
PPAR signaling pathway	Endocrine system	hepatopancreas
Oxidative phosphorylation	Energy metabolism	gill
Antigen processing and presentation	Immune system	hepatopancreas
Biosynthesis of unsaturated fatty acids	Lipid metabolism	hepatopancreas
Arginine biosynthesis	Metabolism	muscle
D-Glutamine and D-glutamate metabolism	Metabolism of other amino acids	muscle
cGMP-PKG signaling pathway	Signal transduction	muscle
Lysosome	Transport and catabolism	gill

Figures

Figure 1

Venn diagram of differentially expressed genes of three tissues in response to high salinity stress in *Macrobrachium nipponense*

Overview of DEGs in 12, 24, 26 and 48 h time points comparisons in muscle (A and D), gills (B and E) and hepatopancreas (C and F), respectively, based on a Veen diagram.

Figure 2

Gene ontology (GO) terms of differentially expressed genes in response to high salinity stress in *Macrobrachium nipponense*

Results of the enrichment analysis of the gene ontology (GO) terms of differentially expressed genes of *Macrobrachium nipponense* exposed to acute high salinity in muscle (A, B, C, D), gills (E, F, G, H), and hepatopancreas (I, J, K, L) in 12, 24, 36 and 48 h time point, respectively.

Figure 3

Enrichment of top 20 KEGG pathways in response to high salinity stress in *Macrobrachium nipponense*

Results of Top 20 KEGG pathways enrichment for *Macrobrachium nipponense* exposed to acute high salinity stress in gills (A, B, C, D), muscle (E, F, G, H), and hepatopancreas (I, J, K, L) at 12, 24, 36 and 48h, respectively.

Figure 4

Relative change of the transcriptome and qRT-PCR data of 15 candidate differentially expressed genes

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