RNA-seq Provides Novel Insights into Response to Acute Salinity Stress in Oriental River Prawn *Macrobrachium nipponense*

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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 identify the genes and pathways in response to acute high salinity stress, *M. nipponense* was 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 a total of 632, 836, and 1246 DEGs with a cutoff of significant twofold change were differentially expressed in the hepatopancreas, gills, and muscle tissues, respectively. Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genome pathway enrichment analyses 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. The results indicated that the *M. nipponense* may regulate essential mechanisms such as metabolism, oxidative stress, and ion exchange to adapt the alternation of environment, when exposed to acute high salinity stress. This work reveals the numbers of genes modified by salinity stress and some important pathways, which could provide a comprehensive insight into the molecular responses to high salinity stress in *M. nipponense* and further boost the understanding of the potential molecular mechanisms of adaptation to salinity stress for euryhaline crustaceans.

**Keywords** *Macrobrachium nipponense* · RNA-seq · Acute high salinity stress · Differentially expressed gene · KEGG pathway

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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 development (Jiménez and Anger 2001; Tantulo and Fotedar 2006; Pan et al. 2007; Huang et al. 2019). 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). Under non-isotonic salinity conditions, aquatic organisms can maintain the homeostasis through osmoregulation (Chourasia et al. 2018). Despite some crustaceans could tolerate 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).

Osmoregulation of crustaceans is complex due to their diverse ranges of salinity (Chen et al. 2015). 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). 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% to 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). A 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), there are relatively fewer studies on salinity adjustment of M. nipponense, particularly at the transcriptomic level of this species exposed to acute high salinity stress. 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. 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 2 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 in July. 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 h (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 Gene 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 log2 fold change (FC) > 2 or FC < 0.5) was considered cutoff criteria for DEGs. Hierarchical cluster analyses 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™ RT reagent Kit (TaKaRa, Dalian, China) according to the manufacturer’s instructions. The qRT-PCR was performed on the CFX96™ 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−ΔΔCT method (Ma et al. 2012). The data were recorded as the mean ± standard deviation of three replicates. The gene expression level was analyzed 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 points under acute high salinity stress (0, 12, 24, 36, and 48 h). Forty-five experimental cDNA libraries were constructed, and three biological repetitions were utilized for three tissues (hepatopancreas, gills, and muscle tissues) in five time points (0 h, 12 h, 24 h, 36 h, 48 h), respectively. 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) were compared with the control group (0 h), respectively. In the hepatopancreas, 6477
DEGs (2813 up- and 3665 downregulated) at 12 h, 7807 DEGs (2696 up- and 5111 downregulated) at 24 h, 5886 DEGs (2208 up- and 3678 downregulated) at 36 h, and 6948 (2261 up- and 4687 downregulated) DEGs at 48 h were identified to be significantly differentially expressed, respectively (Fig. 1C). A total of 632 DEGs were found to be differentially expressed with the fold change (FC) of in all the four time points (FC ≥ 2, \( P < 0.05 \)). In addition, stricter limitations (FC ≥ 4, \( P < 0.01 \)) were set to determine the significant DEGs. The results showed that there were 83 DEGs differentially expressed (Fig. 1F). In the gill tissue, 6015 DEGs (2751 up- and 3264 downregulated) at 12 h, 8783 DEGs (3587 up- and 5196 downregulated) at 24 h, 12,164 DEGs (3904 up- and 8260 downregulated) at 36 h, and 8575 DEGs (3785 up- and 4790 downregulated) at 48 h were identified to be significantly differentially expressed with the fold change (FC ≥ 2, \( P < 0.05 \)), respectively (Fig. 1B). Totally, 836 DEGs were found to be differentially expressed in four treatment time points. In addition, we set stricter limitations (FC ≥ 4, \( P < 0.01 \)), and there were 146 DEGs were significantly differentially expressed (Fig. 1E). In the muscle, 9198 (5561 up- and 3637 downregulated), 9435 (3764 up- and 5671 downregulated), 9207 (4324 up- and 4883 downregulated), and 8524 (3119 up- and 5405 downregulated) DEGs were obtained at 12, 24, 36, and 48 h, respectively (Fig. 1A). Generally, a total of 1246 DEGs were found to be differentially expressed in four treatment time points (FC ≥ 2, \( P < 0.05 \)). In addition, there were 99 DEGs were significantly differentially expressed (Fig. 1D). Based on the annotation information, 126 DEGs were kept regulated in five time points (Table S2). Generally, a total of 16,735, 23,013, and 21,241 DEGs were detected in the 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 was similar in the three tissues (hepatopancreas, gills, and muscle) under acute high salinity stress. These GO terms were involved in cellular process (GO, 0.009,987; level 2, biological process; level 1), metabolic process (GO, 0.008,152; level 2, biological process; level 1), membrane (GO, 0.016,020; level 2, cellular component; level 1), organelle (GO, 0.043,226; level 2, cellular component; level 1), binding (GO, 0.005,488; level 2, molecular function; level 1), and catalytic activity (GO, 0.003,824; level 2, molecular function; level 1) (Fig. 2).

The networks of molecular interactions were identified by mapping the DEGs to the KEGG pathway (Fig. 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 S3). Compared with the control group, the relative expression levels of the five candidate DEGs in the four treatment groups are performed (Fig. 4). The results exhibited that the expression trend of the fifteen candidate DEGs was 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.

Discussion

*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 is above 25 ppt (Huang et al. 2019). Therefore, the salinity of 25 ppt was selected as an acute high salinity stress treatment in group.
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 (Fig. 4). These DEGs were significantly enriched in cellular process, metabolic process, membrane, organelle, binding, and catalytic activity. A previous study illustrated that the catalytic function of enzymes regulated ion changes and osmotic pressure when crustaceans are exposed to salinity stress (Wang et al. 2018). Besides, the fatty acid composition of membrane and metabolic process can also influence permeability to water and ions (Palacios and Racotta 2007; Chen et al. 2015). Hence, those functional changes involved in osmotic regulatory processes enable crustaceans to adapt to new environments with salinity stress. Given that the major role of those processes is to compensate for crustacean osmoregulation systems, crustaceans adjust their metabolism to provide the energy required by those functional changes. Apart from this, the GO term analysis also showed the enrichment in cellular process and binding, which may represent the influence of cellular stress responses (Lou et al. 2019). Therefore, the salinity stress might activate several functional genes of *M. nipponense* to prevent apoptosis through the significant enrichments in binding and cellular 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 chloride (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 (Ca²⁺) from cells and then place 2 Na⁺ or Na⁺ and K⁺ into cells (Hiroi et al. 2008). The expression of NCX was upregulated at 12 h, 24 h, and 36 h, and downregulated at 48 h (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 downregulation in the whole treatment experiments (Figures S1 and S2). This finding corresponded to the previous result (Chung and Lin 2006) that the Na⁺/K⁺-ATPase might not be activated in response to high salinity stress. In addition, the NKCC was down-expressed in *M. nipponense* exposed to acute high salinity from 12 to 48 h (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 downregulated at the 12-h 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 include SOD and CAT. SOD and CAT activities are important indexes of antioxidant capacity. Antioxidant capacity has a strong defense function against oxidative cell damage in the body by transforming hydrogen peroxide into water and by eliminating free radicals, which are generated owing to stress along with other reasons (Mathew et al. 2007). In our study, the expression of SOD3 was kept downregulated, except for 36 h treatment (Figure S3). However, the expression of CAT was not significantly regulated in five time points. 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 downregulated in the whole 48 h 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 normal cellular homeostasis by acting as molecular chaperones for other proteins. Hence, the upregulated 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.

To investigate 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 downregulated in this study, with the exception of HSD17B12 (Figure S7). The HSD17B12, a typical estrogen 17β-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 12 h and then downregulated 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, was 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 upregulated in 48 h 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 upregulated (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 upregulated DEGs also illustrated the 48-h time point 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 in the gill, hepatopancreas, and muscle tissues of *M. nipponense* under acute high salinity stress in 48-h treatment. The results indicated that the osmoregulation of *M. nipponense* was primarily regulated by the energy metabolism and cell membrane regulation and ultimately affect the osmoregulation of *M. nipponense* against acute high salinity stress. In addition, the RNA-seq data also revealed that many other DEGs and KEGG pathways may participate in the osmoregulation of *M. nipponense*. Those significant genes and enriched KEGG pathways indicated that in the short-term acute salinity stress, the *M. nipponense* may regulate some mechanisms such as metabolism, oxidative stress, and ion exchange to adapt the alternation of environment. Therefore, the salinity stress might impact the growth of *M. nipponense* and cause immunity responses. These transcriptome analyses provide novel insights into the osmoregulation and molecular mechanisms of *M. nipponense* under acute salinity high stress.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s10126-022-10151-x.

**Author Contribution** 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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**Declarations**

**Competing Interests** The authors declare no competing interests.

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