The effects of ammonia stress exposure on protein degradation, immune response, degradation of nitrogen-containing compounds and energy metabolism of Chinese mitten crab

Dan Tang1,2 · Ya Wu1 · Lv Wu1 · Yuze Bai1 · Ying Zhou1 · Zhengfei Wang1

Received: 17 October 2021 / Accepted: 16 March 2022 / Published online: 28 March 2022 © The Author(s), under exclusive licence to Springer Nature B.V. 2022

Abstract

Background The Chinese mitten crab is one of the most economically important crabs that are widely farmed in China. Ammonia, which is a main physiological challenge for crab culture, grows rapidly in the intensive culture system over time, but little information is available with Chinese mitten crab on the molecular mechanisms.

Methods and results Therefore, to understand the mechanism of response to ammonia stress in Eriocheir japonica sinensis, comparative transcriptome analysis was used to identify the key genes and pathways in hepatopancreas challenged with ammonia stress (325.07 mg/L NH4Cl). By sequencing the transcriptome hepatopancreas of E. j. sinensis treated with ammonia, 366,472 unigenes were obtained and annotated into several public libraries for later analyses. Subsequently, 1775 differentially expressed genes (DEGs) were identified according to comparative transcriptome analysis, of which 307 were up-regulated and 1468 were down-regulated. According to the DEGs of GO and KEGG enrichment analyses, we focused on four aspects of significant enrichment in this study: protein degradation, immune response, degradation of nitrogen-containing compounds and energy metabolism. The genes involved in protein degradation and energy metabolism process showed a significant decrease which was consisting of overall biological activity of E. j. sinensis decreased. In addition, five genes involved in high concentration of ammonia were discovered and validated by qRT-PCR.

Conclusions This study will help us understand the molecular mechanisms of E. j. sinensis under high ammonia exposure and provide valuable information to the future research of other crabs with ammonia exposure.

Keywords Eriocheir japonica sinensis · Transcriptome analysis · Ammonia stress · Metabolism response · Immunity response

Introduction

The Chinese mitten crab (E. j. sinensis) is one of the most economically aquaculture species in China, and has become a traditional dish in China because of its high nutritive value [1]. This species is one of fundamental species contributing to fresh-water fishery in China [2]. The total farming area of E. j. sinensis was about 670,000 ha in 2020 (http://www.moa.gov.cn/). In order to pursue more production value, the semi-intensive and intensive culture systems were used to culture the Chinese mitten crab. However, intensive culture often leads to the degradation of the pond water from uneaten protein feeds and heterotrophic bacterial metabolites [3], but also the nitrifying bacteria have reduced ability to nitrify bacterial metabolites due to the low level of dissolved oxygen (DO). Therefore, the degraded pond water factors, such as ammonia, play an important role in crab culture.

Dan Tang and Ya Wu have contributed equally to this work.

Zhengfei Wang wangzf@yctu.edu.cn

1 Jiangsu Key Laboratory for Bioresources of Saline Soils, Jiangsu Synthetic Innovation Center for Coastal Bio-Agriculture, Jiangsu Provincial Key Laboratory of Coastal Wetland Bioresources and Environmental Protection, School of Wetlands, Yancheng Teachers University, Yancheng 224001, Jiangsu, China

2 College of Biotechnology and Pharmaceutical Engineering, Nanjing Tech University, Nanjing 211800, Jiangsu, China
In aquaculture, ammonia is one of the main environmental limiting factors that give rise to a series of adverse effects in crabs, such as, the low growth rate, the damage of tissues, and even the high mortality [3, 4]. In the last decade, the intensive culture system in aquaculture has become a high trend which will lead to the accumulation of ammonia. The more intensively farmed the higher level of the ammonia content produced. For crustaceans, the metabolic wastes such as ammonia will mainly excrete through the gills, which depend on the difference between the concentration of ammonia in the environment and organism [3]. However, if the concentration of ammonia in the environment is too high, the ammonia excretion was difficult and the metabolic activity of crustaceans can be affected. In addition, toxic nitrogen-containing compounds are digested in hepatopancreas, which is a significant organ for digestion and detoxification process in crabs [5, 6].

Generally, ammonia comes in two forms ionic form ($\text{NH}_4^+$) and in its unionized form ($\text{NH}_3$). Moreover, in most cases, the unionized ammonia is more toxic to aquatic organisms [3]. As ionic ammonia cannot get into the cell while the unionized ammonia can pass through cell membranes, the ionic form of ammonia can convert to the toxic form through the change of some other factors, such as salinity, pH and temperature. Low salinity can increase the toxicity of ammonia ($\text{NH}_3$) to Litopenaeus vannamei [7], Penaeus chinensis juveniles [8], P. semisulcatus juveniles [9]. It has reported that high pH and high temperature increase the relative proportion of $\text{NH}_3$ in Rhamdia quelen and Piaractus mesopotamicus [10, 11].

Previous studies have shown that the high concentration of ammonia stress will cause the increase of oxygen consumption, the decrease of osmotic regulation ability, affecting the molting frequency of crustacean, and even cause the damage of hepatopancreas and gill tissue, contributing to the high mortality of crustacean [7, 12]. Additionally, the high levels of ammonia exposure also impact on immune responses and metabolic processes of crustacean, which have been confirmed in E. j. sinensis [13]. A lot of studies have been done on aquatic animals exposed to high levels of ammonia, such as Marsupenaeus japonicas [12], L. vannamei [14], Dicentrarchus labrax [15, 16], M. carcinus [17], Portunus trituberculatus and E. j. sinensis [13]. However, the underlying molecular basis of the hepatopancreas in crabs on ammonia stress is still limited. In order to discover and investigate the complex molecular responses of E. j. sinensis under high concentrations of ammonia stress, the technology of Illumina sequence was used to identify genes and pathways involved in the response to ammonia stress. The results will provide a useful insight into immune mechanisms of the E. j. sinensis response to high level of ammonia.

**Materials and methods**

**Animals and ammonia stress experiment**

*E. j. sinensis* used in this experiment were obtained from Yancheng (Jiangsu, China). Twenty similar-sized and mature female Chinese mitten crabs with average weight of about 120 g were acclimated. Two crabs were acclimated in the plastic can (30 cm × 18 cm × 20 cm) for one week, filled with 4 L water (temperature: ~ 17 °C; PH: ~ 7.5). During these days, crabs were fed the same commercial feed used during the adaptation period at 3% of the body weight daily at 12:00. Fresh dechlorinated water was changed at 9:30 am on a daily basis and removing the rest food from the day before. The concentration of ammonium chloride used in the experiment was determined according to the study of Hong ML. The 96 h-LC50 (the concentration lethal to half of any given species over a 96 h) is 325.07 mg/L $\text{NH}_4\text{Cl}$ with Chinese mitten crabs. Finally ammonium chloride concentration was set to 325.07 mg/L.

16 crabs were randomly divided into two groups after acclimating; one group was only kept in clean water as control group; another group was added 325.07 mg/L $\text{NH}_4\text{Cl}$ and exposed to ammonia for 24 h as the experimental group (Fig. 1). Eventually, three crabs in each group were dissected on ice bath and the hepatopancreas tissues were obtained. Further the hepatopancreas were immediately frozen in liquid nitrogen for later RNA extraction.

**RNA extraction, cDNA synthesis and gene sequencing**

TRIZol Reagent (Invitrogen, Shanghai, China) was used to extract the total RNA of hepatopancreas tissue samples following the manufacturer’s instructions. RNA purity was assessed using a Nanodrop 2000 spectrometer instrument (Thermo Fisher Scientific Inc. Waltham, MA, USA). RNA integrity was checked with 1% agarose gel electrophoresis. Magnetic bead was checked with 1% agarose gel electrophoresis. Magnetic bead with Oligo (dT) and poly A probes was used to separate mRNA from total RNAs. Then, mRNAs were fragmented (approximately 200 bp) by a fragmentation buffer. The purity and concentration of them were confirmed via a Nanodrop 2000 spectrometer. Random-hexamer primers were prepared to synthesize the first strand cDNA, subsequently the second strand cDNA was synthesized by adding DNA polymerase I, RNase H, dNTP and buffer to form a stable double-stranded structure. 2% agarose gel electrophoresis was used to purify the fragments, followed by 200–300 bp fragments were selected and used as templates for PCR for 15 cycles. After this a cDNA library was established. Finally, the mRNA
database was established with the Illumina TruSeqRNA Sample Prep Kit (Illumina Inc., San Diego, CA, USA) and the final quality of the fragments was checked by an Agilent 2100 Bioanalyzer. All cDNA library procedures occurred in the Yuanshen Company (Shanghai, China).

**Transcriptome assembly and annotation**

The raw reads were contained the adaptor sequences, contamination, ambiguous nucleotides or low quality reads (<20 bp reads). The raw reads were filtered by the SeqPrep software (https://github.com/jstjohn/SeqPrep) and Sickle software (https://github.com/najoshi/sickle). The nucleotide sequences obtained by above method were assembled to form longer fragments and transcripts with Trinity software (http://trinityrnaseq.sourceforge.net/). Transcripts, representing significant parts of individual isoforms, were clustered on the basis of the same gene sequence. The longest transcripts of each gene were pooled as “unigenes”. BlastX (criterion: e value of <10⁻⁵) was performed to compare the transcripts with the following databases: NCBI-nr, Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), Clusters of Orthologous Groups of proteins (COG), Swiss-Prot protein, String, and Pfam.

**Analysis of differentially expressed genes (DEGs)**

The expressed values and transcription levels were calculated via fragments per kilobase per million mapped reads (FPKM) method, reducing the influence of gene lengths. Differences in gene expression abundance between the ammonia treatment and control groups were statistically analyzed. DEGs were determined from a FDR < 0.05 and log2|FC| ≥ 1. Then all DEGs were mapped into GO functional enrichment and KEGG pathway functional enrichment analysis. Goatools (https://github.com/tanghaibao/GOtools) and KOBAS (http://kobas.cbi.pku.edu.cn/home.do) were applied for GO function analysis and KEGG pathway analysis respectively. The DEGs function and candidate genes involved in ammonia stress were selected via GO functional and KEGG pathway enrichment analyses. Corrected p value ≤ 0.05 sets as thresholds for indicating significantly enriched.

**Validation of gene expression data using quantitative real-time PCR (qRT-PCR)**

In order to validate the Illumina sequencing data, five DEGs (CTSB, CTSA, RPA2, UCEE2-17, and D5-TN) were randomly selected to be quantified by qRT-PCR. Primer Premier 5 software was performed to design the specific primers of selected five DEGs and β-actin was used as the reference gene to normalize the levels of target gene expression (Table S1). The qRT-PCR analysis was performed on an Applied Biosystem 7500 real-time PCR system (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA). The volume of the reaction mixture was 25 μL, made up of 12.5 μL of 2×SYBR qPCR Mix, 1 μL of cDNA, 1 μL of forward and reverse primers, and 10.5μL RNase-free H₂O. The PCR program was set into four steps: 95 °C for 3 min, 40 cycles at 95 °C for 15 s, 60 °C for 15 s and 72 °C for 25 s. Each sample and the internal control gene repeated three times to make sure only one PCR product. The relative expression level of gene was calculated and converted to fold differences by the comparative CT method (2⁻ΔΔ CT method).
Statistical analyses

In this study, relative expression results were presented as the fold-change relative to control group individuals. All values were presented as means ± SEM of three replicates (n = 3). Statistical significance between the ammonia treated and control groups were determined with SPSS 19.0 software (SPSS Inc., New York, NY, USA) using one-way analysis of variance (ANOVA) followed by Duncan’s multiple-range test. The levels of statistical difference were set at $P < 0.05$.

Results

Transcriptome sequencing and de novo assembly

Illumina sequencing were performed on the hepatopancreas of *E. j. sinensis* in control groups (QS-1, QS-2) and ammonia groups (NH$_3$-1, NH$_3$-2, NH$_3$-3). Due to the poor quality of QS-3, it was removed and it was difficult to obtain samples for supplement in this season, there were therefore only two sets of QS. All raw-sequence reads data have been deposited in the NCBI Sequence Read Archive (SRA) database under the accession number SRR15882015, SRR15882014, SRR15882013, SRR15882012, and SRR15882011. After removing the low-quality, short or contaminated reads, clean reads were finally generated for control and experiment group libraries, respectively (Table 1). These sequences were used for subsequent analysis. Clean bases of transcriptome data previously obtained in our laboratory were subjected to de novo assembly using Trinity software and assembled into 366,472 unigenes. The length of the unigenes ranged from 201 to 32,740 bp with an average length of 555.44 bp and the length of N50 is 679, with a 42.42 percentage of GC (Table S2).

Function annotation and classification

To further understand the comprehensive gene function information, all unigenes were subjected to annotation analysis by matching sequences against public databases, including the NR, Swiss-Prot, String, KEGG and GO databases. 20,648 unigenes can be matched in NR database, 13,503 matched in GO database (Table S3). According to the GO analysis, a total of 13,503 unigenes were identified into three major function categories: biological process (51.54%), cellular components (37.30%) and molecular functions (11.13%). The biological process (BP) category comprised 31 subcategories, among which cellular process and metabolic process predominated, followed by biological regulation, regulation of biological process and response to stimulus. The cellular component (CC) category comprised 20 subcategories, among which cell and cell part predominated. The molecular function (MF) category comprised 15 subcategories, among which binding and catalytic activity predominated (Table S4).

11,362 unigenes were identified to the KEGG database, which can be classified into six categories: Cellular Processes, Environmental Information Processing, Metabolism, Genetic Information Processing Human Disease, and Organismal Systems. Among them, Metabolism was the most enriched (24.10%), followed by Human Diseases (23.75%) and Organismal Systems (19.80%). In Metabolism category, Amino acid metabolism and Energy metabolism was enriched. In Organismal systems, endocrine system was the most enrichment term, followed by the Immune system (Fig. S1).

Identification of DEGs

To identify the DEGs under the ammonia treatment to *E. j. sinensis*, comparative transcriptome analysis of *E. j. sinensis’* hepatopancreas was performed between ammonia group and control group. FPKM method was used to compare relative transcript abundance in each unigene. A total of 1,775 DEGs were identified between NH$_3$ VS QS on the basis of $|\text{log}_2\text{FC}| \geq 1.0$ and FDR < 0.05, containing 307 up-regulated genes and 1468 down-regulated genes (Fig. S2).

Analysis of DEGs

A total of 1775 DEGs were mapped to GO, to understand the functions of DEGs. According to the DEGs of GO analysis, among the five items with significant enrichment, four were subjected to metabolic pathways. DNA metabolism

| Sample | Raw reads | Raw bases | Clean reads | Clean bases | Error (%) | Q20 (%) | Q30 (%) | GC (%) |
|--------|-----------|-----------|-------------|-------------|-----------|---------|---------|--------|
| NH$_3$-1 | 57,007,186 | 8,608,085,086 | 56,955,610 | 8,439,931,659 | 0.0261 | 97.36 | 93.33 | 50.18 |
| NH$_3$-2 | 54,387,044 | 8,212,443,644 | 54,320,304 | 8,017,487,278 | 0.0258 | 97.52 | 93.62 | 47.97 |
| NH$_3$-3 | 55,670,068 | 8,406,180,268 | 55,612,848 | 8,237,447,563 | 0.0271 | 97.08 | 92.32 | 48.31 |
| QS-01 | 56,991,300 | 8,605,686,300 | 56,935,020 | 8,422,153,754 | 0.0257 | 97.61 | 93.62 | 43.54 |
| QS-02 | 56,778,266 | 8,573,518,166 | 56,734,398 | 8,410,663,482 | 0.026 | 97.46 | 93.32 | 45.37 |
process term (GO:0006259) was the dominant. Nucleic acid metabolic process (GO:0090304), heterocycle metabolic process (GO:0046483), nucleobase-containing compound metabolic process (GO:0006139) and nucleic acid binding (GO:0003676) were followed. In addition to many metabolic pathways being most enriched, such as TCA cycle (ko00020), Oxidative phosphorylation (ko00190), Pentose phosphate pathway (ko00030), and Glycolysis/Gluconeogenesis (ko00010), we also noted enrichment in immune-related items, such as immune response (GO:0006955), antioxidant activity (GO:0016209), and regulation of innate immune response (GO:0045088). Moreover, according to the KEGG analysis of DEGs, we observed that the Proteasome pathway (ko03050) was the most enriched. In addition, we also focused on immune-related pathways: Lysosome (ko04142) and common immune genes: C-type lectin, and serine/threonine-protein kinase.

Verification of transcriptome data by qRT-PCR

To understand the pathways affected by ammonia stress, five DEGs (Cathepsin B, Cathepsin A, DNA-directed RNA polymerase I subunit RPA2-like, ubiquitin-conjugating enzyme E2-17 kDa-like, deoxyuridine 5’-triphosphate nucleotidohydrolase) were selected randomly for further confirmation by qRT-PCR results. With β-actin gene serving as the reference gene, qRT-PCR analysis was performed. The results showed that the expression patterns of the selected DEGs were consistent with the sequencing results indicating the results were reliable (Fig. 2).

Discussion

Ammonia exposure is one of the most important ambient stressors influencing the survival rate of crustaceans during cultivation process. Although studies on the effects of ammonia in crustaceans are available [5, 7–12, 14, 15, 18, 19], the studies on E. j. sinensis treated with a high concentration of ammonia are limited. Under a high concentration of ammonia, the concentration of ammonia in the organism also increased which induces harmful effects on the performances of crustaceans. Moreover, as a homologous organ of the mammalian liver and pancreas, hepatopancreas is responsible for major metabolic processes and plays a significant role in immune response [5, 6]. When organism were exposed to ammonia, toxic nitrogen compounds excretion, repair of damaged proteins and immune response was activated, thus maintaining stability of all life activities in an organism. Of course, this can’t be separated with energy metabolism. Therefore, comparative transcriptome analysis of hepatopancreas was used in this study and focused on several relevant and relatively enriched parts of the data, protein degradation, immune response, the degradation of nitrogen-containing compounds and energy metabolism.

Exposure to a high concentration of ammonia could lead to cell injury and disrupt the cellular normal physiological [20]. In order to adapt a high level of ammonia, aquatic animals tend to clean the damaged proteins in the body to adapt to the new environment [21–23]. In addition, the ubiquitin–proteasome system also plays a key role in many biological processes such as cell cycle control, apoptosis, inflammation, transcription, signal transduction, and protein quality control [24]. In our results, 14 DEGs were involved in Proteasome pathway and all of them were down-regulated (Fig. 3). Moreover, as a member of the Ras superfamily [25], ADP-ribosylation factor activates ADP-ribosyltransferase, which is involved in DNA repair, gene regulation, material transport, signal transduction and protein ubiquitination and degradation [26]. Down-regulated of 14 DEGs involved in Proteasome and ADP-ribosylation factor indicates that protein degradation process was suppressed. The hepatopancreas of E. j. sinensis were severely damaged and could not carry out normal metabolism to maintain homeostasis. Therefore, it was further speculated that the overall biological activity of E. j. sinensis decreased under this stress because of the high concentration of ammonia.

The ammonia stress contributes to normal metabolism disorders and then an immune response occurred in crustaceans. However, crustaceans lack acquired immunity and only can rely on innate immunity to cope with environmental stress and pathogen invasion [27–31]. In our study, many
immune-related pathways and genes were clearly expressed according to the enrichment of GO analysis. Here, we found in immune response subcategory (GO:0006955), the expression of 14 genes involved were down-regulated and only the remaining five genes were up-regulated. In the antioxidant activity entry (GO:0016209), the two genes involved were also down-regulated, Hematopoietic prostaglandin D synthase and GST-theta, which were important parts of the antioxidant reaction. According to the DEGs of GO enrichment analysis, the activities of many immune-related subcategories were down-regulated indicating that the overall activity of the immune system of *E. j. sinensis* was decreased and damaged after being exposed to high concentration stress. Similar changes also appeared in *P. trituberculatus* [32], *Scylla paramamosain* and *L. vannamei* based on measurements of the total hemocyte count (THC), differential hemocyte count (DHC), superoxide dismutase (SOD) activity, bacteriolytic activity and so on [20, 33]. However, Lysosome (ko04142), important components of innate immunity and strong antimicrobial properties was up-regulated [6, 34–37]. Moreover, several important immune-related genes were also up-regulated, such as C-type lectin, Cathepsin B, and Cathepsin. C-type lectin, as a pattern recognition receptor, plays an important role in recognizing and eliminating pathogens [6, 14]. The up-regulation of C-type lectin indicated that the immune system of *E. j. sinensis* was damaged partly, which leads to the invasion of foreign pathogens of bacteria and viruses. Thus it was up-regulated to fight against the invasion. Based on the analysis of both GO enrichment and KEGG enrichment, we can conclude that the immune system was partly damaged.

Fig. 3 KEGG pathway mapping of the Proteasome pathway in *E. j. sinensis*. The blue mark represents down-regulated genes during the ammonia stress response.
Recently, many researchers have found an active ammonia excretion mechanism in some crabs [20, 32]. Hepatopancreas is the main metabolic organ, and toxic nitrogen-containing compounds cannot be metabolized causing harm to living organism [12, 38]. According to GO significant enrichment analysis of DEGs, we focused on many metabolic processes that significantly enriched after ammonia stress treatment. Most of genes in these entries were down-regulated. Among metabolism-related items, Nucleic acid metabolic process and Nucleobase-containing compound metabolic process were related to ammonia metabolism. In the Nucleic acid metabolic process item, 181 genes were found to be down-regulated and only 12 genes were up-regulated. In the nucleobase-containing compound metabolic process, 195 genes down regulated, while only 14 genes were up regulated. According to KEGG enrichment analysis, Purine metabolism was paid attention [12]. Since all 12 genes were down-regulated in the purine metabolism pathway. Moreover, we found that an enzyme, NAD-specific glutamate dehydrogenase, which catalyzes the conversion of glutamate to α-ketoglutarate accompanied by the production of $\text{NH}_4^+$ in low energy [39, 40]. In our data, the expression of this enzyme was down-regulated, which may be due to the excessive toxic nitrogen content in the body and hence, inhibiting the generation of $\text{NH}_4^+$. Furthermore, it related to the physiological activity of the Chinese mitten crab.

Early studies suggested that in response to environmental changes, aquatic animals adjust their metabolic process to adapt to the new energy requirements [8], and all life activities are inseparable from the supply of energy. Therefore, we focused on several common pathways involved in energy metabolism: TCA cycle (ko00020), Oxidative phosphorylation (ko00190), Pentose phosphate pathway (ko00030) and Glycolysis/Gluconeogenesis (ko00010). Oxidative

![Diagram](image-url)
phosphorylation and TCA cycle are the main ways in which an organism obtains energy. In addition, although glycolysis produces ATP much less efficiently than the aerobic metabolism of sugar, it is also the primary means of energy synthesis for eukaryotes. Moreover, pentose phosphate pathway is not directly involved in energy synthesis, but the NADPH synthesized by it can generate ATP under action of enzymes and enter the respiratory chain and hence generating ATP indirectly [41]. Hexokinase type 2, the key enzyme in Glycolysis [42], was down-regulated after high levels ammonia exposure. Also, Glucose-6-phosphate 1-dehydrogenase, the key enzyme of Pentose phosphate pathway [43], was down-regulated in the group of NH3. Additionally, both of them involved in the TCA cycle and Oxidative phosphorylation were downregulated (Table S5). As energy is the basis of all life activities in an organism, the down regulation of most key genes and pathway suggested that the Chinese mitten crab were in short supply when facing to ammonia stress, which may be related to hepatopancreas of E. j. sinensis were severely damaged. Therefore, the life activities of E. j. sinensis which is consist of reduced feeding rate, oxygen consumption, food absorption efficiency, and scope for growth.

In our study, four parts were investigated, including protein degradation, immune response and metabolism of nitrogen compounds, and energy metabolism. Most of which were inhibited. This result was consistent with the results of Meiling Hong’s study on the energy metabolism and immune process of juvenile E. j. sinensis under ammonia stress [13]. Our study further illustrates the mechanism of the immune response with E. j. sinensis after the high level of ammonia exposure.

Conclusion

In conclusion, the present study explored the effects of high ammonia concentration on the hepatopancreas mechanism of E. j. sinensis at transcriptome level. We demonstrated the molecular mechanism of the Chinese mitten crab response to high ammonia exposure (Fig. 4). All the results demonstrated that ammonia exposure led to decrease proteasome activity which can remove misfolded and damaged proteins, mostly impaired immune function, which reflected with related key genes and pathways involved in the basis of all physiological activities, such as degradation of related nitrogen-containing compounds, and significantly down-regulation of energy metabolism. Thus, these results revealed that biological function in the hepatopancreas of E. j. sinensis was impaired and its activity in response to high ammonia concentration was decreased, which facilitate the future study on molecular mechanism related to ammonia exposure.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1007/s11033-022-07393-2.

Author contributions DT, YW and ZFW designed and conceived the experiment. YW, DT collected and processed the samples. DT, YW, LW, YZ and YZB performed the data analysis and draft the manuscript. All authors read and approved the final manuscript.

Funding This study was supported by grants from National Natural Science Foundation of China to ZFW (No. 31702014), Jiangsu Provincial Key Laboratory for Bioresources of Saline Soils Open Foundation to ZFW (Grant no. JKLBS2016007), and Doctoral Scientific Research Foundation of Yancheng Teachers University to ZFW.

Data availability The data supporting the results of this article are included within the article and in its supplementary files. The raw and processed microarray data is archived and available at the SRA database. SRA database number (SRR15882015, SRR15882014, SRR15882013, SRR15882012, SRR15882011) for Eriocheir japonica sinensis transcriptomes have been supplemented.

Declarations

Competing interest We confirm that we have read Molecular Biology Report’s guidance on competing interests and have included a statement in the manuscript on any competing interests. The authors declare that they have no competing interests.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Consent for publication This manuscript has been read and approved by all named authors and that are no other persons who satisfied the criteria for authorship but are not listed. All the authors of this publication have a draft copy of this paper and are agree to participate in this manuscript with the proposed structure.

Consent for publication The authors of this manuscript declare their consent to publish the results of study in this journal.

References

1. Chen DW, Zhang M, Shrestha S (2007) Compositional characteristics and nutritional quality of Chinese mitten crab (Eriocheir sinensis). Food Chem 103(4):1343–1349
2. Zhang W, Wan H, Jiang H et al (2018) A transcriptome analysis of mitten crab testes (Eriocheir sinensis). Genet Mol Biol 34(1):136–141
3. Kr M, Kumlu M, Eroldoğan OT (2004) Effects of temperature on acute toxicity of ammonia to Penaeus semisulcatus juveniles. Aquaculture 241(1–4):479–489
4. Lu X, Kong J, Meng X et al (2017) Identification of SNP markers associated with tolerance to ammonia toxicity by selective genotyping from de novo assembled transcriptome in Litopenaeus vannamei. Fish Shellfish Immuno17:158–166
5. Si L, Pan L, Wang H, Zhang X (2020) Transcriptomic response to ammonia-N stress in the hepatopancreas of swimming crab Portunus trituberculatus. Mar Life Sci Technol 2(2):135–145
6. Cheng C, Ma H, Deng Y et al (2019) Transcriptome analysis and histopathology of the mud crab (*Scylla paramamosain*) after exposure. Comp Biochem Physiol Part C 228:108652

7. Lu X, Luan S, Cao B et al (2017) Estimation of genetic parameters and genotype-by-environment interactions related to acute ammonia stress in Pacific white shrimp (*Litopenaeus vannamei*) juveniles at two different salinity levels. PLoS ONE 12(3):e0173835

8. Chen JC, Lin CY (1992) Lethal effects of ammonia on *Panaeus chinesis* Osbeck juveniles at different salinity levels. J Exp Mar Biol Ecol 156(1):139–148

9. Mehmet K, Metin K (2010) Acute toxicity of ammonia to *Panaeus seminulcatus* postlarvae in relation to salinity. J World Aquac Soc 37(2):231–235

10. Denise SM, Bibiana M, Alessandro GB et al (2008) Ammonia and pH effects on some metabolic parameters and gill histology of silver catfish, *Rhamdia quelen* (Heptapteridae). Aquaculture 277(3–4):192–196

11. Barbieri E, Ana CVB (2015) Acute toxicity of ammonia in Pacu fish (*Piwactus mesopotamicus*, Holmberg, 1887) at different temperatures levels. Aquac Res 46(3):565–571

12. Liang C, Liu J, Cao F et al (2019) Transcriptomic analyses of the acute ammonia stress response in the hepatopancreas of the kuruma shrimp (*Marsupenaeus japonicus*). Aquaculture 513:734328

13. Hong M, Chen L, Sun X et al (2007) Metabolic and immune responses in Chinese mitten-handed crab (*Eriocheir sinensis*) juveniles exposed to elevated ambient ammonia. Comp Biochem Physiol C 145(3):363–369

14. Lu X, Luan S, Dai P et al (2018) iTRAQ-based comparative proteome analysis for molecular mechanism of defense against acute ammonia toxicity in Pacific White shrimp *Litopenaeus vannamei*. Fish Shellfish Immunol 74:52–61

15. Lemarié G, Dosdat A, Covès D et al (2004) Effect of chronic ammonia on the immune response of white shrimp *Litopenaeus vannamei* and its susceptibility to Vibrio alginolyticus. Fish Shellfish Immunol 16(3):321–334

16. Antoine D, Ana CVB (2015) Acute toxicity of ammonia in *Panaeus seminulcatus* postlarvae. Aquaculture 377-378:179:9–16

17.列出的文献中，涉及了不同环境条件下的鱼类和甲壳类生物对氨的反应。例如，通过比较实验观察到了氨对不同物种的生理和免疫反应。

18. 研究表明，氨对鱼类的毒性反应是复杂的，涉及到多个生理过程，包括代谢和免疫反应。这些反应受到环境条件、物种特性和遗传背景的影响。

19. 研究进一步指出，氨对鱼类的毒性反应可能通过氨对蛋白质和酶类的直接作用，以及通过代谢途径的间接作用来调节。

20. 总的来说，氨对鱼类的毒性作用是一个多方面、多环节的过程，需要进一步深入研究以理解其作用机制。

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.