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RESEARCH ARTICLE

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Adverse effects of chronic ammonia stress on juvenile oriental river prawn (*Macrobrachium nipponense*) and alteration of glucose and ammonia metabolism

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Abstract

Ammonia is one of the common stress factors in aquaculture. However, the effect of chronic ammonia exposure in juvenile oriental river prawn (*Macrobrachium nipponense*) is currently unexplored. This study explored the effects of chronic ammonia on juvenile healthy oriental river prawns. Fifty prawns (0.123 ± 0.003 g) were exposed to 0, 5, and 15 mg/L total ammonia nitrogen (TAN) in triplicates for 28 days. The effects of chronic ammonia challenge were evaluated on growth, antioxidant capacity, hepatopancreas and gill morphology, and glucose and ammonia metabolism. The results showed that, the chronic ammonia exposure reduced significantly survival rate and weight gain of prawns. The prawns exposed to 15 mg/L ammonia had induced oxidative stress. However, the prawn exposed to 15 mg/L ammonia had significantly lower aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and acid phosphatase activities in the serum. Furthermore, exposure of prawns to 15 mg/L ammonia increased the activities of hexokinase, pyruvate kinase, pyruvate and lactic acid content, and glutamine synthase activity. However, the prawns exposed to 15 mg/L ammonia, reduced succinic dehydrogenase, 6-phosphogluconic dehydrogenase, phosphoenolpyruvate carboxykinase, glutamate synthase, and glutamate dehydrogenase activities but increased ammonia content in serum. The exposure of ammonia deformed lumen, damaged basement membrane and decreased secretory cells in the hepatopancreas, disordered gill epithelial and pillar cells, and caused gill filament base vacuolation. Our study indicates that chronic ammonia stress impairs growth performance, tissue morphology, induces oxidative stress, and alters glucose and ammonia metabolism in juvenile oriental river prawns.

KEYWORDS
ammonia exposure, chronic toxicity, growth performance, histological damage, *Macrobrachium nipponense*

1 | INTRODUCTION

Ammonia is a common stressor in aquaculture, which is mainly derived from aquatic animals' protein catabolism and unconsumed organic wastes such as feed and excrement.[1,2] Ammonia exists in two forms in water, which are ionic ammonia (NH_4^+) and molecular (unionized) ammonia (NH_3). NH_3 easily passes through the branchial membrane because of its small molecular weight, lipid solubility, and

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neutral charge.[3,4] Ammonia discharge in aquatic animals relies on its concentration gradient between the body and environment.[5] Aquatic animals have higher ammonia level than their environment, which results in ammonia discharge into the environment. In contrast, a high level of ammonia in the environment reverses the flow.[6] Hence, high ammonia level in water causes stress, which leads to acute or chronic poisoning on aquatic animals. Accordingly, ammonia stress damaged tissues in various cultured organisms such as hyperplasia and interstitial edema hemorrhage of the gill epithelial cells, renal tubule dysfunction, and hemorrhagic hypertrophy in grass carp (*Ctenopharyngodon idella*) and Pengba (*Osteobrama belangeri*).[7,8] Moreover, ammonia stress decreased hemolymph oxygen-carrying capacity, destroyed antioxidant system balance, which resulted into increased oxidative stress in Chinese shrimp (*Fenneropenaeus chinensis*).[9] Furthermore, ammonia stress increased susceptibility to pathogens and inhibited growth in burbot (*Lota lota*).[10] In addition, ammonia stress also decreased weight gain in juvenile blunt snout bream (*Megalobrama amblycephala*) and spotted wolffish, (*Anarhichas minor* Olafsen) affecting individual size.[11,12] These studies indicate that, ammonia stress affects negatively individual harvested size and increase disease susceptibility of various cultured aquatic organisms.

Ammonia stress also causes changes in energy metabolism in animals. For example, pigs exposed to chronic ammonia stress activated glycolysis and lipolysis in the lungs.[13] Aquatic organisms exposed to stress also adaptively change metabolism of energy yielding nutrients such as glucose and lipids.[14,15] For instance, ammonia exposure reduced lipogenic enzymes fatty acid synthase (FAS) and 6-phosphogluconate dehydrogenase (6PGD) activities in juvenile yellow catfish (*Pelteobagrus fulvidraco*) and induced lipid decomposition in pacific white shrimp (*Litopenaeus vannamei*).[16,17] Previous studies reported that glucose is preferentially used under stress in some fish species.[18] Accordingly, lower plasma glucose levels were reported in Atlantic halibut (*Hippoglossus hippoglossus* L.) exposed to salinity stress and juvenile blunt snout bream exposed to chronic ammonia.[11,19] Moreover, starvation stress decreased plasma glucose levels and increased gluconeogenesis activity in lake sturgeon (*Acipenser fulvescens*).[14] Similarly, ammonia exposure decreased glycogen content in the liver and muscle, and increased plasma glucose level in largemouth bass (*Micropterus salmoides*).[18] Interestingly, anaerobic glycolysis was increased after ammonia stress at early stages, while aerobic glucose metabolism was increased during the late stages in pacific white shrimp.[20] These studies suggest that aquatic organisms use glucose to produce more energy to counteract stress.

The oriental river prawn (*Macrobrachium nipponense*) is an important and popular aquaculture species in China.[21] Its annual production has exceeded 228 000 tons in recent years.[22] However, the sustainability and development of oriental river prawn culture is currently limited by disease outbreaks and harvesting of small individual sizes.[23] These challenges have evoked studies on water quality parameters of oriental river prawn culture systems. The water quality for oriental river prawn farming deteriorates because of intensive cultivation and human activities due to industry and agriculture development.[24] Ammonia is a common stressor in intensive culture systems as the main nitrogenous waste excreted by crustaceans.[4,25] To date, the acute toxicity of ammonia on oriental river prawn on immune response and oxidative stress have been reported.[4,26,27] The studies identified the immunity genes for combating ammonia stress and biomarkers for ammonia exposure in oriental river prawn.[4,26,28] However, studies on the chronic toxic effects of ammonia on oriental river prawn are lacking.

Based on previous studies and the toxic effect of ammonia, we hypothesized that chronic ammonia exposure might be linked to the disease outbreaks and small individual sizes harvested in oriental river prawn. These challenges limit the expansion and sustainable development of oriental river prawn aquaculture. Therefore, the present study evaluated the effect of chronic ammonia stress on the growth performance, immunity, antioxidant capacity, glucose metabolism, ammonia metabolism, and tissue morphology of oriental river prawn. Our study further provides a new perspective for alleviating ammonia stress based on the results of glucose metabolism.

2 | MATERIALS AND METHODS

2.1 | Ammonia challenge

Juvenile oriental river prawns were purchased from a local farm (Huzhou, China). The use of prawns in this research was approved by the Animal Ethics Committee of Huzhou University (Huzhou, China). All experiments were conducted according to the guidance suggestions for the care and use of laboratory animals formulated by the Ministry of Science and Technology of China. Before the formal experiment, juvenile oriental river prawns were acclimated for 1 week to adapt to the experimental conditions. After acclimatization, 450 visually healthy, uniform-sized prawns (0.123 ± 0.003 g) were selected and randomly stocked into nine 300 L (100 × 80 × 60 cm) tanks. Each tank contained 50 prawns and each treatment was replicated three times. The oriental river prawns were randomly subjected to the set concentrations of 0 (control), 5, and 15 mg/L of total ammonia nitrogen (TAN) for 28 days. The TAN levels were set based on the 96-h LC50 (36.6 mg/L).[29] During the entire exposure period, the prawns were fed twice daily on a prawn feed containing 40.2% protein and 8.7% lipid at around 07:30 and 17:00 h. One-third of the water in each tank was exchanged daily, and the dead prawns and residual feed were removed to ensure optimum water quality. The water quality parameters were monitored daily by using a multi-probe water quality apparatus (YSI-550A, Xylem Inc., USA). During the entire study period, the water temperature was 27.5 ± 1.5°C, the dissolved oxygen was 6.8 ± 0.5 mg/L and the pH was 7.5 ± 0.5. Prawns were exposed to a natural light cycle during the entire stress period. The TAN concentration in the tanks water was measured every eight hours with Nessler's reagent using a spectrophotometry method (UV-300BPS, Mapada Instruments, Shanghai, China). The TAN was adjusted to the required concentrations by using ammonium chloride solution (NH_4Cl, Sinopharm Group, Shanghai).

2.2 | Sample collection and growth evaluation

After a 4-week exposure to ammonia, the survived prawns were fasted for 24 h before sampling. The prawns from each tank were counted for estimation of survival rate and bulk weighed for calculation of weight gain (WG) using the formulae:

$$\text{Survival rate}(\%) = 100 \times (\text{final number of prawns})/(\text{initial number of prawns}).$$

$$\text{WG}(\%) = 100 \times (\text{final weight} - \text{initial weight})/(\text{initial weight}).$$

Fifteen oriental river prawns from each tank were randomly sampled for hemolymph collection using nonheparinized syringes. The hemolymph was stored at 4°C overnight, then the coagulated hemolymph was centrifuged at 4°C, 3500 rpm/min, for 10 min (Eppendorf Centrifuge 5810 R). Serum was collected and stored at −80°C for later analysis. Concurrently, hepatopancreas were carefully dissected scratched with tweezers, stored at −80°C for enzymes activities analyses.

The cephalothorax parts of three oriental river prawns from each treatment were randomly collected and fixed in 4% paraformaldehyde separately at room temperature for later morphology analysis.

2.3 | Serum and hepatopancreas biochemical parameters analyses

Ammonia level and acid phosphatase (ACP), alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) activities in the serum were determined by using specific commercial kits (Nanjing Jiancheng, Nanjing, China) following the manufacturer's instructions.

The frozen hepatopancreas was diluted nine times using precooled 0.86% (w/v) sodium chloride solution and homogenized at 4 m/s for 15 s (MP FastPrep®-24). The homogenate was centrifuged at 3500 rpm/min at 4°C for 10 min. The resulting supernatant was collected for biochemical analysis. The malondialdehyde (MDA) content, superoxide dismutase (SOD), carbohydrate metabolism enzymes such as hexokinase (HK), pyruvate kinase (PK), succinic dehydrogenase (SDH), phosphoenolpyruvate carboxykinase (PEPCK) activities, and pyruvate and lactic acid (LD) contents were measured by using specific commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) following manufacturer's guidelines.

Moreover, ammonia metabolism enzymes such as glutamate dehydrogenase (GDH), glutamine synthase (GS) activities, and glutamate (GLU) content in the hepatopancreas were also measured by using specific commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) following manufacturer's instructions. The 6-phosphogluconic dehydrogenase (G6PDH) activity as a glucose metabolism enzyme and glutamate synthase (GOGAT) as an ammonia metabolism enzyme were analyzed using specific kits from Comin Biotechnology (Jiangsu, China) according to the instructions of the manufacturer.

2.4 | Histological assays

Gills and hepatopancreas were carefully collected from the fixed prawns. The gills and hepatopancreas were dehydrated using different concentrations of alcohol solution. Dehydrated tissues were made transparent using xylene solution, then embedded in paraffin and cut into 5 μm sections in continuous blocks. The sections were stained using hematoxylin and eosin (H & E). The stained slices were scanned with a digital scanner (KF-PRO-120, Konfoong Biotech International Co., Ltd.) and observed using the K-Viewer software. Changes in the hepatopancreas lumen, gill epithelium and pillar cell were observed, noted and photographed.

2.5 | Statistical analyses

One-way analysis of variance (ANOVA) was used to test for significant differences on measured parameters among the three treatments. When ANOVA showed significant differences, Tukey's post hoc was used for multiple comparisons among treatments. All results are reported as mean ± standard deviation (SD). Results with $p < .05$ were considered statistically significant. All analyses were conducted by using the SPSS 25.0 statistical software (IBM Corp., Armonk, NY).

3 | RESULTS

3.1 | Growth performance and survival rate

The survival rate and WG of prawns decreased with increasing ammonia concentrations in the water (Figure 1). The prawns exposed to 15 mg/L ammonia decreased significantly the survival rate and WG than those exposed to 0 mg/L ($p < .05$). Moreover, the prawns exposed to 5 mg/L ammonia also decreased significantly the survival rate and WG than those exposed to 0 mg/L ($p < .05$). However, the prawns exposed to 5 and 15 mg/L ammonia did not have significant difference in WG ($p > .05$).

3.2 | Tissue morphology

The exposure of prawns to chronic ammonia stress affected the morphology of the hepatopancreas (Figure 2). The prawns in the control treatment had normal hepatopancreas lumen with a star shape in the middle and intact basal membrane with clear borders (Figure 2A). However, the prawns exposed to 5 mg/L (Figure 2B) and 15 mg/L (Figure 2C) ammonia showed irregular tubular structure, indistinct boundaries of hepatic tubules, damaged basal membrane, and reduced secretory cells in the hepatopancreas. The prawns exposed to 15 mg/L ammonia had severe hepatic tubules vacuolation, exfoliated necrotic cell fragments in the lumen, and thickening of the intercellular space (Figure 2C).

The prawns exposed to 0 mg/L ammonia had normal gill epithelium and pillar cell arrangement without cell vacuolation and congestion (Figure 3A). However, prawns exposed to 5 mg/L (Figure 3B) and

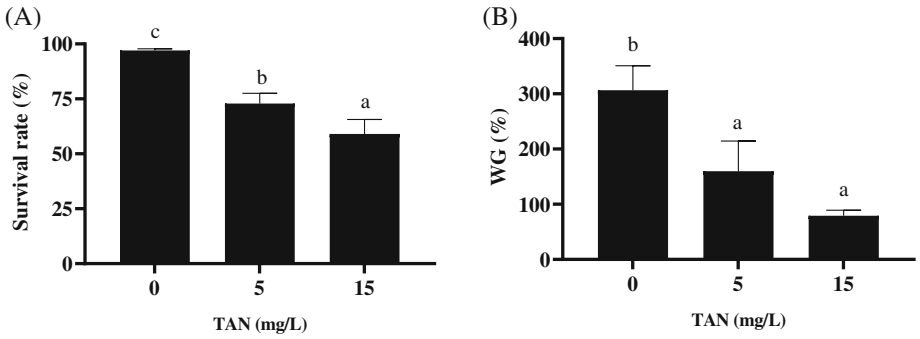


FIGURE 1 Survival rate (A) and weight gain (WG) (B) of oriental river prawn (*Macrobrachium nipponense*) exposed to chronic ammonia. Values are the mean values of three replicates within a treatment ± SD. Bars with different superscript letters are statistically significant ($p < .05$).

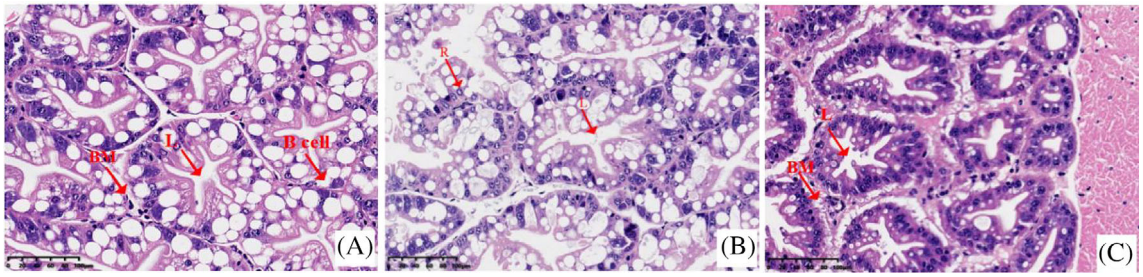


FIGURE 2 Photomicrographs of hepatopancreas sections of oriental river prawn (*Macrobrachium nipponense*) exposure to chronic ammonia. A: control treatment; B: 5 mg/L treatment; C: 15 mg/L treatment. Magnification 20×; scale bar 100 μm; B cell, secretory cells; BM, basement membrane; L, lumen; R, resorptive cells.

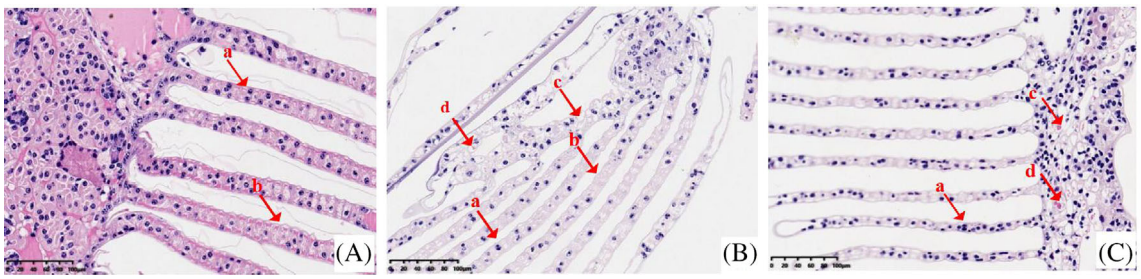


FIGURE 3 Photomicrographs of gills sections of oriental river prawn (*Macrobrachium nipponense*) exposure to chronic ammonia. A: control treatment; B: 5 mg/L treatment; C: 15 mg/L treatment. Magnification 20×; scale bar 100 μm. a, pillar cells arrangement; b, epithelial cells arranged disorder; c, cells vacuolation; d, congestion.

15 mg/L (Figure 3C) ammonia caused gill epithelium and pillar cell arrangement disorders, cell vacuolation and gill filament base congestion.

3.3 | Serum biochemical parameters

Chronic ammonia exposure to prawns affected significantly the ALT, AST, ALP, and ACP activities in the serum (Figure 4). The prawns exposed to 15 mg/L ammonia had significantly lower AST (Figure 4A) and ALT (Figure 4B) activities than those exposed to 0 and 5 mg/L ($p < .05$). However, exposure of prawns to 0 and 5 mg/L ammonia did not affect significantly the ALT and AST activities ($p > .05$). The prawns exposed to 15 mg/L ammonia reduced significantly the ALP (Figure 4C) and ACP (Figure 4D) activities than those exposed to 0 and 5 mg/L ($p < .05$). However, the prawns exposed to 5 mg/L ammonia had significantly higher ALP and ACP activities than those exposed to 0 mg/L ($p < .05$).

3.4 | Antioxidant capacity in the hepatopancreas

The prawns exposed to 15 mg/L ammonia had significantly higher MDA content (Figure 5A) and SOD activity (Figure 5B) in the hepatopancreas compared to those exposed to 0 and 5 mg/L ($p < .05$). However, the prawns exposed to 0 and 5 mg/L ammonia had no significant difference in MDA content and SOD activity ($p > .05$).

3.5 | Glucose metabolism in the hepatopancreas

The prawns exposed to 5 and 15 mg/L ammonia had significantly higher HK (Figure 6A) and PK (Figure 6B) activities in the hepatopancreas than those in the control treatment ($p < .05$). However, the prawns exposed to 5 and 15 mg/L ammonia had no significant difference in HK and PK activities ($p > .05$). Similar to HK and PK, the prawns exposed to 15 mg/L ammonia had higher pyruvate (Figure 6C)

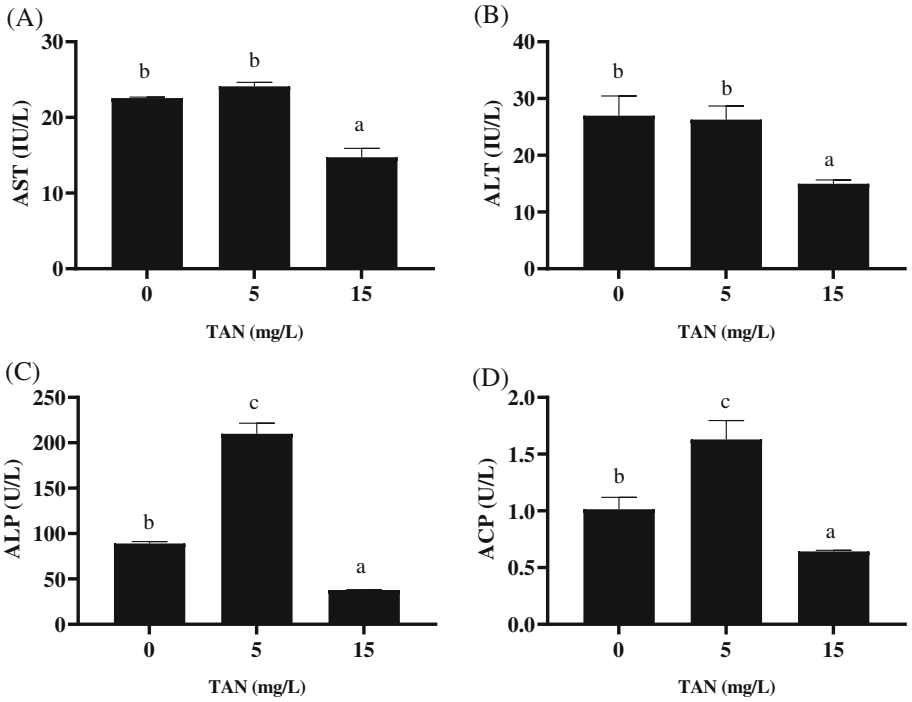


FIGURE 4 Effect of chronic ammonia stress on activities of AST (A), ALT (B), ALP (C), and ACP (D) in the serum of oriental river prawn (*Macrobrachium nipponense*). Values are the mean values of three replicates within a treatment ± SD. Bars with different superscript letters are statistically significant ($p < .05$).

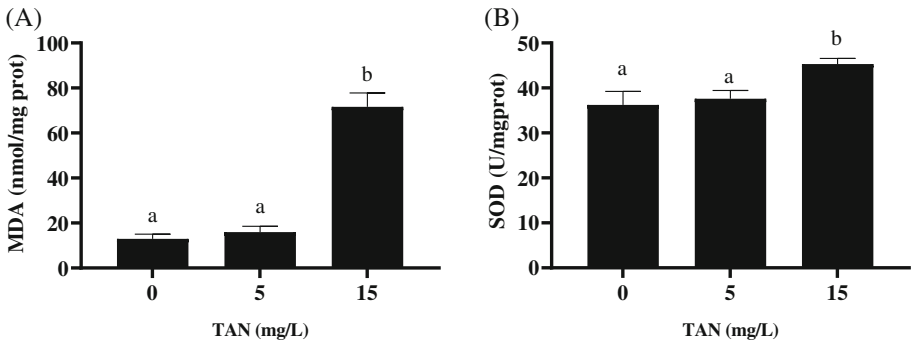


FIGURE 5 Effect of chronic ammonia stress on MDA (A) content and SOD (B) activity in the hepatopancreas of oriental river prawn (*Macrobrachium nipponense*). Values are the mean values of three replicates within a treatment ± SD. Bars with different superscript letters are statistically significant ($p < .05$).

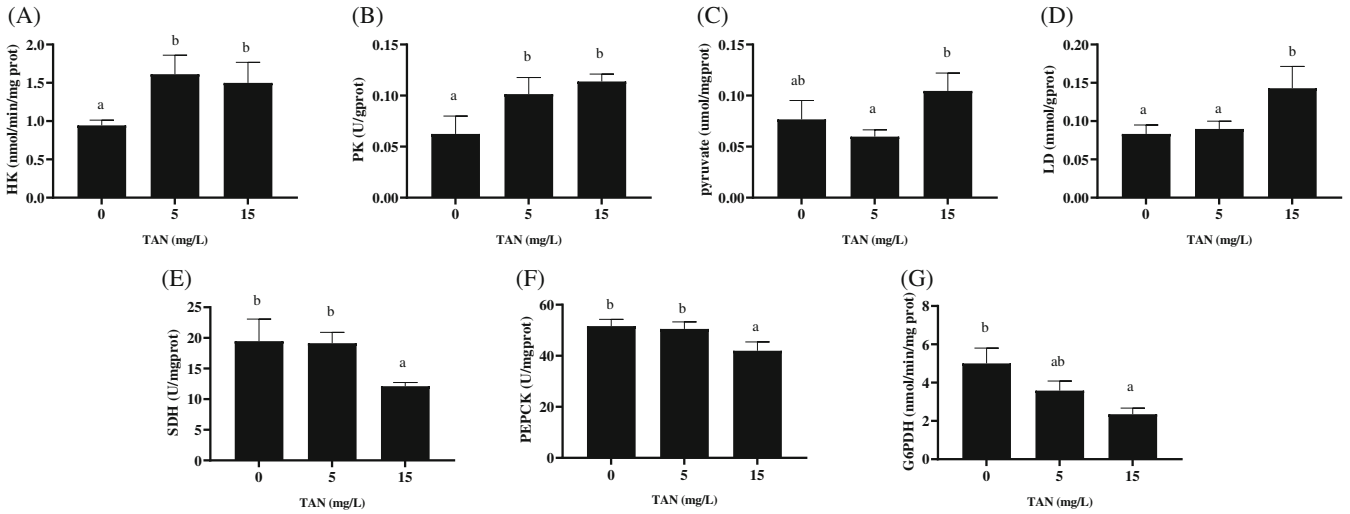


FIGURE 6 Effect of chronic ammonia stress on HK (A) and PK (B) activities, pyruvate (C) and LD (D) contents, and SDH (E), PEPCK (F), and G6PDH (G) activities in the hepatopancreas of oriental river prawn (*Macrobrachium nipponense*). Values are the mean values of three replicates within a treatment ± SD. Bars with different superscript letters are statistically significant ($p < .05$).

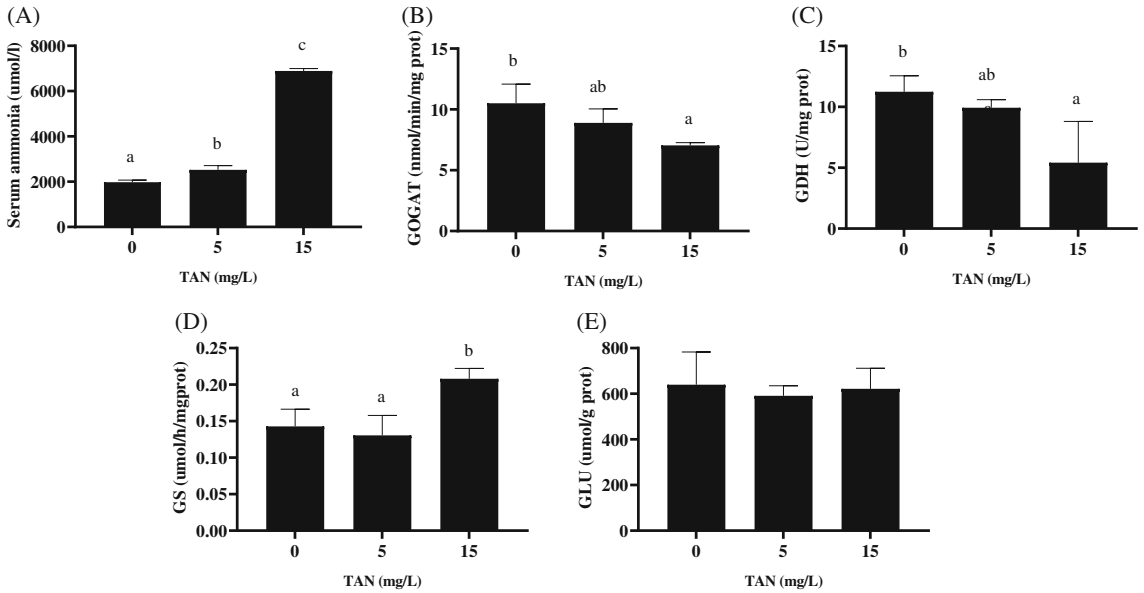


FIGURE 7 Effect of chronic ammonia stress on Serum ammonia (A) content, GOGAT (B), GDH (C) and GS (D) activities, and GLU (E) content of oriental river prawn (*Macrobrachium nipponense*). Values are the mean values of three replicates within a treatment ± SD. Bars with different superscript letters are statistically significant ($p < .05$).

and LD (Figure 6D) contents in the hepatopancreas than those exposed to 0 and 5 mg/L ($p < .05$). However, there was no significant difference between the prawns exposed to 0 and 5 mg/L ammonia had in pyruvate and LD contents ($p > .05$). Conversely, the prawns exposed to the 15 mg/L ammonia reduced significantly SDH (Figure 6E), PEPCK (Figure 6F), and G6PDH (Figure 6G) activities than those exposed to 0 and 5 mg/L ($p < .05$). However, the prawns exposed to 0 and 5 mg/L ammonia had no significant difference in SDH, PEPCK, and G6PDH activities ($p > .05$).

3.6 | Serum ammonia content and ammonia metabolism in the hepatopancreas

Expectedly, the serum ammonia of prawns increased significantly as the ammonia concentration of water rose (Figure 7A; $p < .05$). However, the prawns exposed to 15 mg/L ammonia had significantly lower GOGAT (Figure 7B) and GDH (Figure 7C) activities compared to those in the control treatment ($p < .05$). However, the prawns exposed to 0 and 5 mg/L ammonia had no significant difference in GOGAT and GDH activities ($p > .05$). On the contrary, the prawns exposed to 15 mg/L ammonia had significantly higher GS activity in the hepatopancreas than those exposed to 0 and 5 mg/L (Figure 7D; $p < .05$). However, the prawns exposed to 0 and 5 mg/L ammonia had no significant difference in GS activity ($p > .05$). Exposure of prawns to chronic ammonia at the concentrations used in this study did not affect significantly GLU content (Figure 7E; $p > .05$).

4 | DISCUSSION

The present study showed that chronic ammonia exposure inhibited growth performance and reduced survival rate of juvenile oriental river prawns, similar to results in juvenile Wuchang bream (*M. amblycephala*) and juvenile yellow catfish.[30,31] However, ammonia did not affect growth performance in channel catfish (*Ictalurus punctatus*) and Senegalese sole (*Solea senegalensis*).[32,33] The discrepancy indicated that the tolerance to ammonia in aquatic animals is species specific.[34] More likely, ammonia stress causes the small sized oriental river prawns harvested from aquaculture.

Gills are important respiratory organs in aquatic organisms and play a crucial role in gaseous exchange and nitrogen compound excretion. Gills are in contact with water directly and are easily affected by stress factors in the water.[35] Thus, gill morphology is a sensitive response indicator to environmental toxicity in aquatic organisms. Our results showed that the gills of oriental river prawns exposed to ammonia have a high degree of degeneration, probably caused by cell arrangement disorders and hemocytic infiltration. These results corresponded to the reduced growth of oriental river prawns exposed to ammonia, indicating that ammonia caused gills injury, weakened their ability to exchange gases with the ambient environment, led to tissue hypoxia, and retarded growth performance. Similar results were reported in blue swimmer crab (*Portunus pelagicus*) and estuarine crab (*Chasmagnathus granulata*).[36,37] Hepatopancreas is a digestive organ of crustaceans, which also plays key roles in detoxification and decomposition of toxic compounds.[32] Therefore, hepatopancreas morphology is often analyzed to reflect toxicity in aquatic environment.[38,39] In the present study, ammonia exposure deformed lumen, damaged basement membrane, and reduced secretory cells in the hepatopancreas of oriental river prawns exposed to 5 and 15 mg/L ammonia. The observed thickening of the hepatopancreatic intercellular space in prawns exposed to 15 mg/L ammonia indicates defensive mechanism of the prawn to counteract ammonia toxicity.[38] Hepatopancreas injury might have impaired its digestive and metabolism functions, causing the reduced survival rate and growth performance reported above in this study.

AST and ALT are important enzymes for nutrients metabolism in animals.[15] Liver injury causes AST and ALT release into the blood, increasing their activities.[40] Thus, AST and ALT are sensitive parameters, which reflect the liver status after exposure to stressors.[41] Accordingly, ammonia stress increased AST and ALT activities in common carp (*Cyprinus carpio*) and yellow catfish.[42,43] In contrast, the present study we found that 15 mg/L ammonia chronic stress decreased AST and ALT activities in oriental river prawn serum, consistent to results on hybrid grouper (*Epinephelus lanceolatus* ♂ × *E. fuscoguttatus* ♀).[44] The decreased AST and ALT activities in the present study may be due to hepatopancreas damage, which resulted in reduced AST and ALT synthesis. This also confirms that hepatopancreas damage was more severe for the prawns exposed to ammonia at 15 mg/L.

ALP and ACP regulate protein phosphorylation, and also play functions in detoxification response in organisms.[45] Stressed organisms increase ACP and ALP activities to a certain level as a response to improve immunity.[40,46] Our results showed that, the oriental river prawns exposed to 5 mg/L ammonia increased the ALP and ACP activities, suggesting that ammonia stimulated increase in immunity in order to adapt to the resulting stressful conditions, as previously reported in common carp.[43,47] However, prawns exposed to 15 mg/L ammonia reduced ACP and ALP activities, indicating inability of prawns to tolerance high ammonia stress levels.[48] Combining these results with those on tissue morphology under higher levels of ammonia, we speculate that the reason for the decrease in enzyme activity may be impaired hepatopancreas structure of the oriental river prawns. More likely, ammonia stress reduces the immunity of cultured prawns and causes disease outbreaks.

One of the toxic effects of ammonia on aquatic animals is the generation of oxidative stress, which causes excessive reactive oxygen species (ROS) production relative to antioxidant capacity.[49,50] MDA is the product of lipid peroxidation, which is often used to reflect the degree of oxidative damage.[51] Our results showed increased MDA content in oriental river prawns exposed to ammonia, consistent with results in pacific white shrimp and Chinese mitten crab (*Eriocheir sinensis*).[52–54] These results indicated ammonia exposure induced peroxidation in juvenile oriental river prawns, which might also be responsible for hepatopancreas damage, resulting in hindered digestion and utilization of feed by the prawn, consequently impairing growth performance. This might be one of the reasons for the small size of oriental river prawns harvested from aquaculture.

To understand the response of antioxidant capacity of oriental river prawns under oxidative stress, we measured SOD activity. SOD is the first barrier against oxidative stress, which converts superoxide radicals into hydrogen peroxide (H_2O_2). It is a common antioxidant capacity indicator in most organisms.[17,43,55] We found increased SOD activity in the hepatopancreas of oriental river prawns under ammonia stress are similar to results in ridgetail white prawn (*Exopalaemon carinicauda*) and hybrid grouper.[44,51] These results indicated increasing SOD activity may be an adaptive response of oriental river prawns to oxidative stress to attack ROS, a condition termed "hormesis."[56] The above results indicated that ammonia induced oxidative stress in oriental river prawns, thus increased SOD activity to remove excessive ROS. However, excessive ROS was not removed completely causing oxidative damage, which affected growth performance and metabolism of oriental river prawns.

Aquatic animals adjust energy metabolism in order to respond to environmental stress.[20] Glucose is one of the main energy substrates, preferentially used under stressful conditions in aquatic animals.[18,57] Our study indicated that ammonia exposure affected glucose metabolic enzymes in the hepatopancreas of the oriental river prawn. HK catalyzes the first glucose utilization step, and PK catalyzes the conversion of phosphoenolpyruvic acid to pyruvate in glycolysis.[57] In the present study, oriental river prawns under ammonia stress increased HK and PK activities, similar to results in pacific white shrimp.[20] These results suggested that ammonia induced glycolysis activity in oriental river prawns. Pyruvate is the product of glycolysis activity required for energy production. LD is the product of glucose anoxic metabolism, whose content increases due to enhanced anaerobic respiration as an important adaptation for energy supply.[58] SDH is an important enzyme in the tricarboxylic acid cycle (TCA), which converts α-ketoglutaric acid (α-KG) into succinic acid.[59] In our study, changes in pyruvate and LD concentrations and SDH activity suggest enhanced anaerobic respiration to supply additional energy under ammonia stress to maintain normal metabolism.[60,61] However, it is worth noting that the increased pyruvate content indicates that a required state of energy in the prawn.[62] PEPCK is a key enzyme in gluconeogenesis pathway.[63] Our results showed inhibited PEPCK activity, indicating decreased ability to mobilize noncarbohydrate substances to synthesize glucose. G6PDH plays an important role in the pentose phosphate pathway and is involved in the production of reduced nicotinamide adenine dinucleotide phosphate (NADPH), which is involved in the regulation of intracellular redox state.[64] Our results showed inhibited G6PDH activity in the oriental river prawns exposed to 15 mg/L ammonia stress. In general, chronic ammonia exposure in the oriental river prawns inhibited the conversion of noncarbohydrate to glucose. However, whether the direct energy supplied by noncarbohydrates (e.g., lipid) is inhibited remains to be further studied. Responsively, the oriental river prawns induced glycolysis and enhanced anaerobic respiration to supply energy under stressful conditions. In summary, this study found that the oriental river prawns met the increasing energy demand under ammonia stress by enhancing glycolysis. However, when the ammonia concentration reached 15 mg/L, the hepatopancreas structure of the prawn was seriously damaged, resulting in decreased glucose utilization.

Ammonia stress also affects nitrogenous waste metabolic activities, causing physiological adaptations.[24,65] GDH is involved in α-KG, NADPH, and NH_4^+ forming glutamic acid, while GOGAT catalyzes the conversion of glutamine, α-KG, NADPH, and H^+ to glutamic acid and plays a key role in the nitrogen assimilation regulation.[66] In our study, ammonia exposure increased ammonia content in serum but decreased the GOGAT and GDH activities in the hepatopancreas of oriental river prawns, nevertheless did not affect GLU content. The decreased GOGAT and GDH activities may be related to the decreased reaction NADPH substrate, indicating enhanced ability to synthesize glutamate from other pathways as a response to relieve

ammonia toxicity due to increased ammonia content.[67] Furthermore, we discovered that changes in GS activity in oriental river prawns are comparable to those found in sleeper (*Bostrichthys sinensis*) and gilthead seabream (*Sparus aurata*).[68,69] These results indicate that glutamine synthesis was induced in oriental river prawns exposed to ammonia to accelerate endogenous ammonia metabolism and synthesize nontoxic intermediates to relieve ammonia toxicity.

5 | CONCLUSION

This study established that chronic ammonia stress impairs growth performance and tissue morphology and induces oxidative stress in juvenile oriental river prawns. Ammonia exposure enhanced the ability of juvenile oriental river prawns in converting nitrogenous waste into nontoxic intermediates to mitigate ammonia toxicity. Exposure of juvenile oriental river prawns to chronic ammonia stress inhibited gluconeogenesis, pentose phosphate pathway, and TCA activities but enhanced anaerobic metabolism to supply more energy to counteract stress. Our results shed light on the potential of adaptive changes in glucose metabolism. Further studies are required on the regulatory role of glucose on ammonia toxicity.

ACKNOWLEDGMENTS

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CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that influence the work reported in this article.

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