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Evaluation of short-term toxicity of ammonia and nitrite on the survival of whiteleg shrimp, *Litopenaeus vannamei* juveniles

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Abstract

The effects of short-term toxicity of total ammonia nitrogen (TAN) and nitrite were estimated in juveniles of *Litopenaeus vannamei* under laboratory conditions. In the first experiment, *L. vannamei* juveniles were exposed to different concentrations of ammonia (0, 5, 10, 15, 20, 30, and 40 mg of TAN L^{-1}) or nitrite (0, 5, 10, 20, 30, 40, and 50 mg of NO_2^--N L^{-1}), using the static renewal method at a salinity of 20 ppt and pH 8.2. The survival rates of juveniles significantly decreased when exposed to increased concentrations of ammonia or nitrite during the 96 h bioassays. The 24, 48, 72, and 96 h LC_{50} values of TAN in juveniles were 45.5, 30.1, 13.8, and 6.3 mg L^{-1}, respectively, while the LC_{50} values of NO_2^--N at 24, 48, 72, and 96 h were 37.6, 16.7, 8.8, and 4.8 mg L^{-1}, respectively. Experiment 2 evaluated the tolerance of *L. vannamei* juveniles at various salinities (5, 10, 15, and 20 ppt) under a high concentration of ammonia or nitrite (5 mg L^{-1}). Results showed that the survival rates of *L. vannamei* at 5 ppt and 10 ppt were significantly lower than those at 20 ppt after 72 h and 96 h of exposure.

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Introduction

The whiteleg shrimp, *Litopenaeus vannamei* (Boone, 1931), has become an important commercial species for the aquaculture industry worldwide, especially in Vietnam. With the rapid development of the culture of marine shrimp in low salinity waters since 2000, Vietnam's total whiteleg shrimp production was 632.3 thousand tons in 2020 (Directorate of Fisheries, 2021). Due to increasing export production, intensive and super-intensive systems have been commonly applied in Vietnam. However, these culture systems, where shrimp will be cultured at a relatively high rearing density, are usually related to the degradation of water quality in the culturing environment (Cobo et al., 2012). The accumulation of nitrogen compounds, especially ammonia and nitrite, is an important limiting factor in intensive shrimp farming ponds. In the natural environment, total ammonia nitrogen (TAN) exists in two forms: unionized ammonia (NH_3) and ionized ammonia (NH_4^+) (Wajsbrot et al., 1993), with different proportions, depending on pH, temperature, and to a lesser extent salinity (Randall & Tsui, 2002). In addition, the unionized form of ammonia is extremely toxic to shrimp because of its ability to gain entry through cell membranes (Emerson et al., 2011; Waikhom et al., 2018). Of the nitrogenous compounds, ammonia and its intermediate product of nitrification, nitrite, are more commonly toxic to shrimp compared with nitrate (Cobo et al., 2012). However, some studies have revealed that the tolerances of shrimp to ammonia and nitrite are reduced in low salinity environments (Schuler et al., 2010; Romano & Zeng, 2013; Ramírez-Rochín et al., 2017; Valencia-Castañeda et al., 2018; Nudalo et al., 2020). During the rearing period, the high levels of ammonia and nitrite that are caused by the increase in the concentration of nitrogen compounds from nitrogenous products like feces production, excessive feeding and mineralization of organic detritus may deteriorate water quality, resulting in high mortality and low growth rate in penaeid shrimp (Valencia-Castañeda et al., 2020). Moreover, ammonia and nitrite could injure the gill tissue of shrimp and affect their oxygen transport, causing retarded mortality and growth (Racotta & Hernández-Herrera, 2000; Barbieri et al., 2016; Campos et al., 2015; Lin & Chen, 2003). In the intensive culture systems, the concentrations of TAN and NO_2^--N can reach 46.1 mg L^{-1} (0.87 mg L^{-1} NH_3-N) and 50 mg L^{-1}, respectively (Ciji et al., 2019; Kathyayani et al., 2019). However, Cobo et al. (2012) and Wajsbrot et al. (1993) reported that the sensitivity of the organism to a toxic substance may also vary according to its developmental stages and health status.

Several studies have been conducted to determine the short-term (acute) toxicity of ammonia and nitrite in different life stages of penaeid shrimp, *P. monodon* postlarvae and juveniles (Chen & Chin, 1988; Chen & Chen, 1992), *P. semisulcatus* juveniles (Wajsbrot et al., 1990), *Metapenaeus ensis* juveniles (Nan & Chen, 1991), *P. chinensis* (Lin et al., 1993), *P. paulensis* (Ostrensky & Wasielesky, 1995), *P. setiferus* postlarvae (Alcaraz et al., 1997), *L. vannamei* larvae (Magallón Barajas et al., 2006; Pan et al., 2007; Cobo et al., 2012); *Fenneropenaeus chinensis* (Xue et al., 2017). Although data on the acute effects of ammonia and nitrite on whiteleg shrimp are widely available, researchers have reported median lethal concentrations for ammonia and nitrite in whiteleg shrimp depending on size, age, environmental factors, and time of exposure (Kir & Kumlu, 2006; Cobo et al., 2012; Waikhom et al., 2018; Valencia-Castañeda et al., 2018). Therefore, the objective of this study was to evaluate the effects of short-term ammonia and nitrite exposure on the survival of *L. vannamei* juveniles in low salinity waters under laboratory conditions.

Materials and Methods

Experimental animals

Juvenile shrimp of *L. vannamei* were provided by a private hatchery located in Can Tho, Vietnam. Shrimp were acclimated in 500 l tanks with a salinity level of (20 ppt) and constant aeration for 15 days before the start of the experiments. Water quality parameters were maintained at 29.5±0.4°C temperature, 8.26±0.07 of pH, and 5.13±0.47 mg L^{-1} of dissolved oxygen (DO). Fifty percent of the water in each tank was exchanged daily. During the acclimatization process, shrimp were fed three times a day with commercial feed (40% crude protein, 6% crude lipid, and 5% crude fiber, Charoen Pokphand Group Co. Ltd., Vietnam). The survival rate of shrimp during acclimation was 95%.

Experimental design

In experiment 1, the effects of elevated ammonia or nitrite levels on the survival of shrimp at a constant salinity of 20 ppt were studied. Ammonia and nitrite stock solutions were prepared by dissolving 9.552 g of NH_4Cl (2500 mg L^{-1} total ammonia nitrogen (TAN)), 12.321 g of $NaNO_2$ (2500 mg L^{-1} nitrogen as nitrite (NO_2^--N) in 1000 mL of distilled water, respectively. The selected TAN and NO_2^--N nominal concentrations for single exposure were 0, 5, 10, 20, 30, and 40 mg of TAN L^{-1}; and 0, 5, 10, 20, 30, 40, and 50 mg of NO_2^--N L^{-1}. Each treatment was conducted in four replicates. Groups of 20 acclimated juveniles (±0.22 g wet weight) were randomly selected from the stocking tanks and transferred to the 20 L tanks containing 10 L of the test solution. During the experimental period, each tank was provided with continuous aeration, and water quality parameters included temperature at 28.5 to 29.1°C, pH 8.0 to 8.2, and DO at 5.1 to 6.0 mg L^{-1}. Nominal concentrations of TAN and nitrite were determined every 24 h according to standard colorimetric methods (APHA, 2017). Dead shrimp were immediately removed from the test tanks and each test solution was renewed (100%) every 24 h. The survival of shrimp was checked every 12 hours during the 96-h exposure. Shrimp in all treatments were not fed during the experiments.

Total ammonia nitrogen or nitrite concentration in intensive shrimp ponds is high and often exceeds 5 mg L^{-1} (Boyd 2001), thus this concentration was chosen as a target standard for experiment 2. This experiment was conducted to evaluate the effects of different salinities (5, 10, 15, and 20 ppt) at a high level of TAN or nitrite (5 mg L^{-1}) on the survival of shrimp. Different salinity levels were prepared by diluting high saline water (92 ppt) that was collected and transported from solar saltpans with dechlorinated tap water. Salinity was gradually decreased by 2 ppt per day until the desired salinity levels (5, 10, and 15 ppt) were reached. Each treatment was carried out in four replicates. Therefore, 80 juveniles were used in each treatment. During the experiment, continuous aeration was supplied, and the range of temperature, pH, and DO values were 28.6-29.0°C, 8.1-8.2, and 5.5-5.9 mg L^{-1}, respectively. The survival rate of shrimp was determined as described in experiment 1.

Statistical analysis

The lethal median concentrations (LC_{50}) values of TAN, NO_2^-N, and their 95% confidence limits were calculated by the Probit method (Finney, 1971; Valencia-Castañeda et al., 2018). The safe levels were estimated by multiplying the 96-h LC_{50} value by a factor of 0.05 (Boyd & Tucker, 1998). For un-ionized ammonia (NH_3-N) LC_{50} values, concentrations were presented as mg L^{-1} of TAN and NH_3-N which was calculated according to the equations proposed by (Soderberg & Meade, 1991), based on a temperature of 29°C and pH of 8.2. Differences in the mean survival of shrimp among treatments were evaluated using a one-way analysis of variance (ANOVA). A Tukey test was then applied to identify significant differences between treatments. All statistical significance tests were at the $p < 0.05$ level. The SPSS software (version 22.0) was used for the statistical analysis.

Results

Effects of total ammonia or nitrite at varying levels

Water quality parameters including temperature, pH, and dissolved oxygen were maintained within desired ranges for shrimp survival and growth (Law, 1988; Wyban et al., 1995; Nonwachai et al., 2011). The actual concentrations of ammonia and nitrite in the experiments were within 5% of the nominal concentrations, which is an acceptable difference for toxicity testing (Buikema et al., 1982).

The survival of *L. vannamei* juveniles exposed to different concentrations of TAN at each 24 h interval is presented in **Figure 1**. The higher the concentration of nitrite the shrimp were exposed to, the higher the mortality was observed. During the first 24 h, the survival rates for the control (0 mg L^{-1}), 5, 10, 20, 30, and 40 mg L^{-1} TAN were 100±0.0%, 95.0±2.0%, 85.0±3.5%, 72.5±1.4%, 63.8±4.3%, and 63.8±4.3%, respectively. The survival rates of shrimp in 30 and 40 mg L^{-1} TAN were significantly lower compared to those in the control, 5, 10, and 20 mg L^{-1} TAN, following 48 h and 72 h of exposure. A 100% mortality rate was observed in 30 and 40 mg L^{-1} TAN after 96 h of exposure.

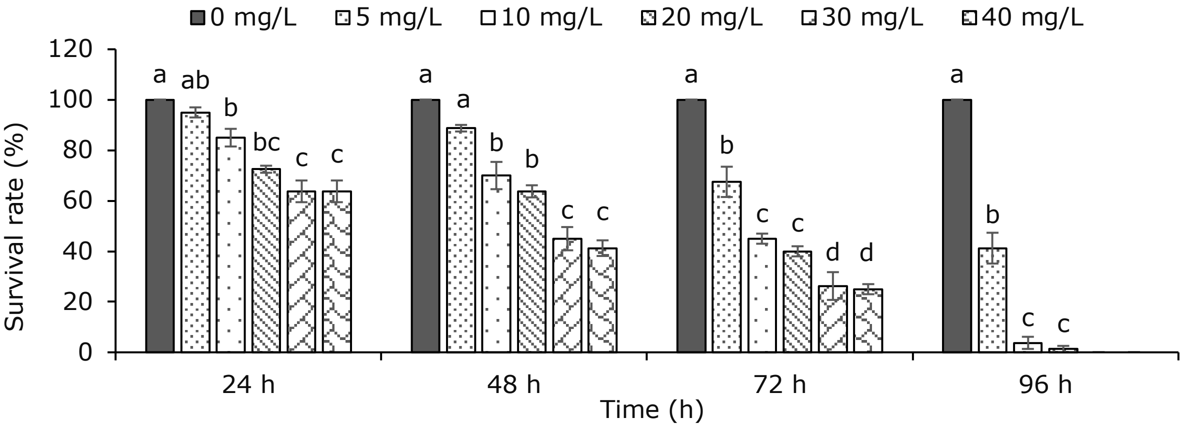

Figure 1 Survival of juveniles of *L. vannamei* exposed to different concentrations of TAN (mg L^{-1}) at 24 h, 48 h, 72 h, and 96 h

[Data are shown as mean ± SE (n = 4). Different letters above the bars indicate significant differences among TAN concentrations at each 24 h of exposure ($p < 0.05$)]

As for the nitrite toxicity test, the average survival values were negatively correlated with increasing levels of TAN ($p > 0.05$). More specifically, the survival of shrimp in the 40 mg L^{-1} NO_2^--N was lowest and showed significant differences compared to other treatments when exposed for 24 h (**Figure 2**). After 48 h, there was a 100% mortality rate in the doses of 30, 40, and 50 mg L^{-1} NO_2^--N, as well as in the doses of 10 and 20 mg L^{-1} NO_2^--N after 72 h. However, no deaths of individuals were recorded in the control group during the 96-h exposure, ensuring that the observed effects were due to the action of NO_2^--N.

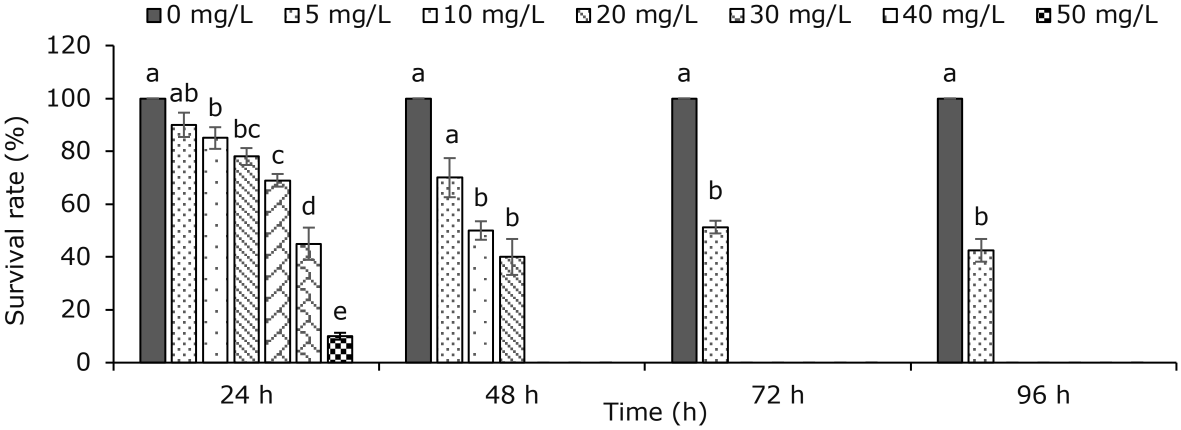

Figure 2 Survival of juveniles of *L. vannamei* exposed to different concentrations of NO_2^--N (mg L^{-1}) at 24 h, 48 h, 72 h, and 96 h

[Data are shown as mean ± SE (n = 4). Different letters above the bars indicate significant differences among NO_2^--N concentrations at each 24 h of exposure ($p < 0.05$)]

The LC_{50} values of TAN, NH_3-N, and NO_2^--N and their 95% confidence intervals at different exposure times for *L. vannamei* juveniles are shown in **Table 1**. The LC_{50} values of TAN and NH_3-N declined sharply after 48 h of exposure. The LC_{50} values at 24, 48, 72, and 96 h of TAN for *L. vannamei* juveniles were 45.5, 30.1, 13.8, and 6.3 mg L^{-1}, respectively, while the LC_{50} values of NH_3-N at 24, 48, 72, and 96 h were 3.93, 2.59, 1.19, and 0.55 mg L^{-1}. The LC_{50} values of NO_2^--N at 24, 48, 72, and 96 h were 37.6, 16.7, 8.8, and 4.8 mg L^{-1}, respectively.

Table 1 LC_{50} values of NO_2^--N, TAN, and NH_3-N (mg L^{-1}) at 24, 48, 72 and 96 h of exposure to *L. vannamei* juveniles at a salinity of 20 ppt.

Time (h)	LC_{50} of TAN (mg L^{-1})	LC_{50} of NH_3-N (mg L^{-1})	LC_{50} of NO_2^--N (mg L^{-1})
24	45.5 (33.9-103.9)	3.93 (2.93-8.97)	37.6 (31.0-52.1)
48	30.1 (22.8-45.2)	2.59 (1.97-3.89)	16.7 (11.3-21.4)
72	13.8 (3.5-22.0)	1.19 (0.31-1.89)	8.8 (4.1-12.0)
96	6.3 (2.1-8.6)	0.55 (0.19-0.74)	4.8 (2.3-6.0)

The 95% confidence intervals are shown within parentheses.

Effects of a high ammonia or nitrite concentration at varying salinities

The survival rates of shrimp were positively correlated with salinity levels at 5 mg L^{-1} TAN, and significant differences among treatments were recorded from 48 h until the end of the experiment (**Table 2**). The survival rates of shrimp at 5 ppt were significantly lower compared to those at 15 and 20 ppt. There were no significant differences in survival rates between 5 and 10 ppt or between 15 and 20 ppt during the 96-h exposure. No significant differences were found among all treatments during the first 24 h of exposure.

Table 2 Survival of juveniles of *L. vannamei* exposed to combined different levels of salinity and a concentration of TAN (5 mg L^{-1}) after each 24 h exposure.

Salinity (ppt)	Survival rate (%)			
	24 h	48 h	72 h	96 h
5 ppt	90.0±2.9^{a}	72.5±2.5^{b}	50.0±2.0^{b}	26.3±3.1^{c}
10 ppt	92.5±3.2^{a}	76.3±2.4^{b}	52.5±3.2^{b}	28.8±2.4^{bc}
15 ppt	93.8±2.4^{a}	91.3±3.8^{a}	70.0±2.0^{a}	38.8±2.4^{ab}
20 ppt (control)	93.8±2.4^{a}	90.0±3.5^{a}	68.8±3.1^{a}	42.5±3.2^{a}

Values shown are mean ± SE. Mean values within a column followed by the same letters show that there is no significant difference among the groups ($p > 0.05$).

The survival rate of shrimp exposed to 5 mg L^{-1} NO_2^--N at different salinity levels is shown in **Table 3**. No significant difference was observed among all treatments during the first 48 h of exposure ($p > 0.05$). After 72 h of exposure, the survival rate was negatively correlated with salinity at a concentration of 5 mg L^{-1} NO_2^--N and significant differences were recorded among treatments ($p < 0.05$). Following 96 h of exposure, the survival rates for the control (20 ppt), 15 ppt, 10 ppt, and 5 ppt were 43.8±3.1%, 45.0±2.0%, 33.8±2.4%, and 27.5±2.5%, respectively.

Table 3 Survival of juveniles of *L. vannamei* exposed to combined different levels of salinity and a concentration of NO_2^--N (5 mg L^{-1}) after each 24 h exposure.

Salinity (ppt)	Survival rate (%)			
	24 h	48 h	72 h	96 h
5 ppt	88.8±2.4^{a}	61.3±2.4^{a}	40.0±3.5^{b}	27.5±2.5^{c}
10 ppt	87.5±2.5^{a}	70.0±3.5^{a}	45.0±2.0^{b}	33.8±2.4^{bc}
15 ppt	90.0±2.9^{a}	70.0±2.0^{a}	58.8±3.1^{a}	45.0±2.0^{ab}
20 ppt (control)	91.3±3.8^{a}	71.3±2.4^{a}	60.0±3.5^{a}	43.8±3.1^{a}

Values shown are mean ± SE. Mean values within a column followed by the same letters show that there is no significant difference among the groups ($p > 0.05$).

Discussion

Ammonia and nitrite are the main nitrogenous products caused by shrimp excreta and metabolic waste and organic detritus. High concentration of nitrogen compounds could affect physiological processes, resulting in a low specific growth rate and even mortality of shrimp (de Campos et al., 2015). The results of this study show that the toxicity of ammonia and nitrite to *L. vannamei* juveniles increased with exposure time. As for the ammonia test, the survival of *L. vannamei* juveniles was reduced sharply with increasing the levels of ammonia concentration and exposure time, but the survival rate in the control group was 100% for 96 h of exposure. TAN treatments (0, 5, 10, 20, 30, and 40 mg L^{-1}) showed the survival of 100%, 90%, 85%, 78%, 69%, and 45%, respectively after 24 h of exposure, which indicated that TAN has a significant role in built-up toxicity, leading to mortality of shrimp. Similar results have been reported in other penaeid shrimps subjected to ammonia toxicity, including the Sao Paulo shrimp *Penaeus paulensis* (Ostrensky & Wasielesky, 1995), white shrimp *P. setiferus* (Alcaraz et al., 1999), green tiger prawn *P. semisulcatus* (Kir & Kumlu, 2006), white shrimp *L. schmitti* (Barbieri, 2010), and the pink-shrimp *Farfantepenaeus paulensis* (Miranda-Filho et al., 2009) and *F. brasiliensis* (de Campos et al., 2015). Han et al. (2017) reported that high levels of ammonia could prevent the synthesis and excretion of digestive enzymes in the hepatopancreas, leading to decreased metabolic energy for survival. In addition, the toxicity of a specific substance to aquatic organisms can vary between species and can change with water salinity, temperature, pH, dissolved oxygen levels, and the size of the organism (Lin & Chen, 2003). In the present study, the survival rate of shrimp tends to decrease with reducing salinity levels under the same ammonia concentration (5 mg L^{-1}) exposure, suggesting that the tolerance of whiteleg shrimp to ammonia is dependent on the levels of salinity. This observation is similar to the report of Barbieri (2010), where the higher mortality of *L. schmitti* is typically observed at lower salinity when shrimp are exposed to the same concentration of ammonia. A possible explanation could be due to the higher uptake of ammonia at low salinity levels (Kir & Oz, 2015).

The nitrite toxicity experiments conducted on *L. vannamei* juveniles in the present study showed that the survival of shrimp was related to concentration, exposure time, and salinity. For example, after 24 h of exposure, the survival of shrimp decreased from 90 to 10% when the concentrations of nitrite increased from 5 to 50 mg L^{-1} at the same salinity level (20 ppt). In addition, the survival of *L. vannamei* juveniles to nitrite decreased sharply after 72 and 96 h as compared to 24 h of exposure, and 100% mortality was recorded in nitrite from 20 to 50 mg L^{-1}. This finding suggests that the nitrite level is the main factor that causes mortality in shrimp. The results are in agreement with those reported by Barbieri et al. (2016), where *L. schmitti* juveniles exposed to high nitrite levels (10-80 mg L^{-1}) showed higher mortality compared to the control. Moreover, this study also found a lower survival rate in *L. vannamei* juveniles when exposed to low salinity levels under the same nitrite concentration. Our finding is similar to the results of other studies on shrimp, such as pink shrimp *F. brasiliensis* (de Campos et al., 2015) and white shrimp *L. schmitti* (Barbieri et al., 2016). A possible explanation could be that nitrite and chloride ions compete for the same transport site at the HCO_3^-/Cl^- exchanger on the apical side of the gill cells (Sowers et al., 2004; Romano & Zeng, 2013). Furthermore, high nitrite accumulation could reduce the amount of oxygen available for metabolism and lead to hypoxia and significant mortality (Barbieri et al., 2014). In the present study, the LC_{50} values (24, 48, 72, and 96 h) of nitrite in juveniles of *L. vannamei* were lower than that of total ammonia at a salinity of 20 ppt, suggesting that nitrite is more toxic than ammonia. This finding is similar to the report of Valencia-Castañeda et al. (2018) wherein the acute toxicity of nitrite in *L. vannamei* postlarvae to salinities of 1 and 3 ppt is greater than that of ammonia, and that its toxicity increases with decreasing salinity. Based on the 96-h values and an empirical application factor of 0.05 (Boyd & Tucker, 1998), the safe levels for rearing juveniles of *L. vannamei* (0.22 g) at 20 ppt salinity were estimated to be 0.32 mg L^{-1} for total ammonia, 0.03 mg/L for NH_3–N and 0.24 mg L^{-1} for nitrite.

In conclusion, our results suggest that *L. vannamei* juvenile is more resistant to ammonia than nitrite toxicity. At low salinity, both ammonia and nitrite showed greater toxicity to *L. vannamei*, which also caused a decrease in survival. The results obtained in this study provide baseline information for future studies and will help shrimp farmers manage their culture systems.

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