



Tolerance of whiteleg shrimp *Litopenaeus vannamei* juvenile to sudden changes of salinity, pH and total ammonia nitrogen

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ABSTRACT

Increasing demand of whiteleg shrimp *Litopenaeus vannamei* promotes aquaculture businesses. However, sudden changes on environmental parameters such as salinity, pH and ammonia concentration can affect the survival of *L. vannamei*. Thus, this study aimed to determine the mortality of juvenile *L. vannamei* to sudden changes of various salinities, pH and ammonia concentrations and the median lethal concentration of those environmental parameters to juvenile *L. vannamei*. The tolerance of the juvenile was tested to different salinities (0 to 40.0 ppt), pH (pH 3.5 to 4.5) and total ammonia nitrogen (TAN) concentrations (0.02 to 200 mg/L). The juvenile showed mortality at 0–7.5 ppt with the highest mortality was recorded at 0 ppt. Meanwhile, they showed mortality at 60 - 200 mg/L TAN concentration, with the highest mortality was recorded at 100 mg/L and 200 mg/L. At pH test, the juvenile showed mortality at pH 3.5–4.5. The highest mortality was recorded at pH 3.5. The LC₅₀ of salinity, TAN and pH on *L. vannamei* juvenile was at 3.53 ppt, 70.47 mg/L and pH 4.11. This study found that *L. vannamei* juveniles able to tolerate sudden changes in salinity (40 ppt to 2.5 ppt), ammonia (as high as 70 mg/L TAN) and pH (as low as pH 4). This study also found that these factors can affect the survival of juvenile without the manipulation of other factors. Thus, this information is important for proper pond management by providing baseline information and eventually minimizing the economic loss of the small-scale business.

Keywords: shrimp farms, aquaculture, environmental parameters, stress.

INTRODUCTION

Shrimp aquaculture is one of the most economically significant sectors in global fisheries, contributing substantially to food security, export revenue, and rural livelihoods across many tropical and subtropical countries. In Malaysia, shrimp industry has the highest animal aquaculture production in brackish water culture system (Annual Fisheries Statistics, 2024). The most commercially important cultured shrimp species in Malaysia is *Litopenaeus vannamei*, commonly known as Pacific white shrimp, with production occupying

around 75% of the total shrimp production (Annual Fisheries Statistics, 2024). Native to the Eastern Pacific coast of the Americas, *L. vannamei* has been widely adopted in Asian aquaculture due to its fast growth rate, high feed conversion efficiency, tolerance to a wide range of environmental conditions, and relatively high disease resistance compared to other penaeid shrimps. These characteristics have made it the preferred species for commercial shrimp farming worldwide.

Due to increasing consumer demand for *L. vannamei* and other shrimp species, intensive shrimp aquaculture practices are currently being

widely adopted (Thean et al., 2016). Intensive systems allow farmers to maximize production yield per unit area by maintaining high stocking densities with supplemental aeration and controlled feeding regimes. However, this intensification has caused various problems including low productivity and disease outbreak (Opiyo et al., 2018; El-Saadony et al., 2022). These challenges are of great concern to the shrimp farming industry as they result in significant economic losses and threaten the sustainability of production.

The low productivity and disease outbreak in shrimp aquaculture have been closely related to environmental factors. Water quality parameters such as salinity, pH, and ammonia concentration play critical roles in determining the physiological condition, immune response, and overall survival of farmed shrimp. Suboptimal or fluctuating levels of these parameters can impair osmoregulation, disrupt acid-base balance, and cause toxic effects on gill function and metabolic processes, ultimately increasing shrimp susceptibility to pathogens and mortality. In Malaysia, the majority of shrimp are cultured in earthen brackish water ponds (Annual Fisheries Statistics, 2024). Pond aquaculture is always exposed and prone to sudden fluctuations of environmental factors (Le et al., 2022) such as salinity, pH, and ammonia concentration. These sudden fluctuations are usually related to weather conditions such as drought, saline water intrusion, and heavy rainfall (Le et al., 2022; Uddin et al., 2025), all of which are particularly prevalent in the tropical climate of Malaysia. Therefore, these environmental instabilities can significantly affect the health and production performance of cultured shrimp.

In shrimp, the effects of environmental factors have been studied; however, most studies were conducted on larvae and post-larvae stages of *L. vannamei* (Valentino-Álvarez et al., 2013; de Lourdes Cobo et al., 2012; Asadi et al., 2017; Asadi et al., 2018), with a notable lack of studies focusing on the juvenile stage, which is a critical phase in the grow-out production cycle. Furthermore, most existing studies were conducted in different regions such as Mexico (Valentino-Álvarez et al., 2013), Ecuador (de Lourdes Cobo et al., 2012), Brazil (Maicá et al., 2014), and China (Wang et al., 2013), where the environmental conditions, water chemistry, and climate may differ considerably from those in Malaysia. The median lethal concentration (LC_{50}) the concentration of a given parameter that causes 50% mortality

in a test population within a specified exposure period is a standard toxicological endpoint widely used to assess the sensitivity of aquatic organisms to environmental stressors. Establishing the LC_{50} values for salinity, pH, and ammonia in juvenile *L. vannamei* under Malaysian conditions is therefore essential to guide farm management decisions and establish safe operational thresholds. Thus, this study aimed to determine the mortality of juvenile *L. vannamei* exposed to sudden changes in salinities, pH, and ammonia concentrations, and the median lethal concentration of those environmental parameters to juvenile *L. vannamei*.

MATERIALS AND METHODS

Animal ethic guideline

Since the Animal Ethics Committee Guidelines of Universiti Malaysia Sabah apply to vertebrates only and there is no official standard for invertebrates, this study adapted its principles to shrimp. This study followed the recommendation by Tangprasittipap et al. (2013) by adopting the guidelines from Australian, New South Wales state government for the humane harvesting of fish and crustaceans (<http://www.dpi.nsw.gov.au/agriculture/livestock/animal-welfare/general/fish/shellfish>) with respect to details regarding the transport of the shrimp and their laboratory maintenance.

Experimental animals and juvenile rearing conditions

Whiteleg shrimp (*L. vannamei*) juveniles were purchased from a local commercial shrimp farm and transported to Shrimp Hatchery of Borneo Marine Research Institute, University Malaysia Sabah (BMRI UMS). The juveniles were reared in round shape and black colour high density polyethylene (HDPE) stocking tank with seawater aerated with 18 mm diameter circular stone aerator at 28–29 ppt, under roofed facilities with natural photoperiod (12 h light and 12 h dark) for acclimatization. Juvenile were fed with *L. vannamei* shrimp feed (Royal Dragon, DT313, Vietnam) and fasted one day prior to experimental tests. Prior the experiment, the shrimp were reared in seawater without any adjustment two days before tolerance test. During the experiment, the juveniles were transferred directly into treatment tank (seawater with adjustment) to facilitate

sudden changes of the environmental parameters. Dead shrimps were removed from the tank during the observation. The observation was recorded daily until the cumulative mortality was constant.

Tolerance of *L. vannamei* to different level of salinity

The experimental tolerance to different salinity levels was conducted according to Bringolf et al. (2005) with some modifications. Juveniles (initial weight: 4.35 ± 0.03 g; $n = 30$ per treatment) were randomly stocked into 80 L of sand filter filtered seawater (salinity of 28–29 ppt, temperature of 29–30 °C, dissolved oxygen of 3.8–5.1 mg/L and pH of 7–9) obtained from Sepanggar Bay in Sabah, Malaysia, aerated with 18 mm diameter circular stone aerator in a 160 L square shape and white colour fibreglass tank, in indoor Wet Laboratory of BMRI UMS with a natural photoperiod of 12 h light and 12 h dark. Semi-static renewal strategy for water was employed in the experimental units. Tolerance of *L. vannamei* was conducted with salinity of 0, 2.5, 5.0, 7.5, 10.0, 35.0, 40.0 ppt and control of seawater without salinity adjustment (28–29 ppt) in triplicates, with the temperature maintained at 29–30 °C, dissolved oxygen at 3.8–5.1 mg/L and pH at 7–9 in every treatment. Preparation of water with salinity of 35.0 and 40.0 ppt were done by adding sea salt (Aquaforest, Poland) to the seawater, whereas preparation of salinity 2.5, 5.0, 7.5 and 10.0 ppt were done by diluting seawater with de-chlorinated tap water. Adjustment of salinity was done with refractometer (Atago, Tokyo, Japan). The juveniles were fed twice daily, 50–60% of water was changed once daily and the cumulative mortality was recorded.

Tolerance of *L. vannamei* to different ammonia concentrations

The experiment on the tolerance to different concentration of ammonia nitrogen (TAN) was conducted according to de Lourdes Cobo et al. (2012) with some modifications. Juveniles (initial weight: 9.52 ± 0.05 g; $n = 30$ per treatment) were randomly stocked and acclimatized for 1–2 days prior to toxicity test, into 80 L of sand filter filtered seawater (salinity of 28–29 ppt, temperature of 29–30 °C, dissolved oxygen of 3.7–5.0 mg/L and pH of 7–8) obtained from Sepanggar Bay in Sabah, Malaysia, aerated with 18 mm diameter circular stone aerator in a 160 L square shape and white

colour fibreglass tank, in indoor Wet Laboratory of BMRI UMS with a natural photoperiod of 12 h light and 12 h dark. Semi-static renewal strategy for water was employed in the experimental units.

Tolerance of *L. vannamei* to TAN concentrations was conducted with 0 (control), 0.02, 0.2, 2.0, 10, 20, 40, 60, 70, 80, 100 and 200 mg/L TAN, in triplicates. A stock solution with concentration 10 000 mg/L was prepared by dissolving 29.67 g of ammonium chloride (NH_4Cl) in 1L of distilled water. The desired TAN concentrations were prepared by diluting the stock solution with seawater. The juveniles were fed twice daily, 50–60% of water was changed once daily and the cumulative mortality was recorded.

Tolerance of *L. vannamei* to pH

The experiment on the tolerance to different pH was conducted according to Morgan and McMahon (1982) with some modifications. Juveniles (initial weight: 7.45 ± 0.01 g; $n = 30$ per treatment) were randomly stocked into 80 L of constantly aerated water (salinity of 28–29 ppt, temperature of 29–30°C, dissolved oxygen of 3.8–5.1 mg/L and pH of 7–9) in a 160 L fibreglass tank. Juveniles were acclimatized for 1–2 days prior to toxicity test with different pH. Static renewal strategy for water was employed in the experimental units. Tolerance of *L. vannamei* on different pH was conducted with pH 3.5, 4.0 and 4.5 in triplicates. The pH of seawater was adjusted with 1M hydrochloric acid (HCl). The pH of water was monitored using pH meter (OHAUS, USA). The juveniles were fed twice daily, 50–60% of water was changed daily and the cumulative mortality was recorded.

Statistical analysis

The data was normalised using arcsine transformation. The data was statistically analyzed with IBM SPSS (version 23). The analysis of variance (one-way ANOVA) was used to determine the difference between the mean values of different treatments at 95% level of significance. Differences in the mean were analysed by Tukey's post hoc test at 95% level of significance.

Media lethal concentration (LC_{50})

Median lethal concentration (LC_{50}) was calculated using Reed and Muench method modified

by Saganuwan (2011), based on the cumulative mortality for each parameter.

RESULTS

Tolerance of *L. vannamei* to different salinity

Mortality of *L. vannamei* juvenile exposed to different salinity for 10 days are presented in Figure 1. No mortality was recorded in the control group and treatment groups of 10.0, 35.0 and 40.0 ppt. Juvenile exposed to 0–7.5 ppt showed significantly higher mortality compared to 10 to 40 ppt. The highest mortality (100%) was recorded at 0 ppt. The result also shows that *L. vannamei* juveniles are able to tolerate from 40 ppt to 2.5 ppt. Mortality started to reduce below 50% when salinity was above 5 ppt. The LC_{50} of water salinity on *L. vannamei* juvenile was 3.53 ppt.

Tolerance of *L. vannamei* to TAN

The mortality of *L. vannamei* juvenile exposed to increasing concentration of TAN for 10 days is presented in Figure 2. No mortality (0%) was recorded in the control group and 0.02–40 mg/L TAN. Juvenile showed mortality at 60–200 mg/L TAN concentration, with the highest mortality (100%) was recorded at 100 mg/L and 200 mg/L. The result also showed significantly higher mortality at 60–200 mg/L TAN concentration compared to 10–40 mg/L. The mortality fell below 50% when the TAN concentration was below 70 mg/L. The LC_{50} of TAN on *L. vannamei* juvenile was 70.47 mg/L.

Tolerance of *L. vannamei* to pH

Mortality of *L. vannamei* juvenile exposed to different pH for 10 days is presented in Figure 3. Juvenile showed mortality at pH level tested. However, the mortality is significantly high at pH 3.5–pH4 compared to pH4.5. The highest mortality (100%) was recorded at pH 3.5. The result shows that *L. vannamei* juveniles able to tolerate pH 4 and above. The mortality was below 50% at pH 4.5. The LC_{50} of pH on *L. vannamei* juvenile was 4.11. Median lethal concentration (LC_{50}) was calculated using Reed and Muench method modified by Saganuwan (2011), based on the cumulative mortality for each parameter.

DISCUSSION

This study reports the tolerance of *L. vannamei* juvenile to different level of salinity, pH and total ammonia nitrogen concentrations. For salinity, the mortality of *L. vannamei* juveniles was higher compare to previous research from Maicá *et al.* (2012; 2014). This might due to the sudden changes of the environment which lead to more stress (Esparza-Leal *et al.*, 2020; Hapsari *et al.*, 2025), and eventually cause more mortality of the *L. vannamei* juvenile. *L. vannamei* juveniles could tolerate salinity from 2.5 ppt to 40 ppt. This finding is comparable to Maicá *et al.* (2012; 2014). According to Chong-Robles *et al.* (2014), juvenile stage of *L. vannamei* is the strongest osmoregulator thus has a wide range of salinity tolerance. Even though the juvenile able to tolerate this range of salinity, the mortality is

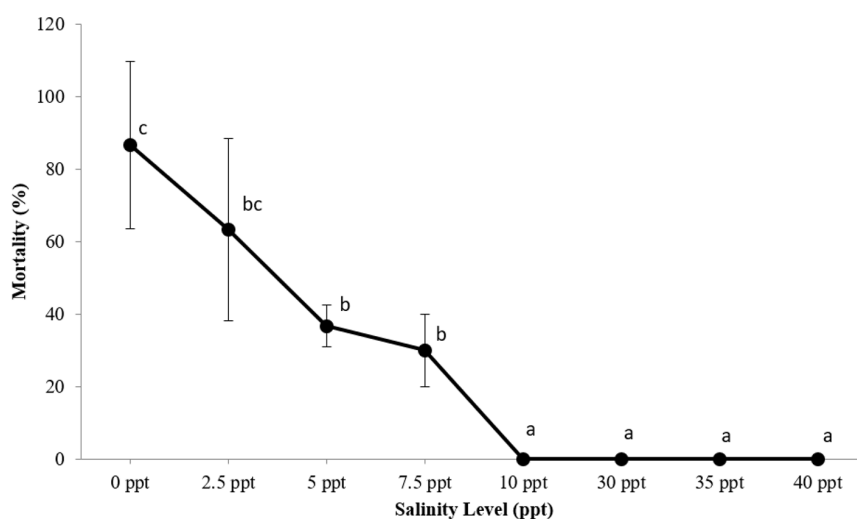


Figure 1. Percentage of mortality of *L. vannamei* juvenile exposed to different level of salinity

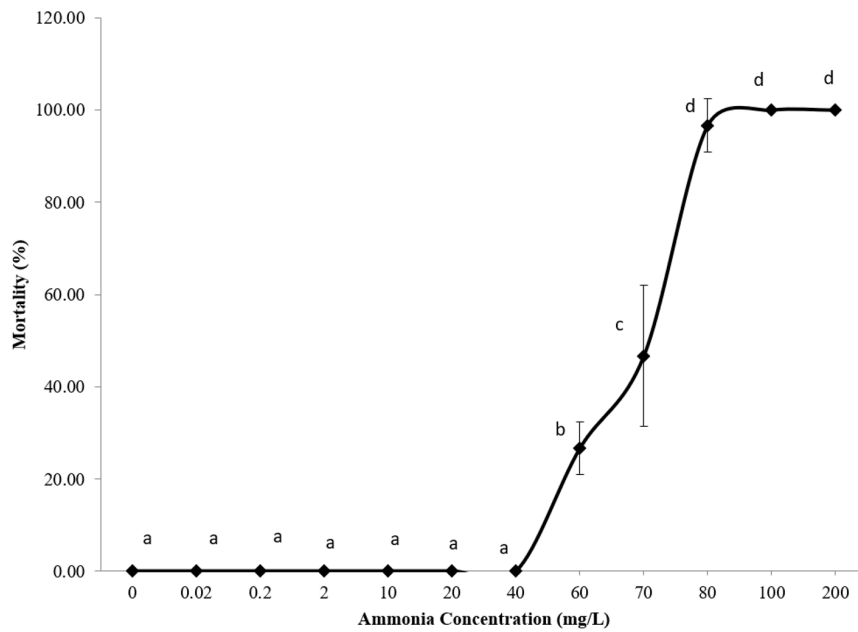


Figure 2. Percentage of mortality of *L. vannamei* juvenile exposed to increasing concentration of TAN

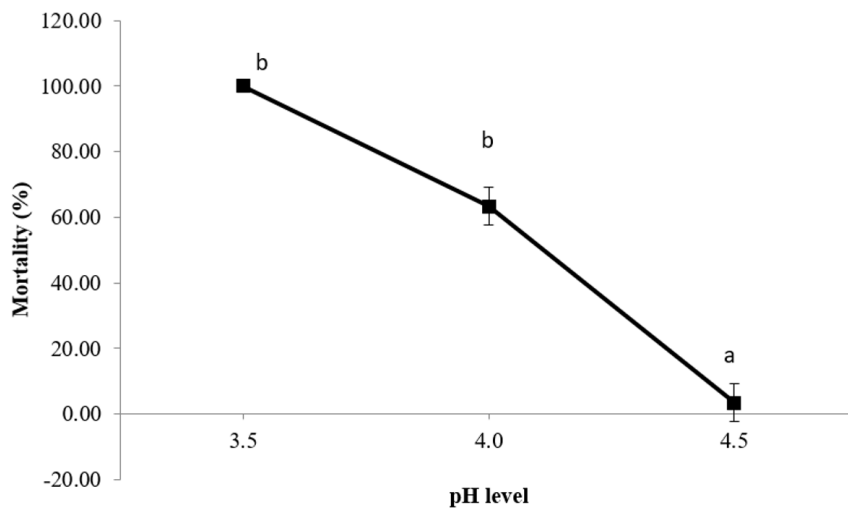


Figure 3. Percentage of mortality of *L. vannamei* juvenile exposed to different level of pH

increases with a decrease of the salinity (Maicá et al., 2012; Decamp et al., 2003; Jayasankar et al., 2009; Hall-Scharf et al., 2025). The LC_{50} of salinity on *L. vannamei* juvenile was in the agreement with Maicá et al. (2014) where they reported that the survival of *L. vannamei* juvenile was 74.5% at 4 ppt. Low salinity of 5‰ induced stress in *L. vannamei*, making *L. vannamei* more susceptible to pesticides and have lower LC_{50} (Wang et al., 2013). However, the finding of this study revealed that at certain level without manipulation of other factors, the salinity itself can affect the survival of *L. vannamei*. The tolerance of *L. vannamei* was lower at high TAN concentration

compared to low TAN concentrations. High level of ammonia concentration can cause mortality in penaeid shrimp (Chen et al., 1992; Kathyayani et al., 2019). Ammonia decreases the haemolymph capability to transport oxygen by damaging the gills, while tissues oxygen consumption increases (Racotta et al., 2000). The *L. vannamei* juveniles tolerate up to 70 mg/L TAN. This finding showed a comparable result to Valencia-Castañeda et al. (2019) where the juvenile *L. vannamei* able to tolerate 80 mg/L TAN, even though they were reared in 3 ppt of seawater. This study might show that the mortality of the shrimp was not affected by the salinity, however, this is different from the report

by Lin and Chen (2001) where salinity affects the ammonia toxicity of the *L. vannamei* juveniles. The LC₅₀ of TAN on *L. vannamei* juvenile was higher compared to study done by de Lourdes Cobo *et al.* (2012) in *L. vannamei* larvae, where their report on LC₅₀ of TAN after 24 h exposure was 4.2-9.9-16.0, 19.0-17.3-17.5 and 13.2 mg/L in zoea (1-2-3), mysis (1-2-3) and postlarvae 1, respectively. Generally, the LC₅₀ of TAN on *L. vannamei* larvae is lower than juvenile. This might be due to juvenile having higher stress tolerance in comparison to larvae.

The survival rate of *L. vannamei* was high at pH 4.5. This finding is in agreement with Furtado *et al.* (2015) where they reported that survival of juvenile *L. vannamei* was over 90% at pH 4.5 to pH 9.5. The pH value corresponding to the LC50 is slightly acidic. This finding was supported by Yu *et al.* (2020) where low pH can adversely affect the growth and health of *L. vannamei*. The tolerance of *L. vannamei* was lower compared to other crustacean such as *Procambarus clarki* and *Orconectes rusticus*. The LC₅₀ of four days exposure to acid were pH 2.8 and 2.5 for *P. clarki* and *O. rusticus* respectively, showing that the crayfish species were acid-tolerant but not long term (Morgan and McMahon, 1982). In *P. chinensis* study, it was revealed that water pH which is slightly acidic (6.0) or basic (8.5) caused metabolic suppression, constraining the activity of Na⁺-K⁺ ATPase (Wang *et al.*, 2002). This deficiency in the active transport mechanism of Na⁺ through the gill epithelium can be the main cause of shrimp deaths (Furtado *et al.*, 2015; Ge *et al.*, 2022). Yu *et al.* (2020) also reported that high pH (pH 9.5) affects the growth and health of *L. vannamei*. Therefore, pH must be maintained in safe level to avoid or reduce mortality of shrimp.

CONCLUSIONS

In conclusion, *L. vannamei* able to tolerate sudden changes of salinity, TAN and pH. Even though they are able to tolerate certain level of salinity, TAN and pH, the survival of the juvenile *L. vannamei* is lower as the condition of the environment parameters shift away from the optimum level. For the sudden changes of the environmental parameters, the LC₅₀ of salinity, TAN and pH to *L. vannamei* juvenile were determined as 3.53 ppt, 70.47 mg/L and pH 4.11, respectively. This information is important for the

pond management and help minimize economic loss of the small-scale business. However, further long-term toxicity studies and synergistic effects investigations would permit the complete evaluation of these environmental factors can affect the survival of the whiteleg shrimp cultured in pond.

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