

Effect of Ammonia at Different pH Values on Survival of Juveniles of *Palaemon adspersus* Rathke, 1837 (Decapoda: Palaemonidae)

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Abstract: The aim of the study was to determine the toxic effect of ammonia concentration on juveniles of *Palaemon adspersus*, a shrimp species important for both fisheries and food webs. Juveniles of *P. adspersus* were exposed to various ammonia concentrations (NH₃) in acute toxicity tests at four pH values (6–9). The 24, 48, 72 and 96-hour LC₅₀ values of total ammonia nitrogen (TAN) ranged from 114.82 to 67.61 mg/dm³ at pH 6; from 120.23 to 69.18 mg/dm³ at pH 7; from 87.10 to 54.95 mg/dm³ at pH 8; from 79.43 to 19.95 mg/dm³ at pH 9. The 24, 48, 72 and 96-hour LC₅₀ values of NH₃-N varied within range of 0.054 to 0.037 mg/dm³ at pH 6, 0.569 to 0.309 mg/dm³ at pH 7, 3.846 to 2.291 mg/dm³ at pH 8 and 24.547 to 6.166 mg/dm³ at pH 9. It was determined that high pH values noticeably decreased the tolerance of juveniles of *P. adspersus* to TAN and NH₃-N. Mortality rates increased in parallel with increasing NH₃ concentrations, pH and exposure time.

Key words: TAN, NH₃-N, lethal concentration, lethal time, mortality, toxicity

Introduction

Environmental pollution is a global problem that has been encountered in many natural water bodies such as seas, lakes and streams. Such pollution is often found in conjunction with the rapid development of industrialisation and technology. Pollutants transported by large rivers and causing eutrophication are among the most significant sources for changes in marine ecosystem. As a result, marine ecosystems are gradually deteriorating (JESSEN et al. 2014, DEMIRARSLAN & AKINCI 2016). Biological waste products of naturally occurring aquatic organ-

isms also release ammonia into water bodies, in addition to ammonia produced by decomposition of organic materials entering the aquatic environment through both anthropogenic and natural processes. If ammonia production exceeds the absorption and adsorption, purification and recycling capacity of the aquatic system, accumulation of ammonia can be observed and aquatic organisms are affected (MIRANDA-FILHO et al. 2009).

Ammonia (NH₃) is the final product of protein digestion of fish, molluscs and crustaceans as well as of the disintegration of organic pollutants, such as faeces and uneaten food (FRIAS-ESPERICUETA et al.

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1999). Due to the increased ammonia accumulation in the intensive culture system, water quality can deteriorate rapidly and oxygen consumption rates can increase. This may lead to harmful changes in the structure of tissues, protein and amino acid levels, blood chemistry, cell function, osmoregulation, resistance to diseases, growth, reproduction and survival rates as well as damage of nervous system (ALCARAZ et al. 1999, KIR et al. 2004). The acute toxicity of NO_2^- and NH_3 for shrimp juveniles has been appraised in dissimilar stages and species in marine and estuarine waters; it has been determined that the toxicity effect depends on the shrimp species, life-cycle stage and age (CHEN & NAN 1991, CHEN et al. 1991). However, the tolerance of shrimps to nitrogen compounds, particularly NH_3 and NO_2^- , indicates an important problem in environments with lower salinity (SCHULER et al. 2010, RAMÍREZ-ROCHÍN et al. 2017). In aquaculture systems, oxygen consumption and NH_3 excretion may increase to the stage where the concentrations of nitrogen compounds reach high levels. This can lower food intake rates and can lead to changes in free amino acid levels and protein concentrations, and even decrease growth and increase mortality (CHEN et al. 1989, LIN & CHEN 2003, VALENCIA-CASTAÑEDA et al. 2018). Total ammonia nitrogen (TAN) consists of the sum of ionised ammonium as nitrogen ($\text{NH}_4^+\text{-N}$) and un-ionised ($\text{NH}_3\text{-N}$) ammonia as nitrogen (CHEN & CHIN 1989). $\text{NH}_3\text{-N}$ induces high toxicity to aquatic organisms. The high toxicity of NH_3 is mainly associated with high diffusion capability through biological membranes in aquatic organisms (BARIMO & WALSH 2005). The ionised form of NH_3 is probably the greatest concern since more than 95% of NH_3 is present as ammonium at physiological pH (MIRANDA-FILHO et al. 2009). Elevated concentrations of NH_3 can be lethal to shrimps. The NH_3 toxicity strongly depends on factors such as pH, salinity, dissolved oxygen and temperature, which all play a major role in the probability of survival (SCHULER 2008). There is a balance between NH_4^+ and $\text{NH}_3\text{-N}$. Factors as pH, temperature and salinity are effective in maintaining this equilibrium. $\text{NH}_3\text{-N}$ ratio decreases with salinity yet increases with pH and temperature. Therefore, TAN is toxic to aquatic organisms (FRIAS-ESPERICUETA et al. 1999, SAMPAIO et al. 2002, KIR & KUMLU 2006, SCHULER et al. 2010). Previous studies have indicated that high NH_3 concentrations can negatively influence the growth and development of organisms (NAQVI et al. 2007, ROMANO & ZENG 2013) and the acid-base balance and gas exchange of the aquatic system as well as osmoregulatory, immunological and histological changes of organisms (JOHANSSON

et al. 2000, HARRIS et al. 2001, LIU & CHEN 2004, HONG et al. 2007, ROMANO & ZEN 2010, CHANG et al. 2015). The effects of NH_3 on immune responses have attracted much attention since immunity is closely related to the emergence and progress of diseases (ROMANO & ZENG 2010, ELLIS et al. 2011, CUI et al. 2017). Various studies were conducted to examine the lethal effects of NH_3 and NO_2^- on various life stages of shrimps such as *Penaeus monodon* (see CHEN & CHIN 1989, CHEN & LEI 1990), *Metapenaeus ensis* (see NAN & CHEN 1991), *P. paulensis* (see OSTRENSKY & WASIELESKY 1995), *Litopenaeus vannamei* (see LIN & CHEN 2001, 2003, VALENCIA-CASTAÑEDA et al. 2018). ALCARAZ et al. (1997) studied temperature toleration of *Litopenaeus setiferus* at diverse NH_3 and NO_2^- concentrations, whereas KIR & KUMLU (2006) investigated acute toxicity of ammonia to *Penaeus semisulcatus* at different salinity levels.

The acute toxicity trials offer valuable information regarding the proportional lethal characteristics of one or more chemicals and different conditions. Such trials are planned to specify the maximum level of a pollutant that may effect a certain percent of survival, e.g., 50% of a limited number of trial organisms (LC_{50}), considering that the period of the toxicity tests in an acute explanation is usually 96 hours or 72, 48 and 24 hours (NEWMAN & UNGER 2003). The conservation of shrimp stocks can contribute to a country's economy and the livelihood of fishermen. For this reason, the dynamic structure of the stocks (e.g., life and death parameters) should be well known before exploitation. *Palaemon adspersus* Rathke, 1837 is an important component in the food chain of the Black Sea and has significant potential in terms of economic yield and ecosystem services.

The aim of the present study is to determine the toxic effect of ammonia on *P. adspersus* at various pH values.

Materials and Methods

As a model species, we used the shrimp *P. adspersus* that is native to Sinop and its surroundings. The trials were carried out at the Faculty of Fisheries Research and Application Unit, Sinop University. Shrimps caught from the coast of Akliman in the Sinop Province were brought to the Research Unit. They were left to adapt to the laboratory conditions for two weeks before used in the trial. The individual wet weights of shrimps ranged from 0.841 to 1.078 g (mean 0.94 ± 0.16 g), and there was no significant difference ($p < 0.05$) between shrimps

placed in various aquariums. The stock solution of NH_3 was prepared by dissolving 0.903 g/cm³ and 25% of ammonium hydroxide (NH_4OH ; Merck) in one litre distilled water to obtain a concentration of 10.000 mg/dm³. Test solutions were prepared with the stock solution of NH_4OH and filtered seawater (17 ppt salinity, which is the normal seawater salinity of the Black Sea). The control and test solutions concentrations were prepared as 0 (control), 25, 50, 100, 150, 200, 250 and 300 mg/dm³ for NH_4OH , and the pH values of these solutions were adjusted to 6, 7, 8 and 9. Each solution was prepared with three replications and the pH settings were made individually in plastic aquariums. A total of 96 test solutions were used in the experiment. The Microsoft Excel program was used to calculate the NH_3 concentration in the contents of these solutions. The amount of NH_3 in the test solutions was 0 (control), 12.1, 24.3, 48.6, 72.9, 97.1, 121.4 and 145.7 mg/dm³. The prepared solutions were put into aquariums in volumes of 4 dm³. The pH values of each solution were adjusted with HCl. The spectrometric method was used for calculating the concentrations of the solutions. The calculated TAN and un-ionised NH_3 -N concentration values of the solutions are presented in Table 1.

The concentrations of un-ionised ammonia as nitrogen (NH_3 -N) and ionised ammonia as nitrogen (NH_4^+ -N) were calculated from ammonia-N, temperature, salinity and pH values as suggested by BOWER & BIDWELL (1978).

When the adaptation period of shrimps was completed, ten shrimps were placed in each aquarium. Shrimps were not fed during the experiment. Observations were made on the first day at the 15th, 30th minutes and at the end of 1, 2, 4, 8 and 12 hours, and later at the end of 24, 48, 72, 96, 120 and 144 hours. Individuals, which did not react or move when touched, were considered dead and were taken from the aquariums by using a glass rod. During 144 hours of the experiment, aquariums

were checked hourly. Temperature, salinity and dissolved oxygen values were measured daily in each aquarium. Water temperature at $23 \pm 1^\circ\text{C}$, salinity at 17 ppt and dissolved oxygen at 7.2 ± 0.4 mg/dm³ were maintained. LC_{50} (mean lethal concentration) test was conducted per the methods designated in the short-dated average deadly concentration toxicity trials (APHA 1989).

LC_{50} and LT_{50} (mean lethal time) values of shrimps were determined (LLOYD 1992, Ünsal 1998). Within 24, 48, 72 and 96 hours, the values of the concentrations (LC_{50}) that killed 50% of the shrimps were calculated by probit analysis (FINNEY 1971). Furthermore, under the same conditions, the same analysis was used to calculate the time (LT_{50}) of 50% of the shrimps' death. Besides, the relationships between survival, pH, TAN concentration and exposure time were compared using the General Linear Model (ANOVA) method of the Minitab (18) Statistical Computer Program.

Results

During the trial, no shrimp death was observed in the control group at any pH level. Although no death was seen at 72 hours of exposure to 7.38 mg/dm³ TAN concentration in pH 7, mortality was only 13.3% after 96 hours of exposure. Shrimp mortality was not observed in 96 hours of exposure at a concentration of 35.43 mg/dm³ TAN at pH 6, while mortality was 3.3% in a 24 hours period at 62.25 mg/dm³ TAN concentration. Although the mortality rate of shrimps exposed to NH_3 -N at pH 6 (13.33%), pH 7 (13.33%), pH 8 (16.66%), pH 9 (43.33%) was low in the first 24 hours, it was found to be higher at other hours. After 96 hours, at the same NH_3 -N concentrations, there was an increase in mortality due to pH in the solutions (pH 6 and 7: 63.33%; pH8: 83.33%; pH9: 96.00%). In the 48-hour period, the mortality rate in NH_3 -N at pH 7 (0.34 mg/dm³) was

Table 1. PH values and calculated TAN and NH_3 -N concentration values of solutions.

Prepared solutions (mg/dm ³)	Calculated concentrations							
	TAN (mg/dm ³)				un-ionised NH_3 -N (mg/dm ³)			
	pH 6	pH 7	pH 8	pH 9	pH 6	pH 7	pH 8	pH 9
25	7.01	7.38	7.26	12.66	0.0031	0.0322	0.3050	3.8611
50	35.43	21.73	35.51	32.69	0.0155	0.0950	1.4921	9.9653
100	62.25	51.85	52.59	54.96	0.0273	0.2264	2.2094	16.7560
150	75.88	77.28	64.42	74.94	0.0333	0.3374	2.7066	22.8477
200	94.55	88.00	80.93	87.23	0.0415	0.3843	3.4005	26.5926
250	108.07	98.65	95.92	97.56	0.0474	0.4308	4.0301	29.7425
300	116.01	120.96	103.85	118.63	0.0509	0.5282	4.3633	36.1670

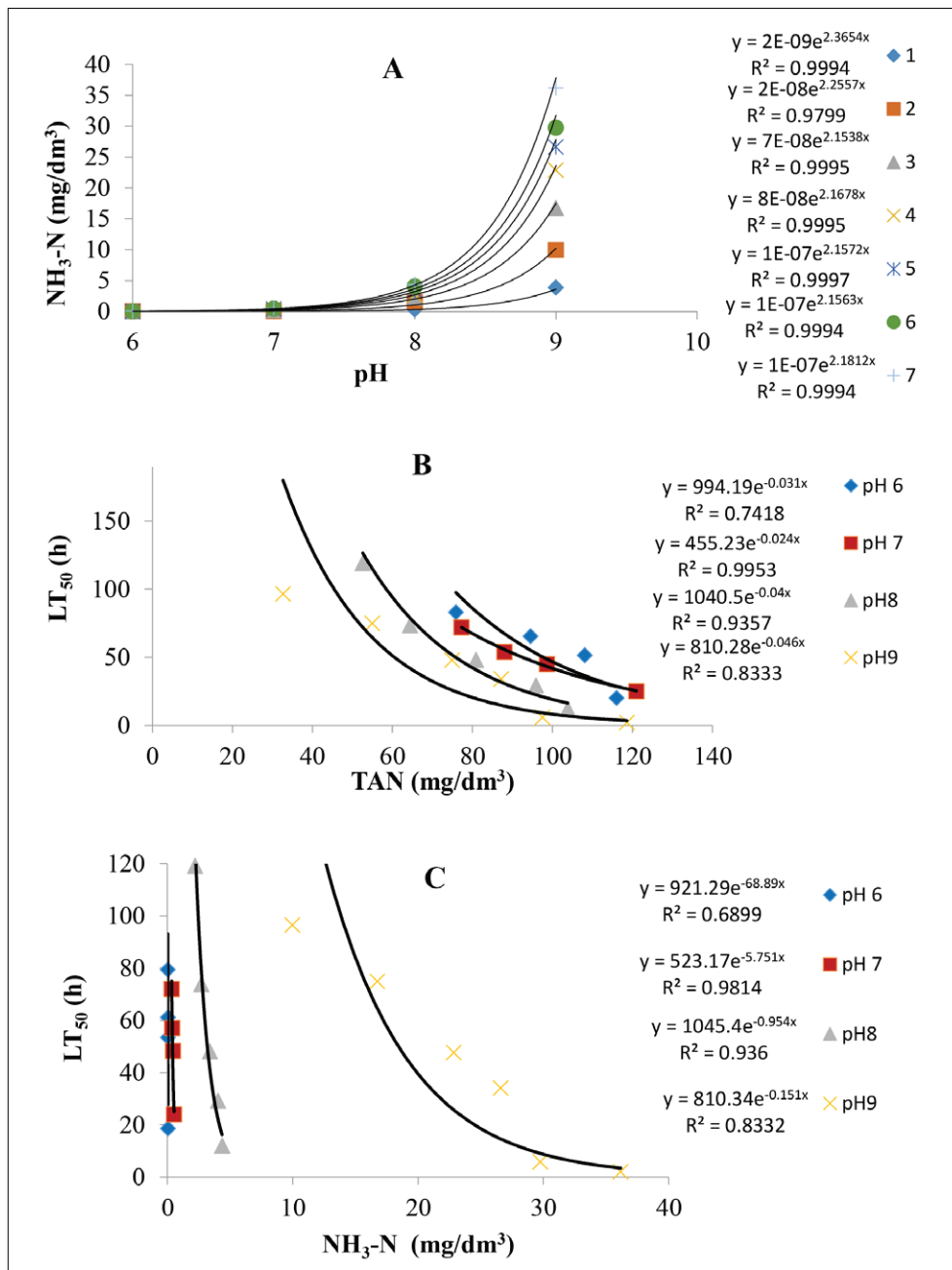


Fig. 1. Relationship between concentration of un-ionised ammonia (NH₃-N) and pH at all concentrations (A). The LT₅₀ (median lethal time to kill half of the population) of TAN (B) and NH₃-N (C) for *Palaemon adspersus* juveniles exposed to different concentrations at various pH levels.

26.66%, while it was 33.33% at pH 6 (0.03 mg/dm³) or pH 8 (2.70 mg/dm³) and 43.33% at pH 9 (22.84 mg/dm³). Shrimps were exposed to 0.0273, 0.226, 2.209 and 16.75 mg/dm³ NH₃-N (pH 6, 7, 8 and 9), and mortality was determined as 10.00%, 23.33%, 30.00% and 80.00% in 96 hours, respectively. In the same procedure, shrimps were exposed to 0.042, 0.384, 3.400 and 26.593 mg/dm³ NH₃-N (pH 6, 7, 8 and 9), and mortality was determined as 66.67%, 73.33%, 90.00% and 100.00%, respectively. The relationship between the concentration of un-ionised

ammonia (NH₃-N) and pH is presented in Fig. 1(A).

The times when 50 % (LT₅₀) of shrimp died at different pH of NH₃-N or TAN concentrations were calculated (Fig. 1 B, C). At pH 6, the LT₅₀ values of juveniles of *P. adspersus* in 75.9, 94.6, 108.1 and 116.0 mg/dm³ were 83.1, 65.6, 51.4 and 20 hours, respectively. At pH 7, the LT₅₀ values in 72.0, 88.0, 98.7 and 120.1 mg/dm³ were 72.0, 54.0, 45.0 and 25.0 hours, respectively. At pH 8, the LT₅₀ values in 52.6, 64.4, 80.9, 95.9 and 103.9 mg/dm³ were 119.1, 73.4, 48.0, 29.2 and 12.0 hours, respectively. At pH

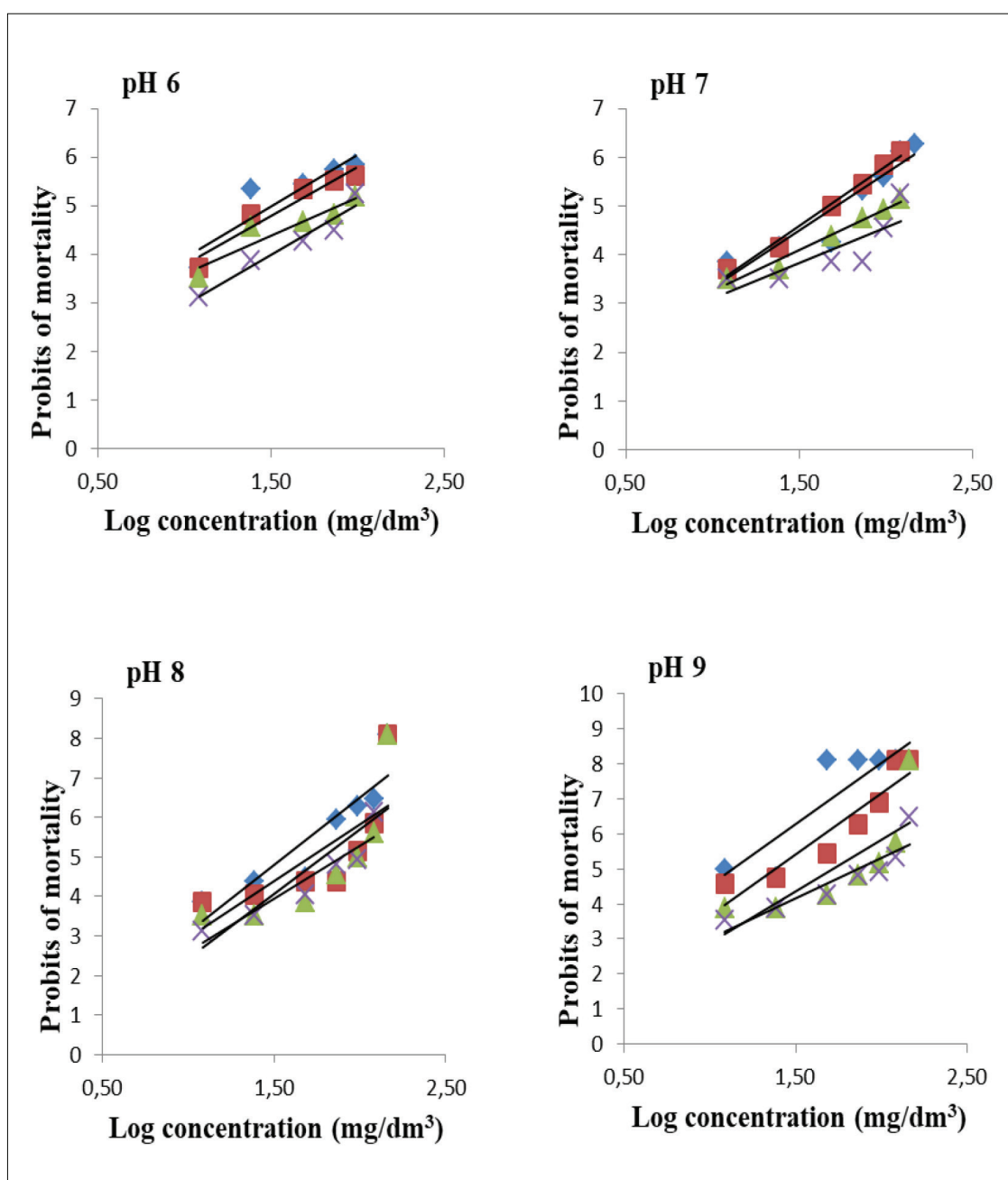


Fig. 2. Relationship between probits of mortality and log TAN concentration (mg/L) for *Palaemon adspersus* juveniles at 24 (x), 48 (▲), 72 (■) and 96 h (◆) of exposure and at four different pH.

9, the LT_{50} values in 32.7, 55.0, 74.9, 87.2, 97.6 and 118.6 mg/dm³ were 96.4, 74.9, 47.7, 34.0, 5.8 and 2.0 hours, respectively (Fig. 1).

At each concentration, the time required for half of the sample's death was determined as LT_{50} (median lethal time). The results of the χ^2 analysis demonstrated that the log TAN concentration as a function of probit mortality had a positive linear correlation with probit mortality, and the calculated values were satisfying at the pH values of water (Fig. 2, Table 2). The LC_{50} values of TAN at different exposure times

for *P. adspersus* and the consequence of χ^2 square test were determined. The survival of juveniles of *P. adspersus* depended on the exposure time, pH and TAN concentration. The interaction between them was found to be statistically significant ($p \leq 0.001$) according to the ANOVA test result (F-Value; Time: 11.21, pH: 18.94, C: 1921.60, C x Time: 8.30, C x pH: 14.81, Time x pH: 4.92, C x Time x pH: 3.42).

In solutions containing different pH and NH₃ (TAN), concentrations causing 50% death (LC_{50}) in 24, 48, 72 and 96 hours were determined. The LC_{50}

Table 2. Relationship between probit mortality (Y) and log TAN as mg/L (x) at various exposure times and at pH 6, 7, 8 and 9 for juveniles of *Palaemon adspersus*.

Time (h)	Y = a + bx	R ²	χ ²	n ^a	χ ² df	0.95
pH 6						
24	Y = 1.639 + 1.5889x	0.850	8.115	5	3	7.815
48	Y = 2.257 + 1.458x	0.913	3.128	6	4	9.488
72	Y = 2.254 + 1.706x	0.995	7.521	6	4	9.488
96	Y = 2.208 + 1.868x	0.975	1.246	6	4	9.488
pH 7						
24	Y = 3.013 + 0.872x	0.887	8.321	5	3	7.815
48	Y = 2.207 + 1.381x	0.956	2.145	7	5	11.07
72	Y = 1.902 + 1.973x	0.950	8.439	6	4	9.488
96	Y = 2.311 + 1.871x	0.932	5.709	5	3	7.815
pH 8						
24	Y = 2.200 + 1.432x	0.860	5.587	5	3	7.815
48	Y = 2.417 + 1.577x	0.855	9.085	7	5	11.07
72	Y = 3.130 + 0.980x	0.793	6.521	7	5	11.07
96	Y = 2.670 + 2.105x	0.998	4.589	4	2	5.991
pH 9						
24	Y = 1.191 + 1.926x	0.885	3.711	7	5	11.07
48	Y = 1.431 + 2.045x	0.882	10.761	7	5	11.07
72	Y = 1.775 + 2.656x	0.986	9.690	7	5	11.07
96	Y = 2.286 + 3.946x	0.922	7.703	7	5	11.07

X² : Chi-square

Table 3. LC₅₀ (mg/dm³) of TAN and NH₃-N for *Palaemon adspersus* and their 95% confidence limits (in parentheses) at four pH values and various exposure times and mean weight.

pH	24	Exposure time (h)				Weight ± SD
		48	72	96		
6	TAN	114.82 (108.75-120.88)	107.89 (102.62-113.17)	93.33 (87.61-99.04)	67.61 (59.16-76.06)	0.912 ± 0.160
	NH ₃ -N	0.054 (0.046-0.061)	0.048 (0.029-0.067)	0.041 (0.021-0.061)	0.037 (0.034-0.040)	
7	TAN	120.23 (113.82-126.62)	108.39 (103.07-113.71)	75.86 (68.73-82.99)	69.18 (62.71-75.66)	1.078 ± 0.180
	NH ₃ -N	0.569 (0.485-0.652)	0.468 (0.450-0.486)	0.363 (0.347-0.380)	0.309 (0.280-0.338)	
8	TAN	87.10 (78.99-95.20)	77.62 (71.23-84.02)	70.79 (64.42-77.17)	54.95 (49.60-60.31)	0.841 ± 0.145
	NH ₃ -N	3.846 (3.464-4.228)	3.020 (3.006-3.034)	2.754 (2.739-2.770)	2.291 (2.066-2.515)	
9	TAN	79.43 (71.59-87.28)	75.86 (68.54-83.18)	25.70 (21.45-29.96)	19.95 (16.10-23.80)	0.934 ± 0.147
	NH ₃ -N	24.547 (22.106-26.988)	23.442 (23.425-23.459)	7.943 (7.915-7.972)	6.166 (4.987-7.345)	

average values of TAN ranged from 114.82 to 79.43 mg/dm³ at 24-hours, from 107.89 to 75.86 mg/dm³ at 48-hour, 93.33 to 25.70 mg/dm³ at 72-hour and 67.61 to 19.95 mg/dm³ at 96-hour in the all pH values. The LC₅₀ average values of NH₃-N ranged from 0.054 to 24.547 mg/dm³ at 24-hours, from 0.048 to 24.442 mg/dm³ at 48-hour, 0.041 to 7.943 mg/dm³ at 72-hour

and 0.037 to 6.166 mg/dm³ at 96-hour in the all pH values (Table 3). With the increasing pH of water and exposure times, it was determined that TAN and NH₃-N decreased LC₅₀ concentrations. It was detected that shrimps' life depends on the NH₃ concentration, pH, and staying time in that environment and is inversely proportional to these conditions (Fig. 1B, C; 3).

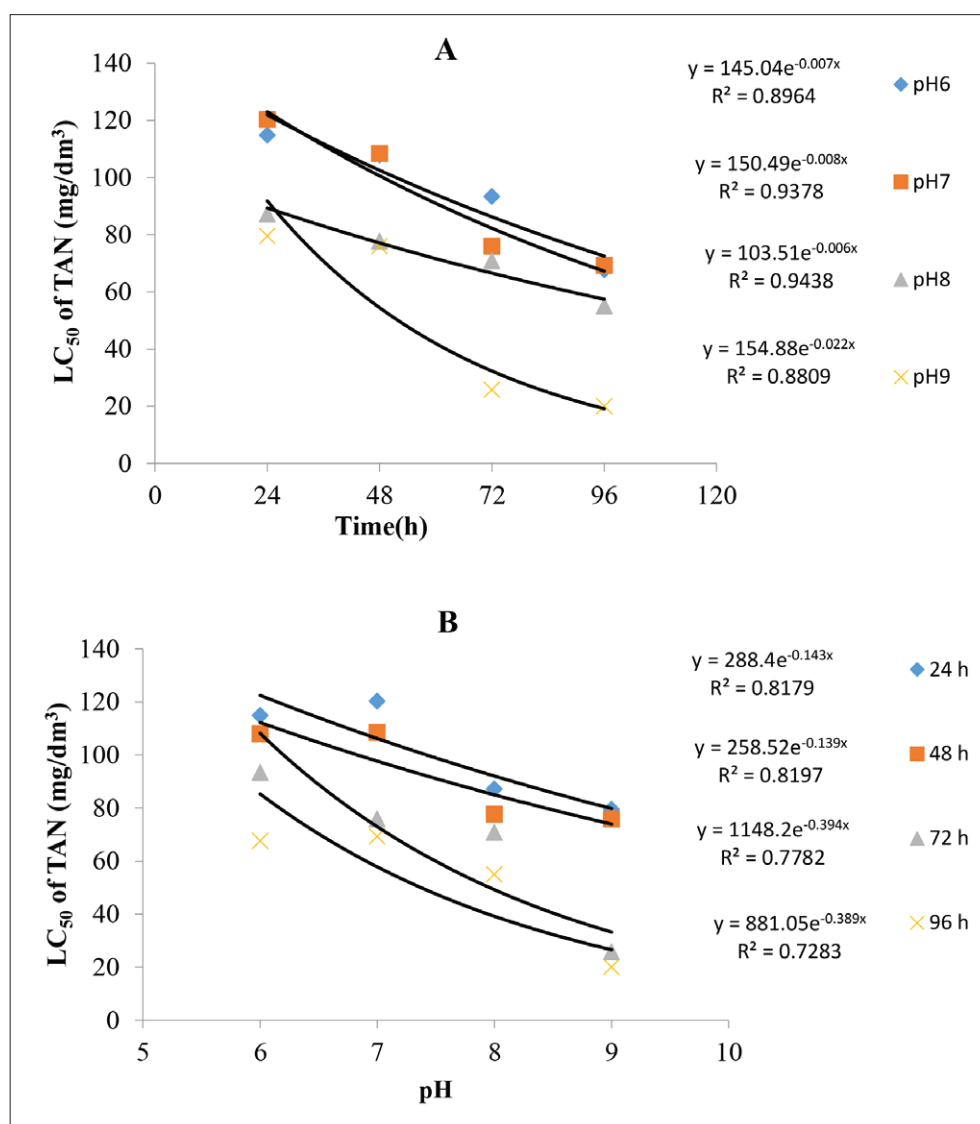


Fig. 3. Relationship between median lethal concentration (LC₅₀) of total nitrogen (TAN) and exposure time at various pH levels (A) and between LC₅₀ of TAN and pH at various exposure times (B).

Discussion

One of the most important water quality problems in aquaculture is NH₃ poisoning (MEADE 1985). Besides, many conditions that cause NH₃ poisoning can also lead to nitrite poisoning as NH₃ is converted to NO₂ by NH₃-oxidizing bacteria. In this study, NH₃-N, pH and time have been found to be statistically significant ($p \leq 0.001$) factors in the survival of juveniles of *P. adspersus*.

ARMSTRONG et al. (1978) explained that the total ammonia concentration consists of two forms, being NH₄⁺ (ionised ammonium) and NH₃ (un-ionised ammonia). The content of TAN depends on physico-chemical conditions such as temperature, salinity and pH (BOWER & BIDWELL 1978). Un-ionised ammonia is more toxic than ionised ammonium (SMART 1978).

LT₅₀ value decreased when pH and NH₃ concentrations increased; thus, there was a negative relationship. A similar negative correlation has been found by LIN & CHEN (2001) by examining ammonia toxicity at different salinity values on juveniles of *Litopenaeus vannamei*. The LT₅₀ values for *P. adspersus* juveniles vary from 5.8 to 51.4 hours when exposed to TAN concentrations ranging from 97.56 to 108.07 mg/dm³ at pH 6-9. The LT₅₀ values for juveniles are from 47.7 to 79.4 hours when exposed to NH₃-N concentration range from 0.03 to 22.85 mg/dm³ at pH 6-9. The results of the present study show that pH affects the tolerance of juveniles of *P. adspersus* against NH₃.

FRIAS-ESPERICUETA et al. (1999) and SCHULER et al. (2010) have reported that mortality has increased with increasing NH₃ concentration and ap-

plication time. Similar results are obtained in the course of the present study. When pH, TAN and $\text{NH}_3\text{-N}$ concentrations of the solutions increase, 50% of the shrimp have shorter death times. CHEN & CHIN (1989) have emphasised that ammonium toxicity increases at high pH levels and that half of *Penaeus monodon* larvae die in less than 13 hours at low (0.07 mg/dm^3) ammonium-N concentration.

NH_3 is a basic molecule in aquatic medium. The increase in the medium's basic feature increases the toxic effect of NH_3 on shrimps. In other words, there is a positive relationship between pH increase and NH_3 toxicity as previously demonstrated (THURSTON et al. 1981, CHEN & CHIN 1989).

In the present study, the 96-hour LC_{50} value for *P. adspersus* has been found 54.95 mg/dm^3 (pH 8) at 17 ppt and 23°C for. The 96-hour LC_{50} values of different shrimps range between 26.72 mg/dm^3 (pH 8.2) for *Penaeus semisulcatus* (see KIR et al. 2004) and 70.9 mg/dm^3 (pH 7.7) for *Penaeus monodon* (see CHEN & LEI 1990). KIR et al. (2006) have reported that the 96-hour LC_{50} value of *P. semisulcatus* postlarvae is 19.06 mg/dm^3 (pH 8.2) at 40 ppt and 25°C . LIN & CHEN (2001) have estimated C_{50} values of $\text{NH}_4^+\text{-N}$ and $\text{NH}_3\text{-N}$ on *Litopenaeus vannamei* juveniles are 24.39 and 1.20 mg/dm^3 at 15‰ and 39.54 and 1.60 mg/dm^3 at 35‰ (pH 8.05 and 23°C), respectively. The impacts of this toxic substance may depend on the species' size or environmental conditions such as temperature, pH and salinity. Compared to the LT_{50} and LC_{50} values of other studies, the present findings demonstrate that *P. adspersus* may be more tolerant of ammonia than other shrimp species. LIN & CHEN (2001) have reported that the tolerance of *L. vannamei* juveniles to ammonia depends on salinity, and they are more resistant as salinity increases. WAJSBROT et al. (1990) and KIR et al. (2004) emphasise that *P. semisulcatus* juveniles are more sensitive to ammonia toxicity than other species.

The ionisation percentage of ammonia increases with pH and temperature (MASSER et al. 1992). In the present study, the 24, 48, 72 and 96-hour LC_{50} values as TAN for *P. adspersus* changes between 114.82 and 67.61 mg/dm^3 at pH 6 and 79.43 and 19.95 mg/dm^3 at pH 9. The 24, 48, 72 and 96-hour LC_{50} at pH 6 is 1.44, 1.42, 3.63 and 3.38 times higher than those at pH 9. The high LC_{50} concentrations at pH 6 show that the shrimps have a high tolerance to the NH_3 concentration. As the temperature is constant in the test solutions, it can be assumed that any difference may depend on the pH value. It can be suggested that *P. adspersus* juveniles have higher toleration to TAN in low pH

waters, as the percent of $\text{NH}_3\text{-N}$ in TAN increases with pH and toxicity. $\text{NH}_3\text{-N}$ is more toxic than $\text{NH}_4^+\text{-N}$ (SMART 1978). Small uncharged particles can easily pass through the lipid membrane of the gill cells of aquatic organisms (SCHULER 2008). As the pH increases, the $\text{NH}_3\text{-N}$ concentration and penetration rate into the cells also increase, leading to increased mortality rates. In their study on *Penaeus monodon* postlarvae, CHEN & CHIN (1989) have reported that ammonia toxicity increases in parallel to pH increase.

Our findings indicate that juveniles of *P. adspersus* are a good test organism to determine NH_3 toxicity in an aquatic environment. NH_3 concentration and pH levels are found to affect shrimp mortality. In conclusion, as the TAN concentration and pH increase, the rate of $\text{NH}_3\text{-N}$ (un-ionised ammonia as nitrogen) in the water increases, the tolerance of shrimps to NH_3 decreases and the mortality rate is much higher; however, the duration of contact with NH_3 is also an effective factor. According to the results obtained by this study, it can be suggested that the biochemical and chronic effects of NH_3 on juveniles of *P. adspersus* should be further investigated.

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