THE EFFECT OF PRE-EXPOSURE AMMONIA ON SURVIVAL AND AMMONIA RESISTANT OF *Macrobrachium lanchesteri* (De Man, 1911)

By
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PENGAKUAN DAN PENGESAHAN LAPORAN
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Adalah ini diakui dan disahkan bahawa laporan ilmiah bertajuk: The effect of pre-exposure ammonia on survival and ammonia resistant of *Macrobrachium lanchesteri* (De Man, 1911), oleh Siti Katijah Bt Mohamad Amin, No.Matrik UK13102 telah diperiksa dan semua pembetulan yang disarankan telah dilakukan. Laporan ini dikemukakan kepada Jabatan Sains Perikanan dan Akuakultur sebagai memenuhi sebahagian daripada keperluan memperolehi Ijazah Sarjana Muda Sains Agroteknologi Akuakultur, Fakulti Agroteknologi dan Sains Makanan, Universiti Malaysia Terengganu.

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DECLARATION

I hereby declare that the work in this thesis is my own except for quotations and summaries which have been duly acknowledged.

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ABSTRACT

This study was conducted in a lab at University Malaysia Terengganu. Acute toxicity test and pre-exposure test with the ammonia were conducted to examine the effect on survival of *Macrobrachium lanchesteri.* Shrimp were submitted for various concentration of ammonia for 96 h on acute toxicity test. 96 h LC_{50} is 13.5 $mg.l^{-1}$ and the safety level for *Macrobrachium lanchesteri* is 1.35$mg.l^{-1}$. Mortalitiy increase with the ammonia concentration in acute toxicity test. Pre-exposure test was conducted by exposing the shrimps to low concentration of ammonia (0.5 $mg.l^{-1}$, 1.0 $mg.l^{-1}$ and a control test for 7days before increasing into 20 $mg.l^{-1}$ ammonia concentrations for 96 h. Shrimp that was exposed under low concentration ammonia (0.5 $mg.l^{-1}$ and 1.0 $mg.l^{-1}$) sensitive to higher concentration of ammonia rather than shrimp in the control test. The shrimp were stressed due to the pre-exposure ammonia compared to the control test that has higher survival rate than the pre-exposure test.

ABSTRAK

Kajian dijalankan dalam makmal di Universiti Malaysia Terengganu. Ujian ketoksikan akute dan pendedahan awal kepada ammonia dilakukan untuk mengkaji kadar kemandirian *Macrobrachium lanchesteri*. *Macrobrahcium lanchesteri* diletakkan dalam kepekatan ammonia yang berbeza selama 96 jam bagi ujian ketoksikan. Keputusan untuk 96 h LC_{50} ammonia ialah 13.5 $mg.l^{-1}$ manakala paras selamat bagi spesis ini ialah 1.35 $mg.l^{-1}$. Ujian pendedahan awal dijalankan dengan mendedahkan udang pada kepekatan rendah (0.5 $mg.l^{-1}$, 1.0 $mg.l^{-1}$ dan ujian kawalan) selama 7 hari sebelum dipindahkan kepada kepekatan yang lebih tinggi iaitu 20 $mg.l^{-1}$ selama 96 jam. Udang yang didedahkan kepada 0.5 $mg.l^{-1}$ dan 1.0 $mg.l^{-1}$ lebih sensitif terhadap kepekatan ammonia yang lebih tinggi berbanding udang yang berada dalam ujian kawalan. Udang menjadi stress disebabkan pendedahan kepada ammonia. Kadar kemandirian udang yang berada dalam ujian kawalan lebih tinggi berbanding udang yang didedahkan kepada 0.5 $mg.l^{-1}$ dan 1.0 $mg.l^{-1}$.

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LIST OF SYMBOLS

DO	-	Dissolve oxygen
LC	-	Lethal concentration
$Mg.l^{-1}$	-	Milligram per litre
NH_4^+	-	Ionized ammonia
NH_3	-	Unionized ammonia
pH	-	Measure of acid or alkaline content
%	-	Percent
°C	-	Degree Celsius

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

Macrobrachium lanchesteri (De Man, 1911) is one of the most common freshwater shrimps occurring in Thailand, Malaysia, Singapore, and in some other countries of the Indopacific region (Suckcharoen, 1980). It is commonly known as rice field prawn. It occurs also in rice fields and has the ability to reproduce in stagnant freshwater (Johnson, 1968). The shrimp forms an important source of protein for the local inhabitants of many Southeast Asia countries, and therefore it has a great potential for development in aquaculture (Guerrero et al., 1975).

In Malaysia, prawns form an important element in the fauna of most freshwater habitats though they are absent from many isolated ponds, some streams and rivers in high mountains, and moderately to highly polluted waters (Johnson, 1968). Their abundance, their role in the ecology of freshwater habitats and their high protein content give them considerable potential importance. *Macrobrachium lanchesteri* was cultured for ornamental use due to its transparent appearance that is unique and as an ornamental organism and was cultured in aquarium as one of ornamental collection among the ornamentals' culturists. Chen and Lai (1992) state that appearance in isolated water mass causes some species is subjected to the fluctuating water quality such as ammonia or nitrite in the water bodies. Oxidation of accumulated organic matter mould lead to the formation of $NH4^{+}$ and NO_2^{-}, which may spoil the living environment of the aquatic animal (Durborow et al., 1997).

Ammonia, the end product of protein catabolism accounts for more than half the nitrogenous waste released by decapod crustaceans (Regnault, 1987). In an intensive culture system, ammonia is released into water primarily from ammonification of organic matter especially unconsumed feed, dead organism, deamination and transamination through the digestion and assimilation of ingested feed by the cultured animals (Jiann and Chi, 1990). When they are consume the food, it is metabolized into the energy, nutrients, and proteins used for survival and growth. As with all animals, there is waste produced by these normal metabolic processes. It has been reported that concentration of ammonia-N (un-ionized plus ionized ammonia as nitrogen) increased directly with culture period, and might reach as high as 46mg l^{-1} in intensive grow-out ponds (Chen et al., 1988).

Ammonia is colorless gas, lighter than air and has a sharp, stinging odor, but concentrated ammonia gas could suffocate and caused mortality. Ammonia occurs in two forms, which together are called the total ammonia nitrogen, (TAN) (Duborow et al., 1997). Chemically, these two forms are represented as NH_4^+ and NH_3. NH_4^+ is called ionized ammonia because it has a positive electrical charge, and NH_3 is called unionized ammonia since it has no charge. Unionized ammonia, NH_3 is usually toxic (Burkhalter and Kaya, 1977). Ammonia is highly soluble in water and forms a solution known as ammonium hydroxide (ammonia water). It will reacts with many chemicals when dissolve in water. The equilibrium between these ammonia forms depends on temperature, salinity and above all the pH of the solution (Chin and Chen, 1987).

Several studies have found the consequence of ammonia level towards crustaceans. In small amounts, ammonia causes stress and exposed to low levels of ammonia over time are more susceptible to bacterial infections, have poor growth and will not tolerate routine handling as well as they should (Floyd and Watson, 2005). Ammonia is a killer when present in higher concentrations, and many unexplained production losses have been caused by ammonia (Wajsbrot et al., 1990). Zin and Chu (1991) on their study on crustacean state that high concentrations of ammonia may result in retardation of shrimp growth and in extreme cases can cause mortality. The build-up of toxic nitrogenous waste, such as ammonia, nitrite and urea in culture system that increase exponentially over time caused the crustacean species stressed, may be decrease in immune defense and increased susceptibility to pathogens (Horowitz and Horowitz, 2001). Toxicity level for unionized ammonia depend on the individual species, however levels below 0.02 ppm are considered safe (Catedral et al., 1977). Catedral et al. (1977) reported that ammonia at 10 ppm is toxic to postlarvae of *P. monodon*. Dangerously high ammonia concentrations (0.6 - 0.2mgl^{-1}) are usually limited to water recirculation systems or hauling tanks where water is continually recycled and in pond culture after phytoplankton die-offs (Le Moullac and Haffner, 2000).

1.1 Significance of study

This species may be abundant in forest streams and rivers but it has excellent flavour. But the waters of their natural habitats tend to be unproductive so it is unlikely that either will ever be exploited on a large scale. *Macrobrachium lanchesteri* can flourish and breed under pond conditions. It is eurytopic with respect to most environmental factors and seems to be well adapted to the soft waters found in most parts of Malaysia. It is not cannibalistic, at least under normal conditions, and appears to be herbivorous. Though it is rather small size, it is larger than many other shrimp. Moreover, it occurs in large numbers and can attain a larger size under especially favorable conditions. All of these features indicate its suitability for pond culture provided that adequate demand (Guerrero et al., 1975) and adequate collecting and marketing facilities are available. In order to understand and optimize production conditions, it is necessary to investigate the effects of pre-exposure ammonia on survival to enable culturists to better manage the husbandry of shrimp. So, this study is important in order to identify the resistance of shrimp toward ammonia level when they are exposing to the ammonia and to study wherever they are able to be culture in ponds without causing high mortality.

1.2 Objectives of the study

The objectives of the study on the effect of pre-exposure ammonia on survival and ammonia resistant of *M. lanchesteri* are:

1.2.1 To study acute effect of ammonia on survival of *Macrobrachium lanchesteri*.

1.2.2 To compare the shrimp survival through the condition of pre-exposure ammonia and the condition without ammonia.

CHAPTER 2

LITERATURE REVIEW

2.1 Taxonomy of *Macrobrachium lanchesteri* De Man, (1911)

Table 2.1: Taxonomy of *Macrobrachium lanchesteri*

Name	Rank
Kingdom	Animalia
Phylum	Arthropoda
Subphylum	Crustacea
Class	Malacostraca
Order	Decapoda
Suborder	Pleocyemata
Infraorder	Caridea
Family	Palaemonidae
Genus	*Macrobrachium*
Species	*Macrobrachium lanchesteri*

2.2 Morpohology of *Macrobrachium lanchesteri*

Macrobrachium lanchesteri is a relatively small species but one which may make a considerable contribution to the biomass of the habitat in which it lives since it normally occurs in large numbers. A good swimmer, it is less strictly a bottom dweller than are many palaemonids. It has a light, compressed build and a relatively large abdomen. This transparent species appears to be indigenous to the swamps and ricelands of Southeast Asia from where it appears to have penetrated south along the Malay Peninsula. It has reached Singapore, probably through human agency, but has not yet been recorded in Indonesian waters (Johnson, 1968). In the Malaysian area, ovigerous females of *Macrobrachium lanchesteri* may have an overall length of only 33 mm. Wild caught specimens seldom exceed 50 mm (Johnson, 1968).

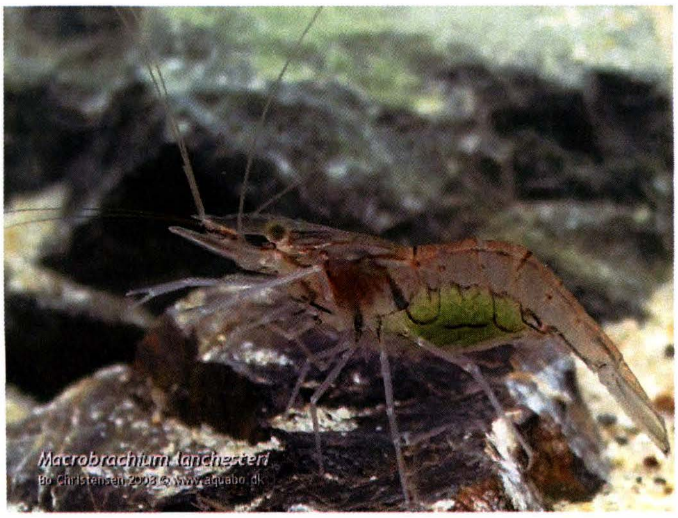

Figure 2.2.1: Adult of *Macrobrachium lanchesteri* (source: Christensen, 2008)

2.1 Life cycle of *Macrobrachium lanchesteri*

In the ponds that inhabited by this species the pH, calcium content, and phosphate content of the water tend to be higher than in many natural habitats, and there is an abundant supply of digestible algae but even where these conditions prevail in natural habitats such large individuals have not been obtained. A probable contributory factor is the rarity of efficient predatory fish which are excluded and eliminated in the ponds. It is quite likely that in uncontrolled habitats shrimps do not survive long enough to attain the maximum size. *M. lanchesteri* flourishes in standing and slowly flowing waters and lives in fresh waters throughout its life cycle (Suckcharoen, 1980). Zoeae and post-larvae can sometimes be obtained from isolated pond habitats, and populations in such habitats appear to maintain themselves. It thus seems clear that breeding as well as growth may occur in stagnant water. Nonetheless populations can become established in streams with a moderate water flow if other conditions are favorable. The maximum flow which the author has recorded for such a habitat was 0.70m/sec (Johnson, 1968)

2.4 *Macrobrachium lanchesteri* and its habitat

Like other shrimps, *M. lanchesteri* appears to be unable to withstand any marked degree of organic pollution though it is not clear just which factors are deleterious. Low oxygen content might be operative in some habitats but the species is absent even where the oxygen content is apparently high. Intolerance to high concentrations of ammonia is another possible limiting factor. No records from habitats with ammonia concentrations greater than 4 mg/l are available. Though there is no precise information it is probable that this species is more tolerant to tin mine pollution than are most species, as evidenced by its common occurrence in mine pools and pools in tin tailings (Johnson, 1968).

2.5 Ammonia

Ammonia is produced as a major end product of the metabolism of protein catabolism and is excreted as unionized ammonia across the gills of aquatic organisms (Duborow et al., 1997). Ammonia, nitrite, and nitrate are all highly soluble in water. In water, ammonia exists in two forms; un-ionized ammonia, NH_3, and ionized ammonium, $NH4^+$ (Hamid et al., 1994). Ammonia occur two forms in the water called Total Ammonia Nitrogen (Durborow et al., 1997). Ammonia in water that can be said in the formula is like this; $NH_4^+ + H_2O = NH_3 + H_3O^+$.

2.6 Source of ammonia in culture ponds

Ammonia enters the culture ponds through excretion from the cultured animals. High density of cultured for the long term will produce more ammonia and dangerous to the prawn cultured (Wurts, 2000). Besides, ammonia also comes from the uneaten food. Food that has not eaten by the prawn will produced ammonia through the decaying process. Debris on the bottom of pond that has undergone decomposition process would contribute to the increasing of ammonia level in water column (Le Moullac and Haffner, 2000). In an intensive culture system, ammonia is released into water primarily from ammonification of organic matter such as unconsumed feed and dead organisms, and from deamination and transamination through the digestion and assimilation of ingested feed by cultured animals (Chen and Lin, 1992).

2.7 Effects of ammonia in aquaculture

In an aqueous solution ammonia is present in the ionized (NH_4^+) and un-ionized (NH_3) forms; the sum of both constitutes the total ammonia ($NH_4^+ + NH_3$). The proportion of each in aqueous ammonia is dependent upon the pH and, to a lesser degree, upon temperature and salinity (Antonio and Wilson, 1995). Un-ionized ammonia is the more toxic form, because it is a neutral molecule and thus is able to diffuse across the epithelial membranes of aquatic organisms much more readily than the charged ammonium ion (Cavalli et al., 2000). The effects of ionized ammonia are considerably less pronounced (Yu and Hirayama, 1986).

Ammonia is unique among regulated pollutants because it is an endogenously produced toxicant that organisms have developed various strategies to excrete, which is in large part by passive diffusion of unionized ammonia from the gills (Floyd and

Watson, 2005). Effect of high external un-ionized ammonia concentrations in water to the aquatic animal is, it will reduce or reverse diffusive gradients and cause the buildup of ammonia in gill tissue and blood. Besides, high ammonia level will affect in the structural development, changes in tissues of gills, liver and kidney of aquatic animals. Moreover, ammonia-N, which is the principal end-product of nitrogenous compounds, accumulates in ponds and has a deleterious effect on fish and crustaceans (Colt and Armstrong, 1981).

Exposing to the ammonia in a long time will cause stress and the aquatic animal can be easily exposed to bacterial infection. In an intensive shrimp culture system, ammonia and nitrite increase exponentially over time in grow-out ponds. High concentrations of ammonia may result in retardation of shrimp growth, and in extreme cases can cause mortality (Wajsbrot et al., 1990; Zin and Chu, 1991). Wickin (1976) and Armstrong et al. (1978) said that high concentrations of ammonia reduces growth and in extreme cases, causes mortality of penaeid and freshwater prawns. The effects of ammonia-N on shrimp or other decapods physiological response or immune resistance are relatively well documented (Wajsbrot et al., 1990).

In seawater, penaeid shrimp hypoosmoregulate, and variation of osmoregulatory capacity (OC), which is the difference in osmotic concentration between haemolymph and surrounding water, was studied. It is a nonspecific indicator commonly used for detecting physiological stress, including in *L. stylirostris* (Lignot et al., 2000). Previous work have shown that total proteins concentration, which can serve as a significant source of metabolic energy for crustaceans (Claybrook, 1983), decreased under ammonia stress (Mugnier and Justou, 2004). Liu and Chen (2004) have said that eventually ammonia caused a depression in the immune response of shrimp. Exposure to high ambient ammonia-N concentrations increases the oxygen consumption in penaeid species (Chen and Lin, 1995). A consequence of shrimp being stressed may be a decrease in immune defense and increase susceptibility to pathogens (Le Moullac and Haffner, 2000; Horowitz and Horowitz, 2001).

Elevated concentration of environmental ammonia has been reported to affect growth and molting (Chen and Kou, 1992) oxygen consumption and ammonia excretion (Chen and Lin, 1992). Ammonia has also been reported to affect the immune response of *L.stylirosyris* (Le Moullac and Haffner, 2000) and *M. rosenbergii* (Cheng and Chen, 2001). Another study has shown that ammonia reduced the intermolt period of *P. japonicus* juveniles during the 60 days of exposure (Chen and Lin, 1992). Studied done by Chen and Kou (1992) resulted that shrimp exposed to 5mg/l ammonia-N showed reduction in growth (length). Ammonia had effect reduction in weight more after 40 to 60 days exposure. Rearing *P. japonicus* juveniles in an environment containing 5 mg/l of ammonia-N (0.35 mg/l of NH_3-N) would significantly affect growth after 50 days of exposure (Chen and Kou, 1992). This paper provides information on the toxicity of ammonia to the rice field prawns in the laboratory.

CHAPTER 3

MATERIALS AND METHOD

3.1 Experimental design

The study was conducted in the laboratory of University Malaysia Terengganu. The experiment was conducted by two stages of experiments. Different concentrations of ammonia were tested on the *Macrobrachium lanchesteri* which is contain of 0.5ppm, 1ppm, 10ppm, 20ppm, 30ppm, 50ppm, 100ppm and one test without ammonia that is used as control. Each test for concentration of ammonia was doing by three replicates. The shrimps were sampled from Tilapia's pond at Maras, Terengganu.

3.2 Glassware cleaning and preparation

All materials and apparatus that were needed to use for the experiments were cleaned especially the plastic cups that were used for experiments. The plastic cup was immersed in the water for few hours before used for the study.

3.3 Preparation of freshwater

Freshwater provided from UMT's Laboratory. The source of freshwater was taken from the tap water and dechlorinated by aerated it in a tank for a day. The parameter of water is 8±1mg.l^{-1} dissolve oxygen, water temperature 27.5±1°C and pH 8±1 mg.l^{-1}.

3.4 Maintained the shrimps

The shrimps were sampled from the pond and transported to the laboratory for maintain in a tank for three days. The size of sampled shrimps were collected uniformly with the range is about 0.24±0.13g. Tank for maintained was filled with freshwater and aerated before inserting the shrimps. The shrimps were fed with catfish pellet and formulated feed during three days.

3.5 Preparing test solution

Concentration of ammonia was prepared by using ammonia solution (Merck GR Grade). Before that, the concentration of ammonia in each g/L was calculated using formula below:

Weight of ammonia solution in 1L : 0.91 kg/L

Concentration of ammonia : 25%

So, the concentration of ammonia after dilution is:

= 0.91kg x 25% x 1000

= 227.5 g/L

The ammonia was prepared under fume hood by diluting 1ml of ammonia solution into 999ml of distilled water. The ammonia was diluting into the freshwater by using the formula; $M_1V_1=M_2V_2$. Ammonia was prepared in a 300ml plastic cup by using the formula to know how many volume of ammonia that should be inserting in the cup according to the determined concentration before placing the shrimps into the plastic cup.

3.6 Acute toxicity test

The experiment to establish tolerance limits were conducted in a 300 ml plastic cup. Each cup was placed with one shrimp to avoid from cannibalism. The study was done by three replicate which ten shrimps in one replicate in four days (96 h). Short term toxicity test were carried out for 0.5ppm, 1ppm, 10ppm, 20ppm, 30ppm, 50ppm, and 100ppm concentration of ammonia. One experiment was carried out for control. Shrimps were sampled randomly from the stocking and exposed to test and control solutions in duplicate plastic cup. During the experiments, the shrimps were not fed.

Temperature, DO, and pH are maintained and check everyday by using YSI meter. Each test solution was renewed daily to prevent from deteriorating water quality since the feces and ammonia secretions will cause increasing of ammonia level. During this period, growth and survival rate of shrimps were observed every day. The observation was done every morning at 10.00am. Died organisms were taken out. The data will be recorded for each died organism at every level of ammonia concentration. Death was assumed when shrimps were immobile and showed no response when touched with a glass rod. During the experiment, water temperature, pH and dissolve oxygen average 27.5±1°C, 8±1, and 8±1.

3.7 Pre- exposure test of ammonia

Pre-exposure experiment were conducted by maintain the shrimps in 0 mg/l (control), 0.5 mg/l and 1.0 mg/l ammonia concentration for seven days. The shrimps were fed with catfish pellet two times per day. Uneaten feed were remove by siphoning it everyday at noon when water was renewed. The maintained shrimps were exposed to the higher concentration of ammonia. Each shrimp was placed in 20 mg/l ammonia for 4 days (96-hours) in the second stage. The data were recorded for each died organism at every level of ammonia concentration. Death of shrimp in the post-exposure test was assumed when shrimp were immobile and showed no response when touched with a glass rod. During the experiment, water temperature, pH and dissolve oxygen average 27.5±1 °C, 8±1, and 8±1.

3.8 Statistical analysis

3.8.1 Acute toxicity test

For the 96-hour acute toxicity test, the data were analyzed with Microsoft Excel by using scatter graph to show the relationship between concentration of ammonia and mortality rate of shrimps. The median concentration of death (LC_{50}) was the concentration at which 50% of animals have died. Log concentration ammonia graph was analyzed to show the relationship with mortality of *Macrobrachium lanchesteri.*

3.8.2 Pre-exposure test of ammonia

The data were analyzed by using paired t-Test to compare the survival of shrimps in control condition between the prawns in pre-exposure test. Data from the two different pre-exposure of ammonia (1.0 $mg.l^{-1}$, 0.5 $mg.l^{-1}$) and data collected from control test were analyzed to compare means between the pre-exposure and post-exposure test. Result from control test was compare with the survival rate in the 0.5 $mg.l^{-1}$ and 1.0 $mg.l^{-1}$.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Acute toxicity test

All shrimps exposed to 30 mg.l^{-1} ammonia died after 72 h, all shrimps exposed to 50mg.l^{-1} and 100 mg.l^{-1} were died within 24 h in ammonia. No test shrimp died in control solution and 0.5 mg.l^{-1} ammonia after 96 h exposure and only one shrimp died in 1 mg.l^{-1} solution after 48 h exposure. Survival of shrimps exposed to different concentrations of ammonia for 96-hours is shown in Table 4.1.

The result from ammonia acute toxicity test of shrimps is shown in the Figure 4.1. Mortality of *Macrobrachium lanchesteri* increase with the ammonia concentration in acute toxicity test after 96 hour exposure. Graph plotted was done to determine the 96-h LC_{50} value. The 96-h LC_{50} value for *Macrobrachium lanchesterii* is 13.5mg.l^{-1}. The LC_{50} value plotted graph was shown in Figure 4.2. The mortality of the shrimps exposed to each test solution had a linear relationship with log ammonia after 24, 48, 72, and 96 h. The shrimps have shown progressive decrease in tolerance to ammonia concentration. As the concentration increase, the survival rate of the *Macrobrachium lanche*sterii decreases. The relationship of mortality with log ammonia concentration has shown in Figure 4.3. These results suggest that ammonia is toxic to *Macrobrachium lanchesteri* at high concentration.

4.2 Pre-exposure test of ammonia

For the second stage, the pre-exposure experiment was conducted for three concentration including control test and was exposed to higher concentration for post-exposure at 20mg.l^{-1} after 7 days pre-exposure. The results of paired T-Test performed with the different between the control with 0.5mg.l^{-1} and 1.0 mg.l^{-1} are significant and their 95% confidence limits for ammonia for 24 h are summarized in Table 4.3. Survival rate of shrimps in the control test compared is higher than the shrimps that have already exposed to the 0.5 mgl^{-1} and 1.0mgl^{-1} after all the three different concentration of ammonia was exposed to higher concentration, 20mgl^{-1} ammonia. This is because the shrimps in the 0.5 mgl^{-1} and 1.0 mgl^{-1} become weak due to the pre-exposure of ammonia compared to the shrimps that in control which is not exposed to the ammonia yet. Exposed to the small amount of ammonia can stressed the shrimps.

Table 4.1: Number of survived *Macrobrachium lanchesteri* exposed to various concentration of ammonia for 96 hours.

Ammonia (mg.l^{-1})	**Time elapsed (day)**				
	0	**1**	**2**	**3**	**4**
Control	30	30	30	30	30
0.5	30	30	30	30	30
1	30	30	29	29	29
10	30	30	30	26	21
20	30	25	15	13	5
30	30	22	6	4	0
50	30	0	0	0	0
100	30	0	0	0	0

*Water temperature 27.5°C and pH 8.5

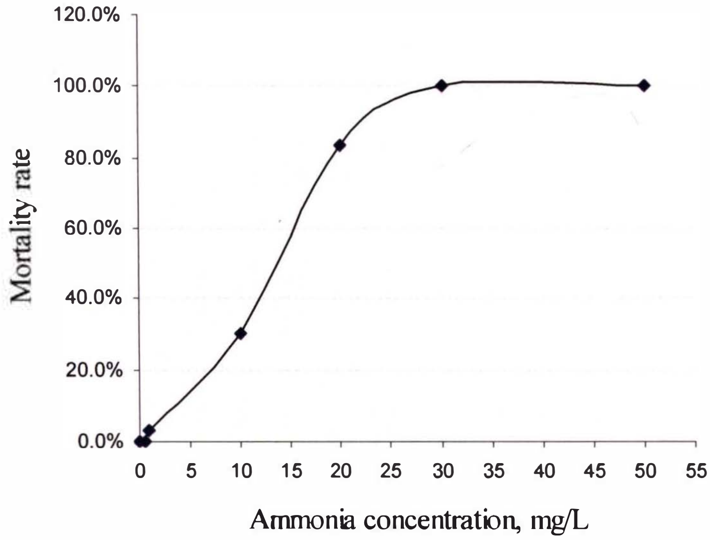

Figure 4.1: Acute toxicity test. Percentage of mortality rate of rice field prawn exposing to various concentration of ammonia in 96 h.

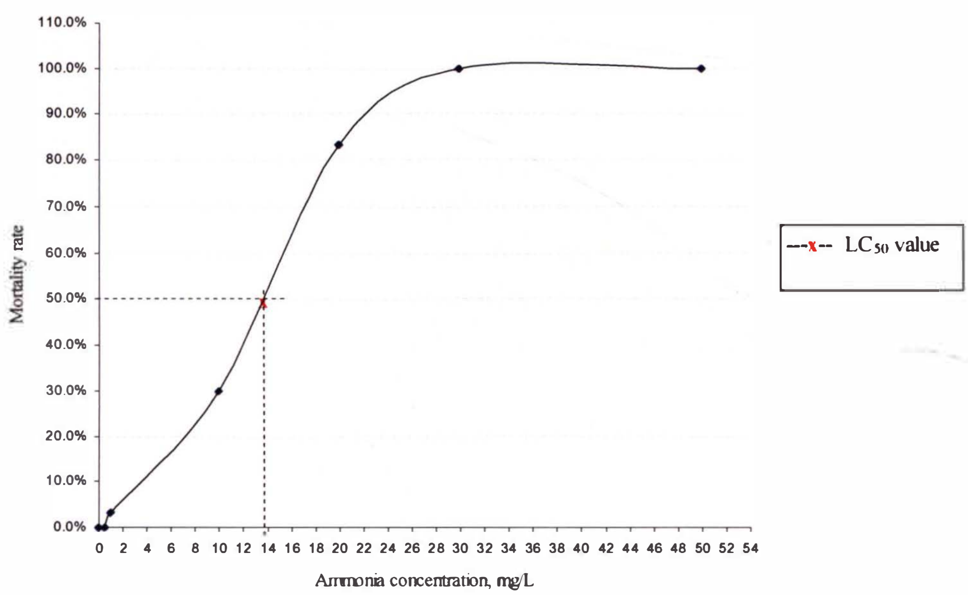

Figure 4.2: Graph plotted 96 h LC_{50} value (13.5 $mg.l^{-1}$)

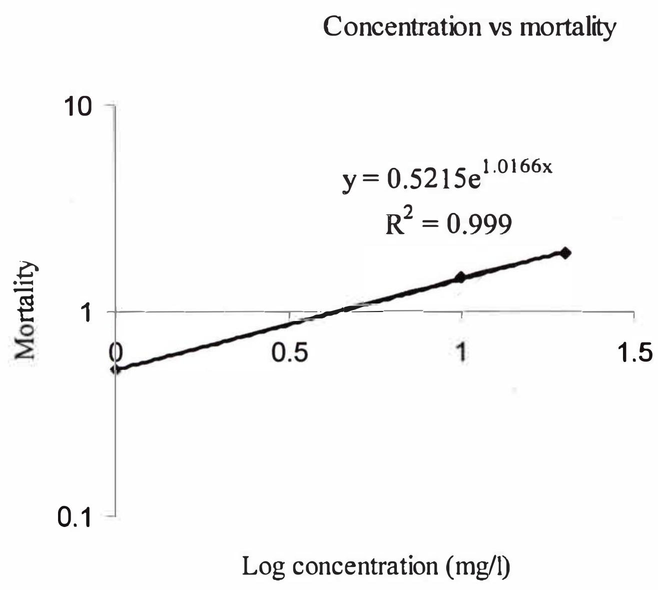

Figure 4.3: Relationship between mortality and log ammonia concentration ($mg.l^{-1}$) exposed for 96 h

Table 4.2: Number of survived shrimps after exposed to higher concentration ($20mg.l^{-1}$) for 96 h

	Time elapsed (hours)				
Ammonia (mgl^{-1})	**0**	**24**	**48**	**72**	**96**
Control	30	12	0	0	0
0.5	30	5	0	0	0
1.0	30	3	0	0	0

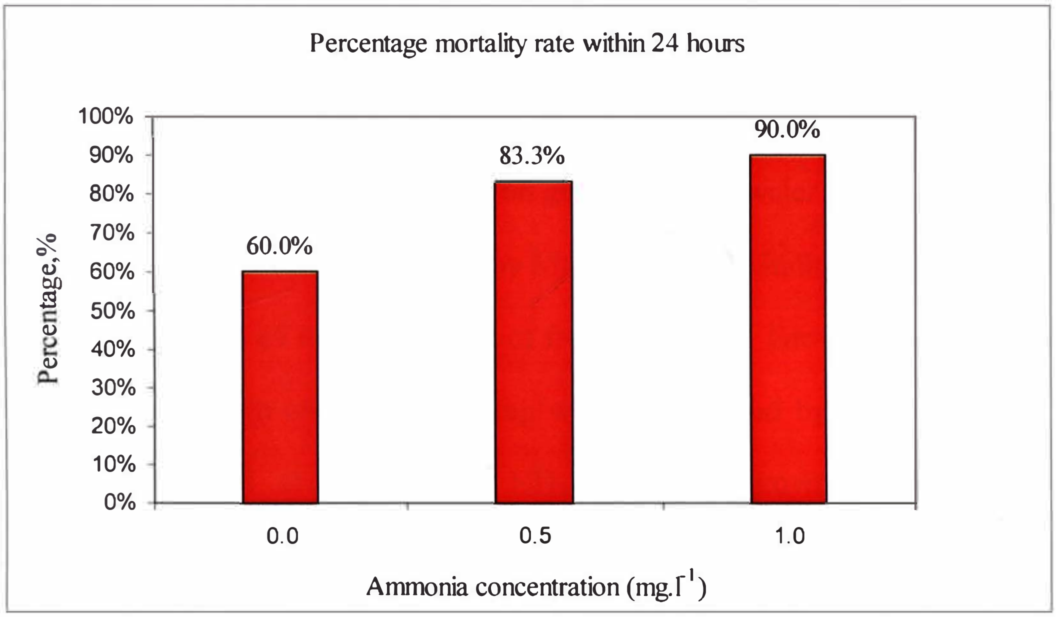

Figure 4.4: Percentage of mortile shrimps in 20 mg.l^{-1} ammonia within 24 hours from difference concentration

Table 4.3.Paired Samples T-Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Control - 0.5mg/l	2.33	4.041	2.333	-7.71	12.37	1.000	2	.423
Pair 2	Control - 1.0mg/l	3.00	5.196	3.000	-9.91	15.91	1.000	2	.423

Pre-exposure and post-exposure test has shown the significant between the concentrations. Shrimps that live in control test more resist than pre-exposure test. This is because shrimps that had exposed did not resist to ammonia. The shrimps became weak due to stress caused by the ammonia. High concentration of ammonia causes increased gill ventilation or hyperplasia and then death. Hyperplasia can blocking the uptake of oxygen efficiently by gill and can cause mortality (Campagna and Cech, 2006).

Data on the effect of ammonia on growth of freshwater fish had been reported by several investigators and reviewed by Muir (1982) who indicates that level of NH_3-N from 0.01 to 1.0 g/l reduces growth of freshwater fish. Growth effect of ammonia on freshwater prawn and paneid shrimp were documented by Wickins (1976) who reported that level above 0.1mg.l^{-1} NH_3-N reduced growth of *Macrobrachium rosenbergii* to 60%-70% of that the control in a salinity of 0.5-4ppt for 6 weeks of exposure. Colt and Armstrong (1981) have reported that the 96-h LC_{50} value for crustacean exposed to NH_3-N ranged from 0.4 mg.l^{-1} to 2.31 mg.l^{-1}.

Ammonia toxicity test on *M. lanchesteri* has not been done yet by researcher. There is similar study that has done on *Penaeus monodon* larval stage. The study deal with the effect of ammonia at different pH levels on the larval. The results indicate that at ammonia concentration of 0-6 mg.l^{-1}, *P.monodon* can survive and develop at pH levels of 7.5-8.0. This study considers pH and ammonia concentration on larval compare to this study that considers the concentration of ammonia towards adult prawn. Adult prawn can survive within 0-10 mg.l^{-1} of ammonia within 96 h compared to the study by Hamid, (1994) that has indicate that the larvae can survive at 0-6 mg.l^{-1} which is the concentration is lower than *Macrobrachium lanchesteri*. The difference between these two studies is by using different stage of prawn and influencing of pH.

Acute toxicity test of ammonia that has done to larvae of *Penaeus monodon* by Chin and Chen (1987) revealed a progressive increase in tolerance to ammonia-N which is LC_{50} value decrease with increasing duration of exposure as the larval develop. Nauplii had the lowest tolerance to ammonia among the four larval stages and the ratio of 24h LC_{50} to 96h LC_{50} of post larvae was 4.53:1. For mysis and postlarvae, the data collected from Chin and Chen (1987) study revealed that the decrease in LC_{50} value with increasing duration of exposure. Post larvae (PL6) were all dead after 6 h in 256 $mg.l^{-1}$, 72 h in 128$mg.l^{-1}$, and 96 h in 64 $mg.l^{-1}$ ammonia-N. A study had been done on fresh water prawn, *Macrobrachium rosenbergii* about the effect of ambient nitrite concentration on larval development by Mallasen and Valenti (2006). The study had shown that increasing ambient nitrite up to 16 $mg.l^{-1}$ delays larval development, reduce larval growth rate and cause mortality. Most of studies on effect of ammonia were done combine with another parameter such as pH, DO, and salinity. Table 4.4 showed the LC_{50} value of ammonia on the shrimp at different life stage.

Table 4.4: The 96 h LC_{50} value of ammonia for several species of shrimps

Species	**LC_{50}**	**Stage**	**Study**
Macrobrachium lanchesteri	13.5 $mg.l^{-1}$	Adult	Present study
Macrobrachium rosenbergii	*2.18 $mg.l^{-1}$	Postlarvae	Straus *et al.* (2007)
Penaeus monodon	11.51 $mg.l^{-1}$	Postlarvae	Chin and Chen (1987)
	1.69 $mg.l^{-1}$	Juvenile	Allan *et al.* (1990)
Penaeus penicillatus	3.25 $mg.l^{-1}$	Juvenile	Chen and Lin (1991)
Penaeus paulensis	1.11 $mg. l^{-1}$	Adult	Antonio and Wilson (1995)
Penaeus semisulcatus	1.43 $mg. l^{-1}$	Juvenile	Wajsbrot *et. al.* (1990)

*Indicator for 72 h LC_{50}

According to the test that had been done to the shrimps and prawns, *Macrobrachium lanchesteri* had the highest tolerance toward the ammonia concentration which is the LC_{50} is 13.5 $mg.l^{-1}$. *Macrobrachium lanchesteri* live in water body that is still or slow moving water and always appear in the pond culture with another cultured species as trash species. Many of the study had been done on the juvenile and postlarvae stage of shrimp species. From the study, there are the differences between the life stage and species that may be influenced the resistant of species toward ammonia. Some of the studies also indicate pH and DO in the experiment.

CHAPTER 5

CONCLUSION AND SUGGESTION

5.1 Conclusion

In conclusion, the result of this study shown that the LC_{50} of *Macrobrachium lanchesteri* is 13.5mg.l^{-1} which is higher than other species. Increasing in ammonia level will cause stress and mortality. Safety level for *Macrobrachium lanchesteri* is below 1.35 mg.l^{-1}.

5.2 Suggestion

It is suggested that further research be undertaken not merely on its biology but also on relevant economic and sociological factors before it can conclusively be decided what use, if any, can be made of this riceland prawn. The important study about water quality should be done to *Macrobrachium lanchesteri* in order to make this species as one of the cultured species in ponds due to its commercial value potential. Thus, will help in promoting new culture species.

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APPENDIX A

Raw data on the number of mortile shrimps for the 96-h LC_{50}

Control
(start on 28/11/08 - 1/12/08)

	Number of mortile shrimps			
Day (hour)	Replicate 1	Replicate 2	Replicate 3	Date
24	0	0	0	28/11/08
48	0	0	0	29/11/08
72	0	0	0	30/11/08
96	0	0	0	1/12/08
Mortality rate	**0%**	**0%**	**0%**	

Survival rate **100%**
Mortality rate **0%**

0.5 ppm
(start on 28/11/08 - 1/12/08)

	Number of mortile shrimps			
Day (hour)	Replicate 1	Replicate 2	Replicate 3	Date
24	0	0	0	28/11/08
48	0	0	0	29/11/08
72	0	0	0	30/11/08
96	0	0	0	1/12/08
Mortality rate	**0%**	**0%**	**0%**	

Survival rate **100%**
Mortality rate **0%**

1.0 ppm
(start on 28/11/08 - 1/12/08)

	Number of mortile shrimps			
Day (hour)	Replicate 1	Replicate 2	Replicate 3	Date
24	0	0	0	28/11/08
48	1	0	0	29/11/08
72	0	0	0	30/11/08
96	0	0	0	1/12/08
Mortality rate	**10%**	**0%**	**0%**	

Survival rate **96.7%**
Mortality rate **3.3%**

10 ppm
(start on 27/12/08 - 30/12/08)

	Number of mortile shrimps			
Day (hour)	Replicate 1	Replicate 2	Replicate 3	Date
24	0	0	0	27/12/08
48	0	0	0	28/12/08
72	2	2	0	29/12/08
96	1	1	3	30/12/08
Mortality rate	**30%**	**30%**	**30%**	

Survival rate **70%**
Mortality rate **30%**

20 ppm
(start on 31/12/08 - 03/01/09)

	Number of mortile shrimps			
Day (hour)	Replicate 1	Replicate 2	Replicate 3	Date
24	2	1	2	31/12/09
48	5	2	3	1/1/09
72	0	1	1	2/1/09
96	3	3	2	3/1/09
Mortality rate	**100%**	**70%**	**80%**	

Survival rate **16.7%**
Mortality rate **83.3%**

30 ppm
(start on 31/12/08 - 03/12/08)

	Number of mortile prawns			
Day (hour)	Replicate 1	Replicate 2	Replicate 3	Date
24	3	2	3	31/12/09
48	4	5	7	1/1/09
72	1	1	-	2/1/09
96	2	2	-	3/1/09
Mortality rate	**100%**	**100%**	**100%**	

Survival rate **0%**
Mortality rate **100%**

50 ppm
(start on 27/12/08 - 30/12/08)

	Number of mortile prawns			
Day (hour)	Replicate 1	Replicate 2	Replicate 3	Date
24	4	5	3	27/12/08
48	6	5	7	28/12/08
72	-	-	-	29/12/08
96	-	-	-	30/12/08
Mortality rate	**100%**	**100%**	**100%**	

Survival rate	**0%**
Mortality rate	**100%**

100 ppm
(start on 14/12/08 - 17/12/08)

	Number of mortile prawns			
Day (hour)	Replicate 1	Replicate 2	Replicate 3	Date
24	10	10	10	14/12/08
48	-	-	-	15/12/08
72	-	-	-	16/12/08
96	-	-	-	17/12/08
Mortality rate	**100%**	**100%**	**100%**	

Survival rate	**0%**
Mortality rate	**100%**

APPENDIX B

Raw data on pre-exposure test. Mortile prawns in $20mg.l^{-1}$ ammonia within 24 hours from difference concentration.

concentration, mg/l	replicate 1	replicate 2	replicate 3	total %
0.0	6	5	7	60.0%
0.5	9	8	8	83.3%
1.0	9	9	9	90.0%

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