Effect of Mercury Exposure on the Growth performance of Giant Freshwater Prawn, *Macrobrachium rosenbergii* Post-Larvae

By

ZURIKA FARHANIS BINTI MOHD ASRI

A report submitted in fulfilment of the requirements for the degree of Bachelor of Applied Science (Animal Husbandry Science) with Honours

Faculty of Agro Based Industry

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DECLARATION

I hereby declare that the work embodied in this report is the result of the original research and has not been submitted for a higher degree to any universities or institutions.

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Student

Name: Zurika Farhanis Binti Mohd Asri

Date:

I certify that the report of this final year project entitled Effect of Mercury Exposure on the Growth Performance of Giant Freshwater Prawn, *Macrobrachium Rosenbergii* Post-Larvae by ZURIKA FARHANIS BINTI MOHD ASRI, matric number F14A0422 has been examined and all the correction recommended by examiners have been done for the degree of Bachelor of Applied Science (Animal Husbandry Science) with Honours, Faculty of Agro Based Industry, Universiti Malaysia Kelantan.

Approved by:

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Supervisor

Name:

Date:

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A study on the Growth Performance of Giant Freshwater Prawn,

***Macrobrachium rosenbergii* Post-Larvae due to Mercury Exposure**

ABSTRACT

The farming of freshwater prawn species, *Macrobrachium rosenbergii* has become one of the most important sectors in Malaysia. However, as human population has been increased, the human activity also increased, thus spreading the heavy metal in the nature. Therefore, the heavy metal contamination has been introduced into aquatic systems which have become the subject of universal concern. Aquatic species such as fish, prawns, and shrimps may accumulate large amounts of metals in their body. Hence this project was aim to identify the effect of mercury on the growth performance of *M. rosenbergii* PL using different concentrations of mercury. In the present study, four treatments were used which are 0.01 ppb, 0.03 ppb and 0.05 ppb and each treatment was replicated three times. The uniform length and weight of *M. rosenbergii* PL were used in the present study are from 1.48 cm to 1.52 cm and from 0.48 g up to 0.52 g. Effects of mercury exposure on the survivability after 96 hours and growth rates were determined. The highest survival rate was from the concentration of 0.01 ppb. The growth performance of the PL was measured for four weeks. And the results shows a significant difference ($p<0.05$) for all treatments groups. Finding from this study provide additional knowledge on the effect of freshwater prawns species against heavy metal contaminants.

Keywords: *Macrobrachium rosenbegiii*, survivability, mercury, growth rates

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Kajian terhadap Perkembangan Pertumbuhan Udang Galah Air Tawar,

***Macrobrachium rosenbergii* Pasca Larva akibat daripada Pendedahan Merkuri**

ABSTRAK

Pada masa kini penternakan udang galah, *Macrobrachium rosenbergii* telah menjadi salah satu sektor yang penting dalam perkembangan ekonomi negara. Walau bagaimanapun, kebelakangan ini, akibat daripada peningkatan populasi telah menyebabkan pelbagai jenis aktiviti harian manusia dijalankan, dan menyebabkan penyebaran logam berat kepada alam sekitar. Dan berikutan itu, pelbagai jenis logam berat telah didedahkan ke dalam sistem akuatik yang mana telah menjadi perkara yang perlu di ambil berat. Pelbagai jenis hidupan akuatik seperti ikan dan udang boleh mengumpul sejumlah besar logam berat di dalam otot badan mereka. Oleh itu, projek ini dijalankan untuk mengenal pasti kesan daripada merkuri terhadap prestasi pertumbuhan udang air tawar pasca-larva, *M. rosenbergii* dengan mengunakan pelbagai jenis kepekatan merkuri yang berlainan. Semasa kajian ini dijalankan, terdapat 4 jenis rawatan yang digunakan iaitu kumpulan untuk mengawal, 0.01 ppb, 0.03 ppb dan 0.05 ppb dan setiap satu rawatan mempunyai tiga replika. Panjang dan berat udang *M. rosenbergii* pasca larva yang digunakan di dalam kajian adalah berukuran 1.48 cm hingga 1.53 cm dan berat daripada 0.48 g hinga ke 0.52 g. Kesan daripada pendedahan merkuri terhadap kadar kelansungan hidup selama 96jam dan perkembagan pertumbuhan Pasca larva (PL) dikenal pasti. Kadar peratusan tertinggi kelansungan hidup telah ditunjukkan oleh rawatan 0.01 ppb. Dan perkembangan pertumbuhan PL diukur selama empat minggu. Hasil melalui kajian ini telah menunjukkan kadar pembesaran pasca-larva menunjukkan bahawa terdapat perbezaan yang signifikan ($p<0.05$) untuk setiap jenis rawatan yang digunakan. Penemuan daripada kajian ini memberikan pengetahuan tambahan tentang kesan terhadap spesis udang air tawar terhadap pencemaran logam berat.

Kata kunci: *Macrobrachium rosenbergii,* kelansungan hidup, merkuri, kadar pertumbuhan

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LIST OF ABBREVIATION AND SYMBOLS

PL	Post-Larvae
LC50	Lethal Concentration 50%
Ppb	Part per Billion
Ppm	Part per Million
mg/L	Milligram per Liter
ml	Milliliter
g	Gram
cm	centimetre
h	hour
%	Percentage
°C	Degree Celsius

CHAPTER 1

INTRODUCTION

1.1 Research Background

The industry of farming giant freshwater prawn, *Macrobrachium rosenbergii* is developing and could potentially boost the income of the indigent and poor farmers. However, the production of the Malaysian giant freshwater prawn, *M. rosenbergii* vigorously was affected by the heavy metals as heavy metals pollution has been a popular issue in aquaculture industry. Heavy metals contamination in the nature is resultant from agricultural, manufacturing industrial, pharmaceutical, and also from domestic effluent. The environment pollution is also noticeable in the point sources for example smelters, mining, foundries and other metal based industries. Although heavy metals are nature elements which can be found throughout the crust of earth, however environment pollution also can occur through the sediment re-suspension, metal oxidization, and soil erosion of metal ions and leakage of heavy metals, atmospheric deposition, and also metal evaporation from the water sources to soil and ground water. Moreover, natural phenomena such as volcanic eruptions and weathering also have been reported to be significantly contribution to heavy metals pollutions. And due to the pollutants from several sources mentioned, it will give an impact on aquatic organisms and thus posing a threat on aquatic species as they constitutes as one of protein sources for mankind.

Akbulut & Tuncer (2011) discussed the term of heavy metals such as some metals for example potassium, calcium; sodium and magnesium are not generally referred as heavy metals. The heavy metals usually referred to mercury, lead,

copper, cadmium, nickel, cobalt, zinc, selenium and manganese. The most hazardous metals are mercury, lead and cadmium.

In recent years, there are supreme courtesy has been compensated to the heavy metals toxicants effect in aquaculture systems. (Rainbow & White, 1989) stated that there are parameters that can be used to measure the effects of exposure to toxics metals. Therefore, the aim of the present study was to investigate the effects of heavy metal of mercury on *M. rosenbergii* Post-larvae. Finding from this study will provide additional knowledge on the effect of freshwater prawn's species against heavy metal contaminants.

1.2 Problem Statement

In 2016, Department of Fisheries Malaysia reported that aquaculture's value in Malaysia was recorded at USD 12.42 million where the production of *M. rosenbergii* devotes USD 3.47 million. One of the strategies to increase more production of *M. rosenbergii is* by increase the production of prawn's seeds. However, the seed production of *M. rosenbergii* has become a major restriction due to the quality of broodstock and prawn seed. Among other factors, water quality was identified to have significant effect on the prawn production. Heavy metals were also acknowledged as a major problem that reduces the water quality thus contributed to many problems on the aquatic species. Hence the present study proposed to investigate the effects of mercury on *M. rosenbergii* PL.

1.3 Research Hypothesis

The growth performances of *M. rosenbergii* will shows a significant difference between the study groups.

1.4 Research Objectives

The objectives of the present study are:

1. To determine the survivability of the *M. rosenbergii* PL towards heavy metal mercury exposure.
2. To study the effects of mercury on the growth performance *M. rosenbergii* PL.

1.5 Research Scope

In this study, *M. rosenbergii* was introduced with three different concentrations of mercury. The survivals of PL were measured and their growth performances were measured.

CHAPTER 2

LITERATURE REVIEW

2.1 General Characteristics of *M. rosenbergii*

M. rosenbergii is known as a prawn where their second pair of legs able to support the name of genus that significance to 'large arms'. Generally, the body form of *M. rosenbergii* is in the form of decapod crustacean where the thorax and head attached together to cephalothorax. (Coyle *et al.,* 2003) justify the rostrum at front of the cephalothorax of the prawn prominently comes within 11-14 dorsal teeth and 8-10 of ventral teeth. There are distinct physical characteristics to differentiate between the males and females. In male prawns, there walking legs will showed a deep colour of blue and the size of leg are twice of the body length. (Holthuis, 1967) recorded the largest males obtain a total length which from rostrum until the end of the telson which is recorded at 320 mm compared to largest female which is 250 mm.

Another feature of male *M. rosenbergii* is the finger of second pair of walking legs or known as cheliped are covered with packed long setae which will gives a greasy appearance of the appendage. Males also will attain bigger size than female and the dominant second pair of walking legs is more lengthy and wider. As for the abdomen, the male have narrower abdomen compared to female where it is much wider and has longer pleura which is the cuticle is perpetual from the exoskeleton and these will join together to produce the chamber which the function is to transport the eggs on the pleopods during the incubation period. The male's genital opening is located at the fifth walking legs while the female's genital is positioned at the third legs.

2.2 Taxonomy Hierarchy of Giant Freshwater Prawn, (*Macrobrachium rosenbergii*)

The giant freshwater prawn belongs to the family of Palamonidae and the genus of Macrobrachium. The taxonomy hierarchy of the genus is as stated below.

Kingdom: Animalia

Phylum: Arthropoda

Subphylum: Crustacea

Class: Malacostraca

Order: Decapoda

Sub-order: Pleocyemata

Infraorder: Caridea

Superfamily: Palaemonodae

Family: Palaemonidae

Subfamily: Palaemonidae

Genus: Macrobrachium

Species: *Macrobrachium rosenbergii*

2.3 Water quality for rearing *M. rosenberjii*

Hashmi *et al* (2002) stated “The route of biological amplification, in which a chemical upsurges in concentration in the bodies of organisms, with subsequent trophic levels (through food chains), increases the properties of metals in the body. The main aspect in aquaculture’s sustainability is water quality. Usually water contamination was often attributed by the poor growth, extensive scale mortality in

hatcheries and grows out farm and anatomical aberrations. The marine shrimp aquaculture is the most species that is facing the peril from water contamination. This is a very likely a serious problem as prawns are a very great values seafood assets where the demands from consumer far exceed for the supply. (Ahsanullah *et al.,* 1981) documented the sensitivity of crustaceans to heavy metals and it is important to study the sensitivity of aquatic organisms to toxicants because by studying that, the effect of heavy metals on the life stage, moulting stage and reproduction cycle can be determined. (McGee *et al.,* 2004) stated even small quantities of immersed trace metals are either deposited in a metabolically accessible form for essential biochemical progressions or detoxified into metabolically inert forms or detained in the body either for the short term or perpetually.

2.4 Common heavy metals from Industrial Activities

Conventionally most prawns' culture farms are found nearby the coastline area and to rearing the prawns without using additional process, the fresh waters is unswervingly used. However, due to human activities at the shoreline area, the seawater was regularly polluted by the various types of waste product and human pathogen (Chua, 1992). Therefore there are some risks such as the dangerous adverse effect on humans and as well the direct lethal effects on the cultured organism by using direct natural coastal seawater. To reduce the influences of heavy metals toxicants on aquaculture, ecologies and human who ingest these products, numerous study have been carried out to discover and also for the direct monitoring of scattering of heavy metals in bay areas, coastline waters and also to investigate the effect of heavy metals toxicants on the tissue of aquatic animals (Paez-Osuna & Tron-Mayen, 1996). In addition, some research also enthusiastic to the prawn's performance of certain parameters as a marker which will reflects the presence and the quantity of heavy metals (Kille *et al.,* 1992)

Cadmium (Cd) is a metal that is used numerous times for industrial applications. On top of that, cadmium is also a by-product of zinc and lead mining and smelting (Faustman & Omenn, 1997). Due to all the applications can raise the possibility for the Cd to barge in into the environment in a very unusual ways and later cause adverse effects to human and ecosystems. (Hidalgo *et al.,*1985) carried out studies of special effects of Cd on countless aquatic organism but only limited studies are handy on prawns which one of the important aquaculture species as toxicants on prawns can cause the adverse effects on consumer and even worse these adverse effects being spread out universally through import and exportation.

Zinc (Zn) is an universal and a very essential metal that played as a factor as the co-factor in over than 200 metalloenzymes and is an efficient element of transcription factor proteins subsidising to the gene expression and regulation (Faustman & Omenn, 1997). Besides that, zinc also plays an essential part in the immune and nervous systems in the optimal metabolism of vitamin A. For aquatic crustaceans like *L.vannamei*, removal of zinc supplements can produces a very significant depression in the tissue mineralization (Davis *et al.,* 1992).

2.5 Type of heavy metals

Heavy metal for example Cadmium, (Cd) is biologically non-essential elements and perceptibly lethal even it is at low concentrations. Only a few metals with a verified hazardous nature are truly omitted in diet for human consumption while some elements such as iodine, copper, manganese and iron are essential for normal body functions. The study from the laboratory and field experiments showed that the accretion of heavy metals in tissue essentially reliant on upon the water concentration of metals and exposure period even though the other factors for example as pH, dissolved oxygen, temperature and salinity play a significant roles in the metals accumulation.

Cadmium is produced as the result of by-product of smelting, mining and refining of zinc and to a lesser degree and also as a by-product of copper and lead production. Cadmium production has almost doubled between the year of 1950 and 1960. However, since the year of 1990 the global consumption has remained persistent which are about 20,000 tons per year. In the body, cadmium is not an element that is used and it is lethal. It can affect kidneys and bones and also carcinogen by the inhalation. In the environment, cadmium is toxic to animals, micro-organism and plant. Being a simple element, cadmium is tenacious. It means, in the environment it cannot be broken down into less toxic substances.

Mercury (Hg) is a volatile element that comes with a very long half-life. Therefore, as a result, it is ubiquitous in the environment and poses a threat to human health. Nowadays, research showed that even the exposure on low concentration of mercury can cause the kidney impairment and long lasting neurological. Mercury is hazardous to aquatic and human. This is because when mercury is dumped in the lakes and streams, the natural bacteria actions will converts it to become methyl mercury which then makes mercury accessible to essence in the fish's tissue, nature and the people who consume aquatic organisms. Flora and fauna that are consistently exposed to Hg are possibly affected in diversity if environmental and human health impacts. The exposure to mercury can results in the long term period for health effects especially in reproduction process.

2.6 Heavy metals and its effects on environment

Generally, aquatic organisms mount up impurities from the surrounding and this manner have been broadly utilized as a part of marine contamination observing programmes((Murk & Koeman, 2016) in many countries, due to developments in the industrial which lead to a spike discharge of the dangerous chemical effluents into the

ecology that can be the factor of the marine damage habitats. The discharged of the heavy metals can destruct the natural ecosystem and the aquatic species due to their accumulative behaviour and (Matta *et al.,* 1999). Normally the nursery and spawning farm of the marine species usually positioned at the aquatic areas and estuarine, hence the aquatic organisms are specifically influenced by such influx of compound contaminations into the aquatic ecosystem (Gibson, 1994). The amassing patterns of toxins in fish and other aquatic organisms depend both on their uptake and removal toxins rates. The different part of the body will be accumulate the heavy metals at the different intensities (Olowu *et al.,* 2010). Under specific conditions,, heavy metals might be accumulated up to lethal concentrations and finally can cause ecological damaged. Thus, heavy metals that is consume through food chain are considering as a potential chemical hazards that is peril to consumers. Therefore, it is essential to check the contamination of chemicals in foods that is acquired from aquaculture industry to figure out their threat levels.

2.7 Types of Mercury, Hg

At ambient temperatures and pressures, Mercury, Hg is a liquid metal. There are two types of mercury in the ionic state that can form salts. And they are known as mercury (I) and mercury (II). Compared to Mercury (I) which is identified as mercurous salts, Mercury (II) or better known as mercuric salts are more typical in the atmosphere. And if these salts are soluble in the water, they are bioavailable and can be considered toxic and lethal. Besides form in the liquid metal, mercury also can be in the forms of organometallic compounds which they are used by agricultural industry and manufacturing sector. Element mercury contributes improvement to the vapour that is soluble in water but they are considered challenging due to easy transference in the air. Insoluble mercuric sulphide that is known as most common form is non-toxic (Du & Niu, 2003).

There are two cycles that is believed in the environmental transportation and mercury spreading. One of them is in world-wide in scope and it is implicates with the atmospheric circulation of elemental mercury vapour from the sources of land to ocean. While the other one, is in local scope where it depends on the methylation of inorganic mercury primarily from anthropogenic sources. Even though the steps from this cycle is can't be understood clearly however it is likely involves with the atmospheric circulation of dimethyl mercury that is moulded by the action of bacteria. Hence, the environmental level of mercury actually depends on balance interaction of bacterial methylation and demethylation.

2.8 Effect of heavy metals on prawn central nervous system

Murk & Koeman (2016) emphasized the induced by the pollutants is the outcome of compound interference or its metabolites with the biochemical circumstance convoluted with the homeostatic control of a physiological progression. Physiological process is frequently integrated with hormones. The changes in the hormones levels can cause the effect on normal balance of the physiological events. After the exposure to a pollutant, the changes in the hormone levels can be expected. Therefore, the hormone change and followed by the biosentinal parameters can be acknowledged by observing for the modifications in the endocrine patterns (Fingerman *et al.,* 1996). The neurohemal organ from epithelial endocrine gland and neuroendocrine structures in the eyestalks of higher crustacean are present. The basic plan of crustacean nervous is the cerebral ganglia, double ventral nerve cord and circumenteric that are connected by commissures. (Chase *et al.,* 2001) issued the series of journals on the accumulation of heavy metals from the adulterated water of the Berettyo River in Eastern Hungary in the central nervous system of the crayfish, *Astacus leptodactylus* where it shows an intense amendment in the hormone levels can dismayed the normal balance of physiological events. And more recently (Chase *et al.,* 2001) described the effect of necrotic from cadmium, lead, and

mercury that had been amassed in the central nervous system of the crayfish, *Astacus leptodactylus*. And the changes from the main neurotic modifications were the disorganisation of the mitochondrial, the development and the breakdown of the Golgi vesicles that is abnormal, the endoplasmic reticulum fragmentation and also the nuclear pycnosis.

The acute exposure produced an increase in the amount of deposited neurosectory material and reduction of the neurosectory cells. Nevertheless, with the chronic exposure the depletion of the neurosectory material and peripheral vacuolization arisen (Orenzon *et al.,* 2001). Mercury, cadmium and lead which accumulate in the brain and also inhibit the sulfhydryl group-containing enzymes (Mandal, 2004). Methyl mercury also seems to owe its high toxicity to the fact that the compound is complete to be passes to the cell membranes by using passive diffusion and once it is inside the cell, it will wields its toxic action. There is also an indication that the inorganic mercury can intermingles with the cell membrane's phospholipids to form the stable complexes. And these complexes can do the impairment or modified the physical properties of the cell membrane and can lead to cell dysfunction (Ribeiro *et al.,* 2016). Inside the cell such heavy metals, in addition to inhibiting enzymes are identified to modify the function of mitochondria and delay the mitosis. The effect on the mitosis is because of the interruption pf the mitotic spindle. With the respect to cell membrane itself, heavy metals are alleged to inhibit the enzyme's bound membrane such as Na+/K + ATPase and can cause the disruption of the polyunsaturated lipids which are present in the membrane. And lead to the production of the superoxide anion radicals and then later converted to hydroxyl radicals, the substances that is known to be lethal to cells.

2.9 Mercury in environment

One of the most toxic elements in our environment is mercury. According to (Barbieri *et al.,* 2010), mercury occur in the trace of soil, atmosphere, water and organisms in many forms. The environment's concentration is reported that the mercury occurred from a few of Nano gram to more than the 7mg/1 (Minh *et al,* 2010). The ocean also has been reported to contain more than 108metric tons of mercury which the most common reason for it is because of the natural weathering. And 20,000metric tons of mercury was produced widely in 2000, where approximately 35% of it was processed in United State of America. And about 12000 tons of mercury was released in to the environment worldwide.

According to (Frı & Voltolina, 2001), a 5 ppb(parts per billion) of mercury is limited on drinking water. And usually surface waters are less than 0.1 ppb. Even many epidemiologic studies has been carried out, however due to insufficient information of the biological effects of mercury has avert the establishing adequate of water quality standards. Thus, the long term effects of mercury toxicant less than 1 ppb should be resolute. (Lal, 2004) reported that there are more 1000 cases of mercury poisons on humans has been reported. And some of the mercury poisoning occurred there are some cases with death have been reported. These cases are endorsed to contamination of seafood. Aquatic organism, for example fish, shrimp, and prawns are identified to distillate the mercury due to their direct uptake through the trophic levels. (McGee *et al.,* 2004) reports mercury concentrations in the aquatic organisms are more 1000 times greater than the seawater. Mercury retention and its toxicity is greater than before when inorganic mercury in transformed into the organic form by the organisms. There are many type of organisms can transformed inorganic mercury into organic mercury compounds. The conversions do occur anaerobically

but more effective in aerobic environment. (Kaushik *et al,* 1998) has also shown that mercury do inhibit methane formation. In man, the biological half-life of mercury can reach up to 70 days and up to 36-95 days for the inorganic mercury. In fish, the biological half-life of methyl mercury for fish is 200 days. Mercury toxicity is reliant on the chemical environment whether it is inorganic or organic mercury. Mercury toxicity also hinges on its own reaction with the essential sulfhydryl groups. Even at a small level of mercury, the enzyme can occur to meddling in the cellular malfunction. The inorganic mercury's action befalls in two phases which one is rapid and the other one is slow.

The rapid phase has a half-life with only a few minutes with achievement are not less than one half hour. In rapid phase, mercury is easily to eliminate by using various complexing agents. In the slow phase, it occurs with higher concentration of mercury and the biding is vital long-lasting. The low binding seems to affect the cellular structure and not the enzyme properties. Mercury has been recognised to interfere with the respiration, potassium metabolism and other cellular processes. The methylation of mercury always benevolences a more stern peril to biosphere than the inorganic form. In sediment water, methyl mercury content is less than 1 % of the total mercury concentration and the aeration encouraged the release of fundamental mercury from the sediment water system.

3.0 Acute and Chronic Effects of Mercury Exposure

Previous studies have reported acute and chronic effects of mercury exposure on the freshwater prawns *M. rosenbergii*. Toxicity normally increased both with the treatment concentration and length of exposure (Gebhardt, 1976). As Mercury is mould to the cell walls or cell membranes of microorganisms, the side effect of

mercury can be expected associated with the both cell density and mercury's concentration in the solution. (Thongra-ar et al., 2003) studied the 96 h LC50 values for freshwater prawns and indicate that aquatic organisms are demethylase or methylate mercury. Some research also stated that organic mercury also lethal so birds. As mercury vapour has a very extensive atmospheric residence period and mercury contamination seems organically universal, mercury contamination is always regarded as a universal problem that confronts state or nation. Mercury ascends from earth crust degassing that come through volcano and also by evaporation of the sea (Tao Yuan et al., 2012). The indication from atmospheric pollutants that is from industrial production has been diminished over the years but however water pollutants by pit tailings still remains the same.

Mercury that emits smelting lead, zinc and copper to atmosphere has been estimated been emits annually at 5,000 to 8, 0000 metric tons globally and Southeast Asia and East has been reported emitted about 40% of the global anthropogenic emissions and is reported that about 75% mercury emitted from this region comes from China (Koz, 2010). Mercury also been produced by the burning of fossil fuels. Industrial supplements such as paint, electric equipment, chloralkali, and wood mashing are the biggest patrons of mercury. Nowadays, mercury also has been widely used in agriculture primarily used as fungicides. Other than that, mercury also has been used in military equipment, medicines, batteries and dentists. Even though, recent years industrial of mercury has been abridged due to stricter regulations however there are still higher concentration of mercury that is still existent in residues still linked with the industrial use of mercury.

CHAPTER 3

MATERIALS & METHODS

3.1 *M. rosenbergii* PL samples collection.

A total of 360 prawn samples of *M. rosenbergii* post larvae (PL) size about 0.5 g were used in the study. PL samples were obtained from Pusat Peternakan Udang Galah (PPUG), located in Setiawan, Perak. During transportation, 1/3 of durable transparent plastic bags were filled up with water from original post-larvae ponds where the temperature was in range of 28-31 °C, pH within 7.0-8.5 mg/L, dissolved water at 3-7 ppb and salinity less than 10 while 2/3 of that plastic bag was filled with oxygen

3.2 Experimental Design

Uniform length of PL which is from 1.48 cm to 1.53 cm with weight from 0.48 g to 0.52 g was used in the study and they were divided into the aquariums accordingly. The entire PL was transferred into the aquariums with gentle care. This is because to minimize the chances of any injury or stress on PL (Hoang *et al.,* 2002) The entire PL was transferred Prawn samples were divided into three groups based on the concentration of heavy metals. Prawn samples without additional of mercury was used as control group. Each group was replicated three times. Then, second group was exposed to mercury concentration that is below than the optimum range which is 0.01 ppb. The next group was exposed to mercury concentration that is within the optimum range, 0.03 ppb while the last group that is was exposed to mercury concentration which is higher than the optimum range, 0.05 ppb.

3.3 Preparation of heavy metal solution

10 ml from 1000 ppm mercury were used in this study. By using serial dilution technique, 10 ml of mercury was exactly measured and was transfer into the

volumetric flask. 190 ml of distilled water were added into the volumetric flask to make 200 ml of the solution. The flask was shaken thoroughly so that all the solution was mixed well. After that, the 1800 ml of water was added into the volumetric flask so that the solution becomes 5 ppm with 2000 ml of solution. To make 2 ppm, 800 ml of the 5 ppm solution were taken and was placed into the volumetric flask and 1200 of distilled water were added and the flask was shaken well. Next to prepare 2 ppm to become 0.1 ppm, 750 ml of 2 ppm were placed into the volumetric flask and 1425 ml of distilled water were added. Therefore, to prepare 0.05 ppb, 750 ml of 0.1 ppm were placed into volumetric flask and 750 ml of water was added. And to prepared 0.03 ppb, 900 ml of 0.05 ppb were placed into the volumetric flask and 600 of distilled water were added. The solution was shaken thoroughly until they mixed well. And to prepared 0.01 ppb, 500 ml of 0.03 ppb were placed into the volumetric flask and 1000 ml of distilled water was added. The solution also was shaken thoroughly.

3.4 Preparation of the prawn samples

The cleaning of the aquarium tanks (12cm x18cm x12cm) were carried out using standard protocol. Later, the anti-chlorine was used to neutralize harmful chlorine from tap water. The aquariums also were prepared with filtration to clean and to keep healthy water. The aquariums also were prepared with aeration that functions to oxygenate and circulates water. Lastly, the substrates also were provided for hideaways or cover for prawns.

Water quality such as temperature, salinity and dissolved oxygen were measured daily by using YSI ProDSS handheld multiparameter water quality system by following protocol of American Public Health Association (APHA). *M. rosenbergii* PL were acclimated for three days. PL were fed commercial pellet two times a day.

3.4.1 Toxicity

The acute toxicity was observed for 96 h and they were fed two times a day. The toxicity and the survival rate were calculated and recorded.

3.4.2 Growth rates

Growth of PL was measured by using Relative growth Rate method for 30 days until PL reached juvenile stage. All the length of PL was measured from day 1 until day 30 after the exposure on mercury concentration. The growth performance of PL was recorded once a week. Relative growth rate (RGR) was calculated by followed as (García-Trejo *et al.,* 2016) and the formula given as;

$$\mathrm{RGR}(\%) = \frac{(\mathrm{Wf} - \mathrm{Wi})\mathrm{x}100}{\mathrm{Wi}}$$

Where,

Wf = Final average weight at the end of the experiment.

Wi = Initial average weight at the beginning of the experiment.

3.5 Data analysis

All the data was measured and calculated and also statistically analysed by using ANOVA (Analysis of Variance) by using Statistical package for the social sciences software version 23.0 to understand the significant difference in growth performance of *M. rosenbergii PL* with different mercury concentration.

CHAPTER 4

RESULT

4.1 Acute toxicity

Figure 4.1 shows the survival ability of PL to survive in the mercury exposure. The survival rate shows a decrease in the treatment by day except for control group. From the graph, 0.01 ppb shows a higher survival rate compared to others treatments.

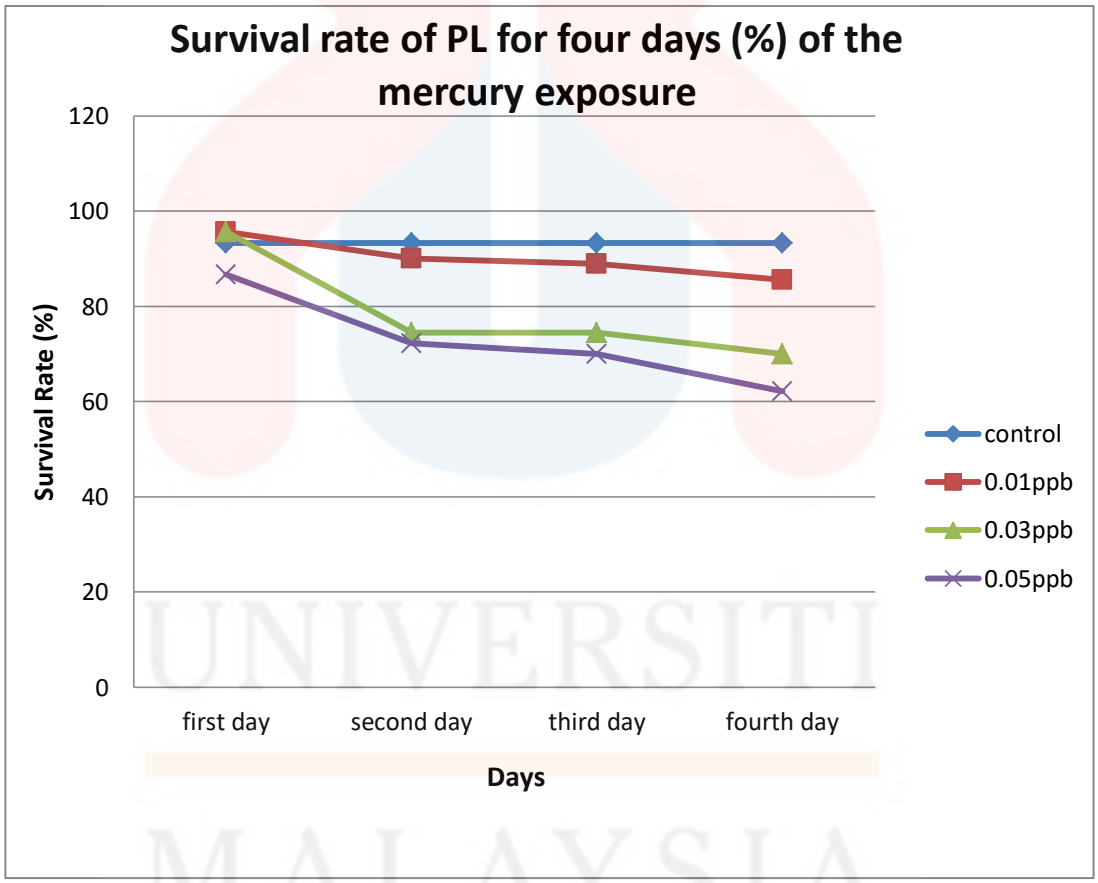

Figure 4.1 Survival rate of PL after four days of treatment of the mercury exposure. 0.01 ppb shows the highest survival rate (%) compared to other treatments.

Figure 4.1.1 shows the statistical analysis of the survival rate of the PL after the 96 h of exposure of mercury concentration. And it is showed that a significant difference is shown by the control group with 0.05 ppb. Besides that, 0.01 ppb also showed a significant difference with the 0.05 ppb group.

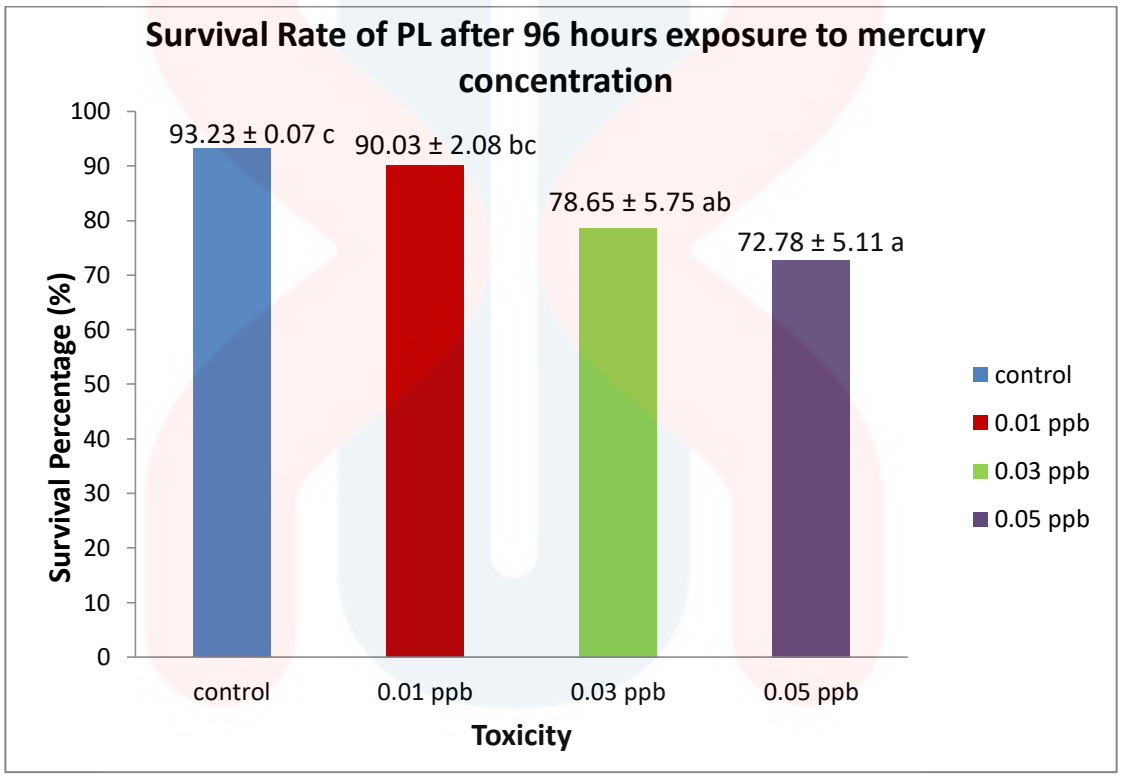

Figure 4.1.1 showed that toxicity has a significant difference ($p<0.05$) impact of the survival of PL. Survival percentage of PL on the treatment 0.01 ppb found to be higher.

4.2 Effect of mercury exposure on length of PL

Figure 4.2 shows the average of length of PL for five weeks. For the following five weeks, it can be seen that the highest length of PL was shown by the 0.01 ppb which is 2.53 cm. As illustrated by graph, the average of length of PL steadily increase for the past five weeks except for the fourth week, where 0.05 ppb showed slowly increase from 1.75 cm to 1.86 cm.

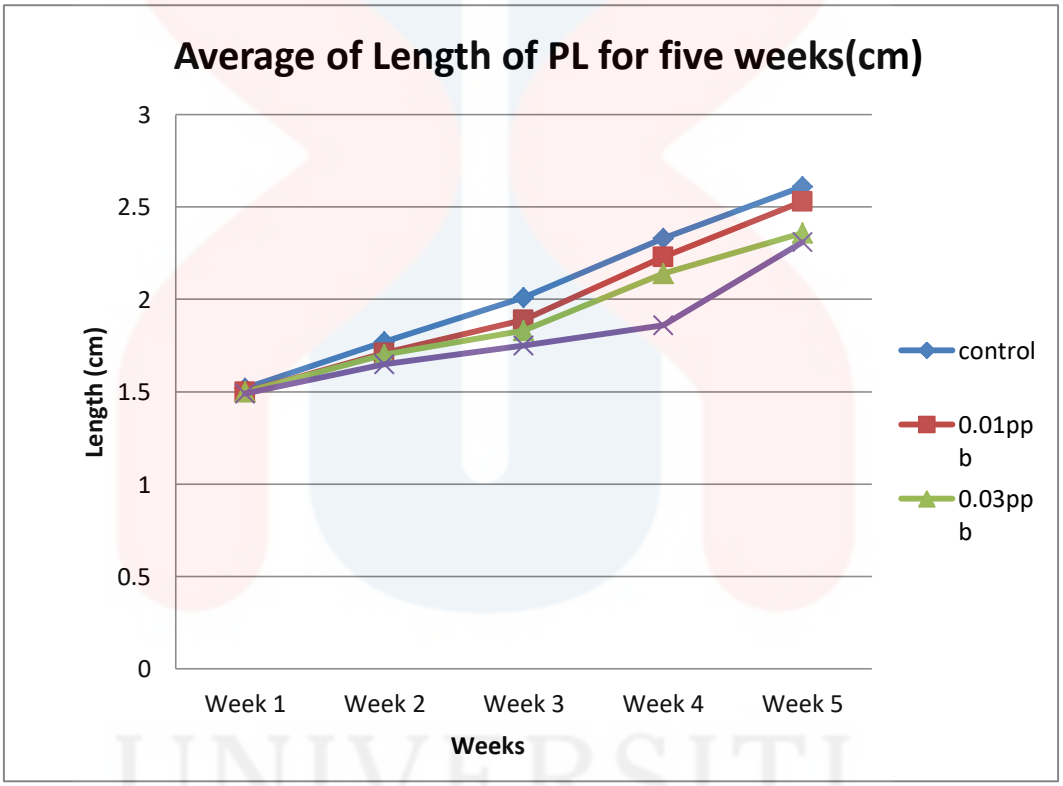

Figure 4.2 Average of Length of PL for 5 weeks for each treatment (cm). It is shown that 0.01 ppb has the highest average of length compared to 0.03 ppb and 0.05 ppb.

4.3 Effect of mercury exposure on weight of PL

Figure 4.3 shows the average of weight of PL for five weeks. The largest rise is shown by the 0.01 ppb treatment which is 1.44 g. Over the five weeks, all the treatments show an increment as the weeks increased. At week 2, 0.01 ppb showed an increased greatly from 0.53 g to 0.92 g. And however, increase slowly from week 3 to week 4 from 1.07 g to 1.19 g. On the other hand, the lowest average of weight is shown by the 0.05 ppb.

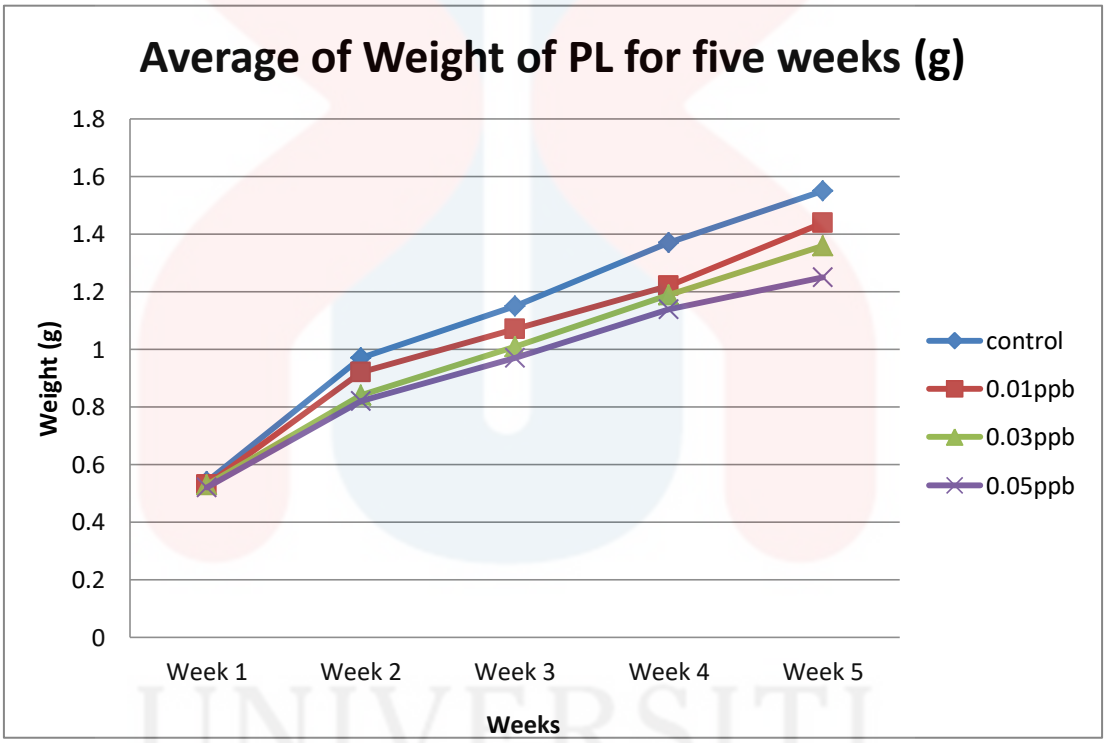

Figure 4.3 Average of Weight of PL for 5 weeks for each treatment (g). From the graph, it is shown that every concentration shown an increment every weeks and the highest weight is shown by 0.01 ppb.

4.4 Relative Growth Rate (%)

Table 4.4 shows the RGR of the PL after one month. From the table, it is shown that the best Relative Growth Rate is shown by the 0.01 ppb treatment where the Relative Growth Rate is the highest compared to others. The lowest Relative Growth Rate is belongs to 0.05 ppb where the length and the weight is 55.03 % and 140.38 % which is smallest among the groups.

Table 4.4 shows the Relative Growth Rate (%) of the study groups. The highest RGR is shown by the 0.01 ppb treatment.

Treatments	Relative Growth Rate (%)	
	Length	Weight
Control	71.71	187.03
0.01 ppb	68.67	171.70
0.03 ppb	57.33	156.60
0.05 ppb	55.03	140.38

CHAPTER 5

DISCUSSIONS

5.1 Concentration of mercury for toxicity study

Mercury can be considered as highly toxic heavy metal to all living organisms and the toxicity is because it inhibits the active transport mechanisms through the dissipation of the normal cation gradient and destroys the mitochondrial apparatus. And also due to inhibition of a high variety of enzyme system (Thongra-ar *et al.,* 2003). Therefore, it is important to understand the effect of mercury exposure on PL growth performance. In the present study, the effect of mercury concentration shows a significant difference ($p>0.05$). The reason for this result is because the mercury concentrations tested are still within the optimum range of toxicity. According to (Sivaperuma *et al.,* 2007) , all mercury ≤ 0.056 ppb, no lethal effect occurred within the 96h. This explains why there is not 100% mortality when acute toxicity experiments on PL were carried out. Besides that, as the experiment were carried out by using static rather than using the continuous flow methods which cause the loss of mercury during the experiment. This is because, one of the problems with mercury is due to its own rapidly tendency to be lost from the solution through the naturally reducing agents that are present in the water. Hence throughout the acute toxicity, it is actually essential to continuously supply mercury in the solution to avoid any mercury loss.

Based on the result, it shows that the effect of mercury exposure was not obvious at lower mercury concentration where after 96 h of exposure and we can see the toxicity effect at highly toxic concentration, 0.05 ppb. (Sivaperumal *et al.,* 2007) stated low mercury exposure increase the survival rate. The heavy metal accumulate

to high levels in tissue thus cause the death or organisms (Glicksl, 1998) . this is because as during the proses ecdysis, PL use water to upsurge their size which resulting the hydration in their tissue and increase in the blood volume. This may cause the lethal effect that decrease the survival rate of PL. As Hg is considerably toxic to all living organisms, we can expect were the survival rate from high level of mercury concentration, 0.05 ppb were low compared to the other concentrations as high level of merucry concentration mean higher level of toxicity. The toxicity from the mercury was because it constrains active transport mechanisms through the dissipation of the normal cation gradient thus detroys mitochondrial apparatus.

It is also because of the inhibtion of the high variety of enzyme system which is with thiol and ligands (Ah, 1992). The impact of aquatic ecosystems from the toxicants promotes an essential concern about the discharged that is from manufactures and chemicals into the river ecosystems as side effect of the manufacturing society. This is an auxiliary exaggerated by the facts that the toxicants residues released into the ecosystems may substantially alter their physical and chemical structure (Renzoni *et al.,* 1998). From the present study, even though 0.05ppb was a high level of mercury concentration however the survival rate was still above 50%. This maybe because of the relatively high resistant due to the ability of the PL to withdraw their bodies into shells thereby reduce the dissemination of the mercury into the soft part of the PL.

5.2 Effect Mercury Exposure on the *M. rosenbergii* Post Larvae

Mercury concentration was proven to be highly toxic to PL which can inhibit their growth performance. The present study shows that growth performance of PL was significantly affected in mercury concentration. As we can see from the result Table 4.2 and Table 4.3, body weight and body length of PL at 0.03 ppb and 0.05 ppb were relatively smaller than 0.01 ppb. In this study, the effect of mercury concentration shows significant different ($p<0.05$) of growth performance. According to (Nadu, 2013), effects to the mercury exposure was influenced by the relative growth rate of the species. A significant decrease of length as well as the reduction of the weight after the exposure to even lower concentrations 0.01 ppb also was observed in the study of Blue Crayfish, *Procambarus alleni* by (Sarabia *et al.,* 1998) . (Hansen *et al.,* 2002) reported a slow rate of the growth is an effect of heavy metal action in marine organisms. The action of inhibitory regarding towards growth which exposed to higher mercury concentration of PL that is observed in present study also could be due to the effect of food intake and assimilation. This is because due to mercury exposed, it can decreased the propensity of the food intake and assimilation which later lead to decreasing growth performance in PL.

James *et al* (1992), observed the declination of feed conversion rate in marine organisms at sub- lethal level of heavy metals might be due to the tissue burden of more heavy metals which leading to increasing the metabolic cost. (Payle *et al.,* 2005) studied the effect of cadmium, mercury and lead on the growth parameters and concluded that, heavy metal exposures tainted the quality of health quality of marine organisms correlating with the result of the present study. (Hogstrand *et al.,* 1995) showed normal growth processes could be divert from the increased metabolic demand. Due to heavy metal exposure, it also could affect the swimming stamina which indicates a reduction available energy to detoxification process for survival.

Aquatic organisms require movement which is exceedingly ecology relevant with their behaviour marker as movement is needed to find food and find substrate to avoid predator.

Wright (1995) reported that Hg may also be confiscated within the cell membranes and react profligate with the sulfhydryl group. As growth was found to be more sensitive measure from the adverse effect of Hg, the inhibitory effect of Hg on growth performance of PL was probably due to the impact of Hg on prey hunting capability. The decreasing in feeding also may be due to the loss of the coordination and earlier satiation of starvation from neurotoxic effect of Hg. Mercury also inhibits the intestinal absorption of nutrient such as sugars and amino acid in PL which also may cause the fall in growth. Sub lethal of the concentration of Hg also can affect the ion transport and osmoregulatory function in aquatic organisms by the inhibition if Na+/K+ ATP ase activity. According to (Manahan, 1992) the earlier stage of aquatic organisms are more sensitive to metal toxicity compared to older stages.

The exposure of heavy metals pollutions can also effected the health of aquatic organism may involve with ecological death where when the animals have inability to function in the ecological, leading to death. In the aquatic toxicology, toxicants can give directly affect aquatic organisms but does not kill it instantly. Instead of that, the toxicants impaired the aquatic organisms' standard ecological function and behaviour which then will lead to death or lack of defence system (Scott and Sloman, 2004). This is because the aquatic organism requires defence and repair mechanisms that are hinged on the energy requiring process for example synthetic activity and active transport. Therefore, confronting stress is likely to be energetically costly for the stressed organisms (Smolders *et al.,* 2005). As pollutants are unavoidably eternally immobilized in residues establishing a possible threat and are proficient of exerting considerable biological effects due to their ubiquity and

perseverance nature even they are low levels. In marine organisms, the accumulation of heavy metals for a more extended timeframe can eventually prompt death thereby jeopardizing the biodiversity of aquatic biota. The present study clearly shows that even such short term exposure to mercury concentration at 96 h could have significant effect on PL, with the rigorousness of the effect mostly reliant upon the mercury concentration.

CHAPTER 6

CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

The outcome from the present study showed a significant effect on the growth performance of PL. Concentration of mercury was contrary the growth performance. Similarly, mercury concentration was also inversed with the survivability rate. The highly toxic mercury concentration which caused the highest mortality and the slowest growth rate is shown by the 0.05 ppb. Hence the objectives of the study which were aims to investigate the toxicity of mercury and the effect of the mercury exposure on the PL were achieved.

6.2 RECOMMENDATION

As for the recommendation, effort to limit the mercury exposure and other heavy metals contamination into the aquatic system should be taken into consideration as it can effect on the aquatic organisms performance. According to this study, it is affirmed that PL that has been exposed to mercury concentration showed a slower growth rate performance and also shows that mercury exposure can cause mortality on PL. Therefore, the finding from this study can provide additional knowledge on the effect of freshwater prawns species against heavy metal contaminants.

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APPENDICES

Table A.1: The Survivability of PL throughout 4 days of mercury exposure

Treatments	Survival of PL throughout 4 days exposure to mercury concentration (%)			
	R1	R2	R3	Average
Control				
First day	96.7	93.3	90.0	93.3
Second day	96.7	93.3	90.0	93.3
Third day	96.7	93.3	90.0	93.3
Fourth day	96.7	93.3	90.0	93.3
0.01 ppb				
First day	96.7	96.7	93.3	95.6
Second day	93.3	96.7	80.0	90.0
Third day	90.0	96.7	80.0	88.9
Fourth day	86.7	90.0	80.0	85.6
0.03 ppb				
First day	96.7	93.3	96.7	95.6
Second day	76.7	70.0	76.7	74.5
Third day	76.7	70.0	76.7	74.5
Fourth day	73.3	63.3	73.3	70.0
0.05 ppb				
First day	93.3	76.7	90.0	86.7
Second day	93.3	53.3	70.0	72.2
Third day	90.0	50.0	70.0	70.0
Fourth day	83.3	40.0	63.3	62.2

Table A.2: The Significant Difference of Survivability of PL after 96h

Survivability of PL throughout 4 days exposure to mercury concentration (%)	
Treatment	Mean survival ± SE (%)
Control	93.23 ± 0.07 c
0.01ppb	90.03 ± 2.08 bc
0.03ppb	78.65 ± 5.75 ab
0.05ppb	72.78 ± 5.11 a

Table A.3: Analysis of Variance (ANOVA) on the toxicity of PL for 96h of exposure

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1102.342	3	367.447	5.784	.011
Within Groups	762.332	12	63.528		
Total	1864.674	15			

Table A.4: Multiple comparisons of Post Hoc Test for survival *M. rosenbergii* Post-larvae

					95% Confidence Interval for Mean	
Treatments	Days	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound
control	4	93.2250	.15000	.07500	92.9863	93.4637
0.01ppb	4	90.0250	4.16043	2.08021	83.4048	96.6452
0.03ppb	4	78.6500	11.49739	5.74870	60.3551	96.9449
0.05ppb	4	72.7750	10.22688	5.11344	56.5017	89.0483
Total	16	83.6688	11.14951	2.78738	77.7276	89.6099

Table A.5: Weight of *M. rosenbergii* Post-larvae for five weeks (g)

Treatments		Weight of PL of one month (g) (%)					
		Week 1	Week 2	Week 3	Week 4	Week 5	Average
Control							
i.	R1	0.53	0.93	1.12	1.33	1.55	1.09
ii.	R2	0.53	0.95	1.15	1.33	1.53	1.10
iii.	R3	0.55	1.02	1.18	1.35	1.58	1.14
0.01ppb							
i.	R1	0.53	0.92	1.08	1.25	1.48	1.05
ii.	R2	0.53	0.93	1.07	1.22	1.45	1.04
iii.	R3	0.52	0.90	1.05	1.20	1.40	1.01
0.03ppb							
i.	R1	0.53	0.85	1.02	1.20	1.38	1.00
ii.	R2	0.53	0.84	1.02	1.18	1.35	0.98
iii.	R3	0.52	0.82	1.00	1.18	1.35	0.97
0.05ppb							
i.	R1	0.52	0.82	1.00	1.15	1.28	0.95

ii.	R2	0.53	0.82	0.97	1.13	1.23	0.94
iii.	R3	0.52	0.82	0.95	1.13	1.25	0.93

Table A.6: The length of *M. rosenbergii* Post-larvae for five weeks (cm)

Treatments		Length of Pl of one month (cm) (%)					
		Week 1	Week 2	Week 3	Week 4	Week 5	Average
Control							
i.	R1	1.50	1.72	1.96	2.30	2.60	2.02
ii.	R2	1.53	1.80	2.03	2.35	2.62	2.07
iii.	R3	1.53	1.80	2.04	2.35	2.62	2.07
0.01ppm							
i.	R1	1.50	1.72	1.92	2.26	2.55	1.99
ii.	R2	1.50	1.70	1.91	2.25	2.53	1.99
iii.	R3	1.50	1.70	1.85	2.18	2.50	1.95
0.03ppm							
i.	R1	1.48	1.67	1.82	2.15	2.38	1.90
ii.	R2	1.52	1.71	1.83	2.14	2.35	1.91
iii.	R3	1.50	1.72	1.83	2.12	2.35	1.90
0.05ppm							
i.	R1	1.50	1.65	1.78	1.90	2.32	1.83
ii.	R2	1.48	1.62	1.74	1.85	2.30	1.80
iii.	R3	1.50	1.67	1.74	1.83	2.30	1.81

Table A.7: Homogenous subsets for weight of *M. rosenbergii* Post-larvae for each treatment

Treatment	N	Subset for alpha = 0.05
control	3	1.1100
0.01 ppb	3	1.0333
0.03 ppb	3	0.9833
0.05 ppb	3	0.9400
Sig.		1.000

Table A.8: Homogenous for subsets of length of *M. rosenbergii* Post-larvae for each treatment

Treatment	N	Subset for alpha = 0.05
Control	3	2.0533
0.01	3	1.9767
0.03	3	1.9033
0.05	3	1.8133
Sig.		1.000

Table A.9: One Way of ANOVA of weight of *M. rosenbergii* Post-larvae

	Sum of Squares	df	Mean Square	F	Sig.
Between groups	.048	3	.016	43.576	.000
Within groups	.003	8	.000		
Total	.051	11			

Table A.10: One Way of ANOVA of length of *M. rosenbergii* Post-larvae

	Sum of Squares	df	Mean Square	F	Sig.
Between groups	.095	3	.032	77.224	.000
Within groups	.003	8	.000		
Total	.098	11			

Table A.11: Multiple Comparisons of Post Hoc test of growth performance of *M. rosenbergii* Post-larvae for each treatment

						95% Confidence Interval for Mean	
		N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound
length	control	3	2.0533	.02887	.01667	1.9816	2.1250
	0.01ppb	3	1.9767	.02309	.01333	1.9193	2.0340
	0.03ppb	3	1.9033	.00577	.00333	1.8890	1.9177
	0.05ppb	3	1.8133	.01528	.00882	1.7754	1.8513
	Total	12	1.9367	.09432	.02723	1.8767	1.9966
weight	control	3	1.1100	.02646	.01528	1.0443	1.1757
	0.01ppb	3	1.0333	.02082	.01202	.9816	1.0850
	0.03ppb	3	.9833	.01528	.00882	.9454	1.0213
	0.05ppb	3	.9400	.01000	.00577	.9152	.9648
	Total	12	1.0167	.06800	.01963	.9735	1.0599

Figure A.12: The preparation of the Aquariums for the experiment

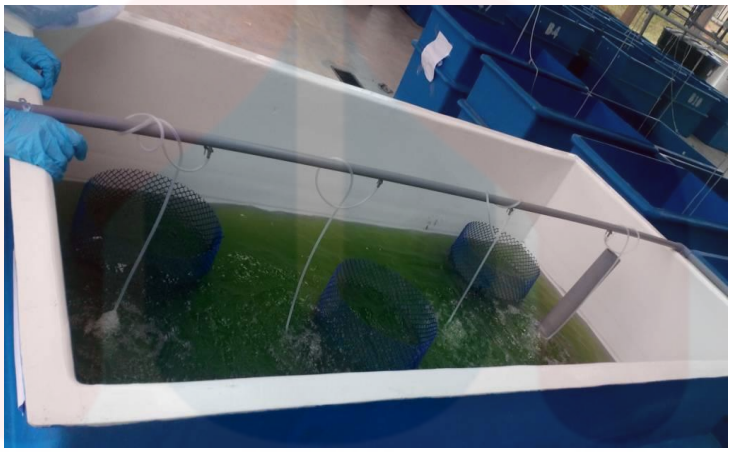

Figure A.13: The PL was placed in the tank

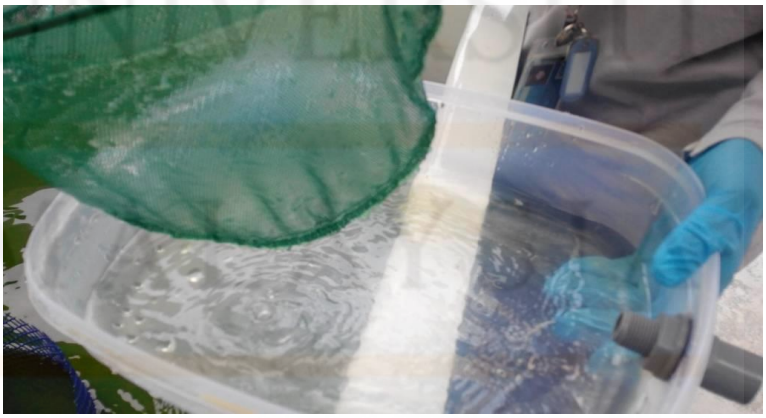

Figure A.14: The PL was transferred from tank to aquariums

Figure A.15: The PL was weighed by using weighing scale

Figure A.16: The experiment were run in Politeknik Jeli laboratory

Figure A.17: The dissolved oxygen, Salinity and pH were checked every day to ensure there is no high ammonia