The Effects of Salinity on the Development of Giant Freshwater Prawn, *Macrobrachium rosenbergii* Larvae

By

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

Faculty of Agro Based Industry

UNIVERSITY MALAYSIA KELANTAN

2018

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THESIS 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 highger degree to any universities or institutions.

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I certify that the report of this final year project entitled " The Effets of Salinity on The Development of Giant Freshwater Prawn, *Macrobrahium rosenbergii* Larvae" by Muhammad Danial Khaleq Bin Mohd Ali, matriks number F14A0149 has been examined and all the correction recommended by examiners have been done for the degree of Bachelor of Applied Sciene (Animal Husbandry Science), Faculty of Agro Based Industry, Universiti Malaysia Kelantan.

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ACKNOWLEDGEMENT

"In the Name of Allah, the Most Gracious and the Most Merciful"

Thanks to Allah, finally, I have succeeded in completing my final year project. First and foremost, I am very thankful and grateful to Allah for giving me the opportunity to complete my project on time. This project cannot be complete successfully without the support from my family, supervisor, aquaculture lectures, husbandry science lectures, friends and others respective help lender.

I would like to thank my pretty supervisor of this project, Dr. Hasnita Bt Che Harun, for all the advice and guidance. She inspired me greatly to work on this project. Her willingness to motivate me gives the best for this project. Also, I would like to thank to student master, Cik Amira and all aquaculture and husbandry science lectures, for the entire valuable information, ideas and support that motivated me to withstand the challenges and finish my final year project.

My deepest gratitude goes to all my family members especially my beloved parents, Mr. Mohd Ali Bin Abdullah and Mrs. Norriha Bt Abd. Rahman, for the endless support, love, encouragement and motivation that enabled me to perform the best in this final year project until I managed to complete my thesis. Without them, I might quit and give up from doing this project.

Last but not least, I want to thank to all my team mates, classmates and friends that always give moral support and help me throughout the process of completing my project. Thanks for all the hardship that all of you went through with me.

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LIST OF ABBREVIATIONS AND SYMBOLS

PPUG	Pusat Penternakan Udang Galah
FAO	Fisheries of Agriculture Organisation
PL	Post Larvae
ppm	Part per million
ppb	Part per billion
Mg/L	Milligram per litter
ºC	Degree Celsius
nm	Nano meter
µg/l	microgram per litre
kW	kilowatt
L	Liter
Vol	Volume
Ppt	Part per thousand
%	Percentage

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The Effects of Salinity on the Development of Giant Freshwater Prawn,

***Macrobrachium rosenbergii* Larvae**

ABSTRACT

Macrobrachium rosenbergii is a giant freshwater prawn and is the largest family in the genus *Palaemonidae.* This species has high demand and high market value. It is also commonly used in aquaculture industry. Eggs *M. rosenbergii* are round shaped with a size of 0.6 mm to 0.7 mm and it is orange colored during lay-out and turn into dark gray 2 or 3 days before hatching. Eggs *M. rosenbergii* will take 1 or 2 nights in the process of hatching with optimum salinity levels to produce larvae. *M. rosenbergii* larvae are planktonic and swimming with his body in reverse. It needs brackish water to survive. The aims of this study were to investigate the optimum water salinity rate for growth *M. rosenbergii* larvae and to observe the larvae development by stage 1 to stage 11. There were 11 stages of *M. rosenbergii* larvae and each stage change was preceded by skin replacing. Each stage has a recognizable characteristic. Stage 1 of larvae is 2 mm long while at stage 11 is 7 mm. This process takes 20 to 40 days. The study was conducted in 15 litter plastic tank (12cmX18cmX12cm) with maintain the temperature at 28 degree celcius (room temperature) and different rate of salinity (8, 10 and 12 ppt). The level of survivality of larvae it was observed and monitored manually. Finding from this study showed that the optimum water salinity in development and resistance for *M. rosenbergii* larvae were 10 ppt. However, the optimum rate of water salinity for larvae survivability was 8-12 ppt. In addition, the optimum salinity needed to be maintaining for the larval development.

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Kesan Kemasinan Air untuk Tumbesaran Rega Udang Gergasi Air Tawar,

Macrobrachium rosenbergii

ABSTRAK

Macrobrachium rosenbergii merupakan udang air tawar gergasi dan merupakan yang terbesar di dalam genus *Palaemonidae*. Spesis ini mendapat permintaan yang tinggi dan nilai pasaran yang tinggi. Ia juga selalu digunakan di dalam industri akuakultur. Telur-telur *M. rosenbergii* berbentuk bulat panjang dengan saiz 0.6 mm ke 0.7 mm dan ia berwarna oren terang sewaktu peneluran dan bertukar menjadi kelabu hitam 2 atau 3 hari sebelum menetas. Telur *M. rosenbergii* akan mengambil masa selama 1 atau 2 malam dalam proses menetas dengan kadar kemasinan optimum untuk menghasilkan rega. Rega-rega *M. rosenbergii* adalah berbentuk planktonic dan berenang kepala dahulu dengan badannya berkeadaan terbalik. Ianya memerlukan air payau untuk hidup. Tujuan kajian ini dijalankan adalah untuk menyiasat kadar kemasinan air yang optimum untuk tumbesaran rega *M. rosenbergii* dan melihat perkembangan peringkat rega dari Peringkat 1 sehingga Peringkat 11. Terdapat 11 peringkat rega *M. rosenbergii* dan pertukaran setiap peringkat didahului dengan menyalin kulit. Setiap peringkat mempunyai ciri khas yang boleh dikenali. Rega di Peringkat 1 berurukuran 2 mm panjang manakala di peringkat 11 pula ianya berurukuran 7 mm. Proses ini mengambil masa dari 20 ke 40 hari. Kajian ini dijalankan di dalam tangki saiz 15 liter (12cmX18cmX12cm) dengan menggunakan suhu yang dikekalkan 28 darjah celcius (suhu bilik) dan kemasinan air yang berbeza (8, 10 dan 12 ppt). Kemudian, di peringkat daya tahan rega diperhatikan dan dipantau secara manual. Hasil daripada kajian ini, menunjukkan bahawa kadar kemasinan air yang optimum untuk tumbesaran dan daya tahan rega *M. rosenbergii* adalah 10 ppt. Walaupun begitu, kadar optimum kemasinan air bagi daya tahan rega adalah 8-12 ppt. Sebagai tambahan, kemasinan optimum perlu dikekalkan untuk peringkat pembentukan rega.

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CHAPTER 1

INTRODUCTION

1.1 Research Background

Macrobrachium rosenbergii is a large freshwater prawn native to the Indo-West Pacific from northwest India to Vietnam, Philippines, New Guinea and northern Australia. It has been introduced into many countries for aquaculture. Adverse impacts have not been reported so if there are effects they have so far not been noticeable. However, impacts of escapes in tropical river systems might not be noticed that readily. There is the potential for viruses to be introduced via aquaculture stock (Peng *et al.,* 1998, Flegel, 2003) and a possible risk of interbreeding with local species. This seems to be a very small risk since it has been demonstrated that in the case of *M. carcinus* and *M. rosenbergii* (Graziani et al., 2003) no successful pairings occurred. When using artificial insemination interspecifically, although zygotes were produced they did not progress past the gastrula stage.

The first recognized output of farmed giant river prawn (*M. rosenbergii*) production recorded in (Food and Agriculture Organization) statistics appeared in 1970. Global production of the prawn in 2009 was 229,419 tonnes, of which 552 tonnes came from Malaysia. Malaysia has a high potential for aquaculture development due to the country's favourable condition in terms of natural habitats such a pond, rivers, lakes, estuaries and coastal areas. Since the beginning of the 2000s, the aquaculture sector in Malaysia has been expanding in terms of culture area, production, targeted species and degree of management intensity. Production based on aquaculture has been increasing rapidly in comparison with that from capture fisheries. Recently, some species, and certain culture system types, have contributed significantly to the growth of the aquaculture sector in terms of production and export values.

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Although, the global production of *M. rosenbergii* in 2007 was over 221,000 tonnes, 2.7 times greater than a decade earlier, the production in Malaysia has decreased almost half in 2011 compared 2010. The limiting factor is said to be the supply of quality juveniles. In addition, many causes contribute to decrease the production of *M. rosenbergii* in Malaysia tremendously for the last decade. Although, the production of freshwater aquaculture system has decreased in 2013 compared to 2012, the production of freshwater prawn has increased again in 2013 compared to 2012.

The physical parameters of the culture conditions are of critical importance for the successful rearing of any type of aquatic organism (Anger, 2001, Maciolek, 1972, Zheng *et al.,* 2008). Two fundamental aspects of the rearing environment for marine crustaceans are those of salinity and temperature; once the optima for these parameters have been established, further work can then be carried out to determine appropriate ranges of other environmental parameters, such as pH, dissolved oxygen concentration, light etc. Most crustacean larvae actively regulate internal ion concentrations by osmo-ionic regulatory processes, and salinity tolerances are often closely related to their ability to maintain internal media largely independent of external conditions (Anger, 2001, Brown *et al.,* 2010).

In *M. rosenbergii* world production, Malaysia is far behind compared to Vietnam, Taiwan and Thailand to become a major producer of giant freshwater world. Contribution to giant freshwater aquaculture industry in Malaysia is also low. For example, in 1995, the industry accounted for only about 0.06% of the value of aquaculture production only. Generally, the species starts and completed its larvae phase in brackish water with salinity range of 6-19 ppt by Ling (1969). Steele, (2001) reported in his study that salinity affect the reproduction and embryonic development. Previous studies by Lee & Wee, (2010) claimed that the water rearing of *M. rosenbergii* starting at PL stage must be maintained at a salinity of 4-6 ppt.

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1.2 Problem Statement

Based on the observation, the population of giant freshwater prawn was endangered. The aquaculture industry of giant freshwater prawn has expanding. By the way, the production and activation the aquaculture industry of giant freshwater prawn is very slow especially at nursery phase in east cost area because of limited brackish water source.

1.3 Research Hypothesis

1) Optimum salinities for development of *M. rosenbergii* larvae have on 8 ppt, 10 ppt and 12 ppt.
2) Different level of salinities will have significant effect on the survavility rate of *M. rosenbergii* larvae.

1.4 Research Scope

The present study will discuss the effects of different salinities on the survival and development of *M. rosenbergii* larvae.

1.5 Significant of the study

This study will provide knowledge on the optimum salinity for the growth and survival of different larvae stages of *M. rosenbergii*.

1.6 Limitation of the study

The present study required cost for transportation to Pusat Penternakan Udang Galah, PPUG in Setiawan, Perak. Expensive to buy *M. rosenbergii* berried female and availability of berried female is only at certain period.

1.7 Research Objective

The aims of this study was to determine the optimum salinity of *M. rosenbergii* larvae development and survivability.

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CHAPTER 2

LITERATURE REVIEW

2.1 Background of *Macrobrachium rosenbergii*

Giant freshwater prawn or *Macrobrachium rosenbergii* is one of the most economically important aquaculture species. *M. rosenbergii* is also produce in other countries such as Americas and Africa (Michael, 2002). *M. rosenbergii* is the largest freshwater prawn and also known as giant river prawn. *M. rosenbergii* species that possess many interesting characteristic for an artificial prawn culture (John, 2009). The gravid female will migrate across saline gradients to the estuaries where the eggs hatch and develop (Ismael and New, 2000). The advantage of all male culture is that the prawns will reach market size at a faster rate (Hartnoll, 1982)

There are many factors that affect the growth of *M. rosenbergii* including sexual maturity, gender, and age of the animals (Hartnol, 1982; Botsford, 1985; Aiken & Waddy, 1992). Some of the crustacean species displays bimodal growth patterns, where males show supreme growth to female or vice versa (Hartnoll, 1982). The bimodal growth pattern is also observed in *M. rosenbergii* where the size of male is bigger than female, reach about 12g. This has made it more favourable to be culture compared to female *M. rosenbergii* (Aflalo, 2016).

In prawn hatchery, the berried female is commonly transferred from freshwater to brackish water to improve their eggs hatching rates. The adults *M. rosenbergii* will migrate to the brackish water for the spawning because larvae need brackish water during their growth development (New and Shingholka, 1982). It is because, larvae required brackish water to grow and develop otherwise the larvae will die and eventually affect the population of giant freshwater at that area. This species can

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tolerate with large range of temperature and salinity which are 14-35^{o}C and 0-25 ppt (New, 1995)

The nature of *M. rosenbergii* is also to defend themselves. Their defense mechanism is they will move backwards quickly and retreat when disturbed (Nadhal, 2006). Other than that, they will also use their claw to clamp their enemy. It prefer to hide in shade and under shelter in the shallow areas during the day to avoid sunlight but are very active at night. In flight, special during soft shell period after molting, the species is very vulnerable and cannibalism often occurs. Shell hardening may be delayed by acidic water or lack of calcium in the diet.

According to Brock (1993), the optimal salinity for the rearing *M. rosenbergii* larvae is 8 to 15 ppt. According to New (2002), there will be no morphology changes from PL towards juvenile stage but the juvenile stage prawn body will gradually turn into light brown and bluish color as they grow into adults. FAO (2003) stated that as adult *M. rosenbergii* can reach approximately 100 mm of total body length within 4-6 months during the PL stage until adult. In Lalrinsanga *et al.* (2012) studies, the species reach maturity at around 250 mm total body length, the females begin to divert more energy into egg production and less into growth.

The species usually is found in lakes, rivers, swamps and irrigation canals with preference for turbid conditions. They are capable of climbing waterfalls and can also traverse land where there is plenty of moist vegetation (New 1990). There are many method developed for effectively rearing the *M. rosenbergii*. Initially, the easiest method proposed by Fujimura and Okamoto, (1972) in rearing PL is the green water method for seed production in the hatchery. A few years after, new method, recirculation systems becomes popular because it gives consistent production of quality post larvae than the previous method.

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2.2 Life Cycle of *Macrobrachium rosenbergii*

The life cycle of the prawn consist of four main stages, namely eggs, larvae, post larvae and adult. FAO (2003) reported that crustaceans allow further growth when it expend its body size by moulting or shed off its hard shell (exoskeleton or exuvium). The new soft shell will eventually harden. Chowdhury (1993) claimed that the number of moults and the durations of intermoults are not fixed and depend on the environment, particularly temperature and the availability of food. High quality food and the right environmental conditions, especially in terms of dissolved oxygen and water temperature increase the growth rate of the species.

The culture of *M. rosenbergii* in Malaysia is being brought up since the studies by Ling (1969). Generally, the species starts and completed its larvae phase in brackish water with salinity range of 6-19 ppt. The growth rate will differ only after they start developing sexual characteristic (Fatema, *et al*., 2011). Larvae in a hatchery take a minimum of 26 days to metamorphose into PL. The time taken to complete metamorphosis depends mainly on optimum mainly, water, temperature and salinity. As well as of the supply of food, it takes 35-40 days for larvae metamorphosis into post larvae to be completed.

In a tank situation, it is up to the breeder to change the salinity of the water accordingly so that it does not give stress to the PL. FAO (2002) mentioned in a paper that the young PL will slowly move to the less salinity environment. These PL favour to be reared at the bottom and in vigorous and flowing water. Instead of swimming in the water, they crawl along the wall or bottom of the tank (New 2002). According to FAO (2003), there is a rapid growth in the juvenile stage between PL and adult stage. However, there is no specific indicator that the prawn has reached that stage. It is said that PL reached juvenile after 1 month period.

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2.3 Stages of *Macrobrachium rosenbergii* Larvae

All larvae stages are active swimmers and are planktonic in habit. The larvae attracted by light, but direct sunlight and other strong light are avoided. They swim tail first, ventral side up, with the head rather lower than the tail at an oblique angle. At early larval stages tend to swim close together in large groups, usually close to the surface of the water. The thousands of young larvae in each group swim swiftly in a continuous stream, milling and churning up and down repeatedly. All larval stages require brackish water, corresponding to 20 to 40 % sea water. All larval stages eat continuously as long as suitable food is available.

There is some identification that can be observed during the metamorphosis of *M. rosenbergii* larvae (Satya and Timothy, 2005). There is 11 stages of larvae before it turn into post larvae stage. For the stage 1, larvae only have sessile eyes. Then for the stage 2, the stalked eyes will occur. Next stage is stage 3, one dorsal teeth of rostrum will seen. After that, two dorsal teeth of rostrum at stage 4. For stage 5, telson elongated and narrower at the larvae body. At stage 6, pleopod buds only will produce. For the stage 7, after pleopod buds, the pleopod will bramous. And for the stage 8, seven segments of rostrum will see. Before turn to post larvae, it known as young seed at stage 9 and stage 10, endopods with appendices internae and 3-4 teeth on the anterior edge of the rostrum. Last is stage 11, many teeth on the dorsal edge of the rostrum.

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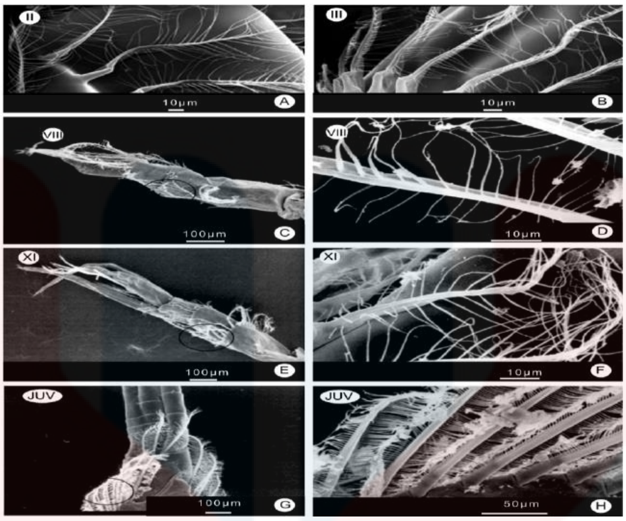

Figure 2.0 : Scanning electron microscopy of antennules of *M. rosenbergii*

(Source : https://www.researchgate.net/figure/281129866_fig1_Figure-1-Scanning-electron-microscopy on November 2014)

2.4 Salinity of *M. rosenbergii* Larvae

Salinity is one of important environment factors affecting survival, growth and distribution of giant freshwater prawn, *M. rosenbergii* (Kumlu and Jones, 1995). The effect of salinity on the growth and survival of *M. rosenbergii* have also been extensively study (Kumlu *et al.,*1999). Salinities between 15 ppt and 25 ppt are considered optimal for *M. rosenbergii* culture. Adult *M. rosenbergii* can tolerate salinity ranging from 0 ppt to 25 ppt and for larvae *M. rosenbergii* can tolerate to 10 ppt to 15 ppt (Kumlu *et al.,* 2000). Salinity plays critical role on egg, embryo and larval development takes place in brackish water.

This current study is basically using different parameters to determine the suitable optimum salinity on the growth rate of the *M. rosenbergii*. Steele (2001) reported in his study that salinity affect the reproduction and embryonic development. However, it is not stated that the salinity affect the growth rate. The optimum salinity for reproduction of *M. rosenbergii* is 12ppt. Water with same temperature as the tank water during rearing should be added to maintain the salinity around range of 8-12 ppt for larvae until post larvae stage. Salinity should be checked weekly and freshwater is added as required.

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Lee & Wee (2010) claimed that the water rearing of *M. rosenbergii* starting at PL stage must be maintained at a salinity 8-15 ppt. Goodwin & Hanson (1975) who indicated that *M. rosenbergii* juvenile grows more rapidly in fresh water or slightly brackish water which is less than 5 ppt compared to more brackish water of up to 15 ppt. There 8-15 ppt are the most optimum level of salinity and required for high growth and survival of the larvae. Usually the instrument used for salinity observation is either refrectometer or multiparameter.

2.5 Feeding

Feed for each stage of *M. rosenbergii* life cycle is different. Freshwater prawn require feed for their growth developments. Young larvae fed on their own egg sac for about 3 days. Later, they need live feed such as *Artemia nauplii* and high protein feed such as egg custard. Hence, zooplankton and oligochaete worms are important nutrients intake in *M. rosenbergii* especially when they are grown in pond. Besides, the uniform production cycle of large prawn can be obtained when they are given the quality feeds (Tidwell *et al,* 2005).

Shailender (2012) experiment reported that feeding the larvae with *Artemia nauplii* has higher survival rate compared to the ones that is fed with egg custard. The reason of that statement is the movement of the live feed helps the larvae to capture their feed rather the stationary egg custard. If the larvae are not given enough feed, they tend to eat each other because of their carnivorous behaviour (Satya and Timothy, 2005). Lavens & Sorgeloos (1996) claimed that freshly hatched *Artemia nauplii* can ensure proper ingestion for the larvae.

At larvae stage, they can be given eggs custard as feed. Egg custard diet is one of the formulated diets that are suitable for larvae and it contains chicken eggs, milk powder and squid meat. If egg custard is feed to the larvae along with the *Artemia nauplii*, it given the better growth and survival of *M. rosenbergii* larvae (Rao, 1997). However, instead of giving *Artemia* or egg custard, PL can now consume commercial

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pellet as their digestive system are already developed. Other than that, some other studies such as from Abdel-Hakim et al. (2013), stated that PL can be fed with commercial diet.

2.6 Mortality

In early larvae life cycle, the death of larvae is common because they are very small and fragile. Ou (2011) also explained that numbers of larvae lost are due to some factors such as the siphoning, change of water quality in tanks that cause stress. Uneven feed intake or improper diet feeding can lead to difference in larvae growth. Thus, cause cannibalism among larvae. The mortality caused by cannibalism is high when the larval population increases (Uddin et al., 1998). This case was maybe due to insufficient nutrition or high stocking densities. Some cases showed mortality that are cause by lack of dissolved oxygen in water and above water level when larvae jump at times and stranded on tank walls. Jain et al. (2008) documented that high concentration of ammonia causes osmo-regulatory imbalance, and also interferes with oxygen and carbon-dioxide exchange in the prawn blood.

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CHAPTER 3

METHODOLOGY

3.1 Sample Collection

A total of 1,000 samples of *M. rosenbergii* larvae were purchased from the hatchery at Pusat Penternakan Udang Galah (PPUG) in Setiawan, Perak. It took about 6 h from Pusat Penternatakan Udang Galah (PPUG) located in Setiawan, Perak to Fish Propagation House (FPH) in Politeknik Jeli where the research took place.

3.2 Location of Study

This research was done at Fish Propagation House (FPH), Politeknik Jeli and University Malaysia Kelantan (UMK), Jeli Campus. This experiment was running about 5 weeks which are everyday of data collection and analysis.

3.3 Transportation

During the transportation, the water temperature maintains at 25°C to reduce the metabolism of *M. rosenbergii* larvae and make it inactive The plastic bag contained *M. rosenbergii* larvae were transported to Fish Propagation House (FPH), Politeknik Jeli.1/3 of durable transparent plastic bags were filled up with water from original larvae ponds where the temperature will be in 25˚C, pH within 7.0-8.5, dissolved oxygen at 3-7 ppm and salinity was at 10 ppt while 2/3 of those plastic bags were filled with oxygen. The samples were acclimitised about three days before the investigation started.

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Safety measurement such as securing the rubber band at the open end and at both edges is applied for the packaging. Then, it was better to not feed the larvae during transportation to control the ammonia content in water. The stocking density in the plastic bag is 500 larvae each. At the end of the transport, the larvae were transferred into a basin to provide suitable condition after transportation. The transported larvae being introduced to the freshwater a while in the basin bit by bit before transferring to the rearing tank to reduce stress and allow the larvae to adapt to the surroundings. The important parameter used during transportation are explained in Table 3.0.

Table 3.0 : Transportation condition of *M. rosenbergii* larvae

Parameter	Treatment
Salinity	10 ppt
Ice	Present
Temperature	25-28^{o}C
pH	6.5-8.5
Dissolved Oxygen (DO)	>1.5 ppm
Ammonia	<0.01 ppm
Stocking density	500 larvae / 4L

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3.4 Experimental Design

3.4.1 Treatment of the Sample

The size of the tank can be concluded as 12 cm x 18 cm x 12 cm which can contain about 15 litter of water overall. The tank has filled with 10 litter of diluted water. The tank that used as shown in Figure 3.0.

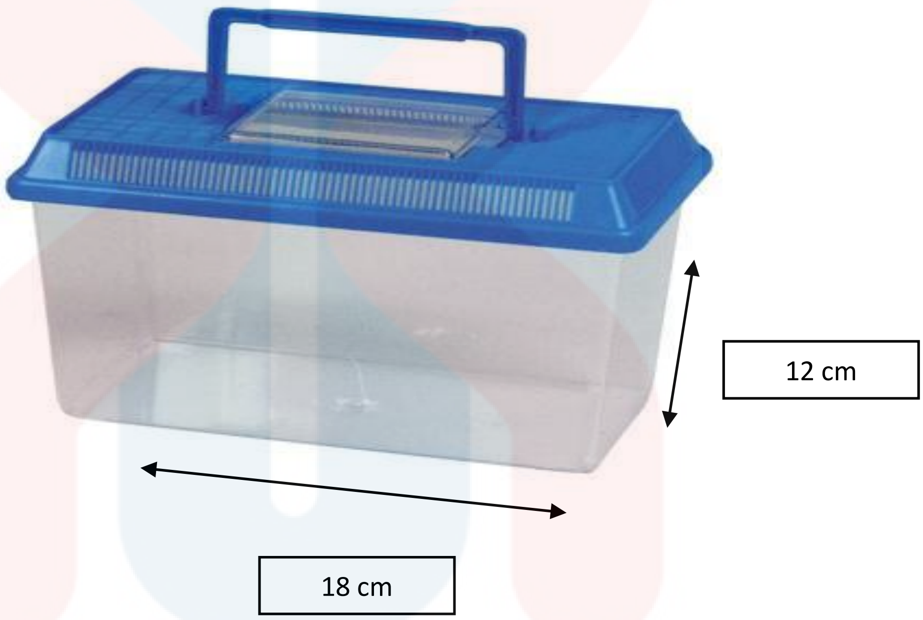

Figure 3.0 : Measurement of the rearing tank

The brackish water was prepared by mixing the sea water and the tap water with the ratio 2:1. After that, the tanks have been provided with different water salinity according to the study. Each tank has been marked with A, B and C according to the water salinity that has been provided. At water salinity with 8 ppt was provided with 3 tanks that marked with A, B and C. Same with 10 ppt and 12 ppt was provided with 3 tanks that marked with A, B and C. In the meanwhile, the rest of the parameter are kept at the studied optimum condition as shown in Table 3.1.

Table 3.1 : Constant optimum condition in the tank

Parameter	Treatment
Salinity	8 ppt/10 ppt/12 ppt
Temperature	28°C
pH	7.0 – 8.5
Dissolved Oxygen (DO)	>1.2 ppm
Ammonia	<0.01 ppm

The temperature was maintained for room temperature 28 °C and the salinity was measured using multiparameter and refrectometer. The pH, dissolved water and ammonia also maintained every week and was measured using multiparameter. The stocking density treatment each tanks consisted 50 larvaes. Each treatment included 3 replicate. The development and survivality of larvae has been observed and recorded every day. In the meanwhile, the summary of treatment for rearing *M. rosenbergii* larvae approximate total larvae needed were 50 x 9 = 950 as shown in Table 3.2.

Table 3.2 : The summary of treatments for rearing *M. rosenbergii* larvae

Salinity	Label Tank	Total Larvae
	A	50
8 ppt	B	50
	C	50
	A	50
10 ppt	B	50
	C	50
	A	50
12 ppt	B	50
	C	50

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3.4.2 Data Collection

The data were collected daily for survival percentage and for growth performance within 38 days of larvae stage. The survival percentage was recorded by calculating mortality daily. The growth performance were observed from larvae morphology. 3 random larvae out of all the others in the rearing tank are taken out to be observed. Average data of each treatment were recorded.

3.5 Life Feed Preparation

Artemia were prepared using seawater. About 3 mg of *Artemia* cyst was weight and aerated in 1 litter of seawater inside the beaker. The beaker was then exposed with light and aerated vigorously to trigger the hatching. After 24 hours, the *Artemia nauplii* were collected and given as feed to the *M. rosenbergii* larvae.

3.6 Data Analysis

Data of survivability rate of larvae and development of larvae was analysed using Analysis of Variance (ANOVA) available form Statistical Package for the Social Science (SPSS) version 22. The confidence intervals of 95 % ($P< 0.05$) was set for each parameter.

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CHAPTER 4

RESULT

4.1 Mean Development of *M. rosenbergii* Larvae

4.1.1 Salinity 8 ppt

In water salinity 8 ppt, development of larvae at range day-23, recorded the highest mean, 38.99% development performance while at range day-38 showed the lowest mean, 3.33% development performance. The second highest mean development performance was shown at range day-18, 28.89 % followed by range day-28, 19.39 % and range day-33, 9.39 %. The range day-38 were significant.

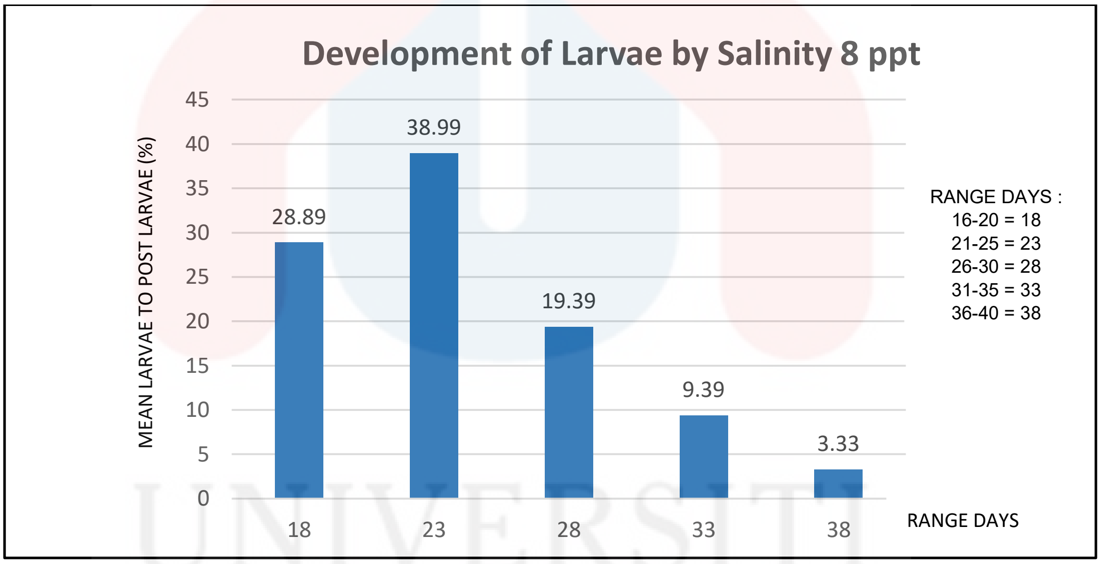

Figure 4.1 : Development larvae performance at salinity 8 ppt.

Table 4.1 : Mean (%) for Development of Larvae at 8 ppt.

Range Days	Mean (%)	Standard Deviation
16-20 = 18	28.89	± 34.2125
21-25 = 23	38.99	± 22.3638
26-30 = 28	19.39	± 20.0276
31-35 = 33	9.39	± 9.10517
36-40 = 38	3.33	± 5.77350

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4.1.2 Salinity 10 ppt

In water salinity 10 ppt, development of larvae at range day-23, recorded the highest mean, 48.01% development performance while at range day - 38 showed the lowest mean, 6.27% development performance. The second highest mean development performance was shown at range day-28, 23.64% followed by range day-33, 11.38% and range day-18, 10.68%. The range day-38 were significant.

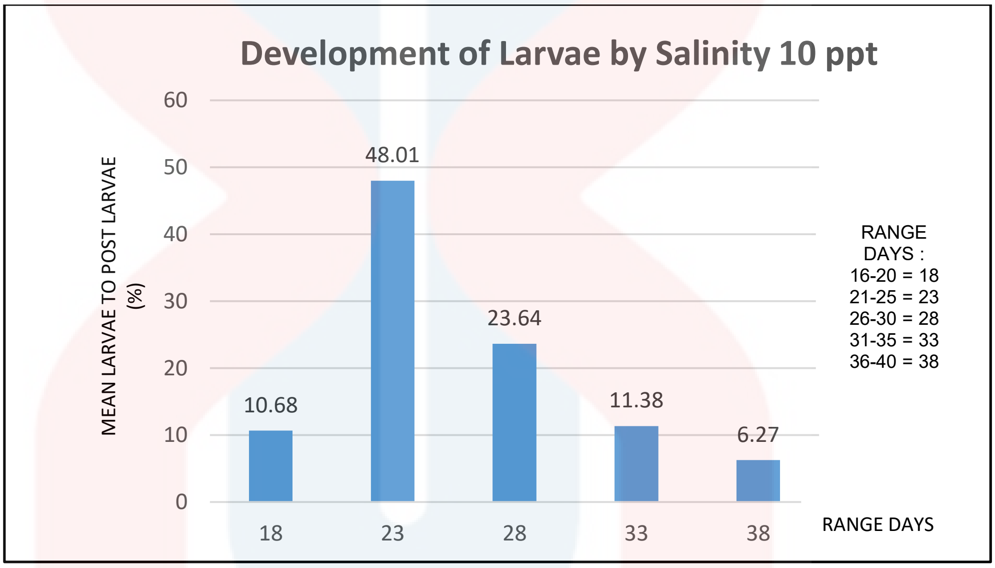

Figure 4.2 : Development larvae performance at salinity 10 ppt.

Table 4.2 : Mean (%) for Development of Larvae at 10 ppt.

Range Days	Mean (%)	Standard Deviation
16-20 = 18	10.68	± 9.27449
21-25 = 23	48.01	± 8.72253
26-30 = 28	23.64	± 9.05925
31-35 = 33	11.38	± 11.5173
36-40 = 38	6.27	± 5.69137

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4.1.3 Salinity 12 ppt

In water salinity 12 ppt, development of larvae at range day-23, recorded the highest mean, 51.55% development performance while at range day-18 showed the lowest mean, 0.0% development performance. The second highest mean development performance was shown at range day-33, 19.76% and range day-38, 19.76% followed by range day-28, 8.93%.

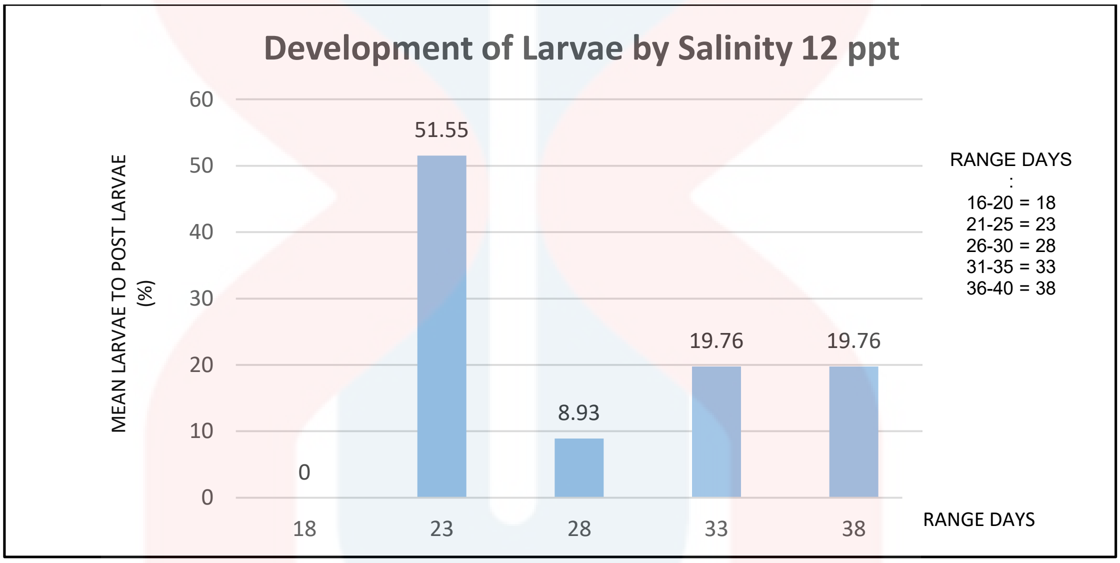

Figure 4.3 : Development larvae performance at salinity 12 ppt.

Table 4.3 : Mean (%) for Development of Larvae at 12 ppt.

Range Days	Mean (%)	Standard Deviation
16-20 = 18	0.0	$\pm .000000$
21-25 = 23	51.55	± 12.2485
26-30 = 28	8.93	± 7.78522
31-35 = 33	19.76	± 5.35892
36-40 = 38	19.76	± 5.35892

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4.1.4 Comparison Development of Larvae by Different Salinities

Development of larvae by different salinity was showed that only at range day-38 were significant for development performance while at range day-18, 23, 28 and 33 was not significant for development performance.

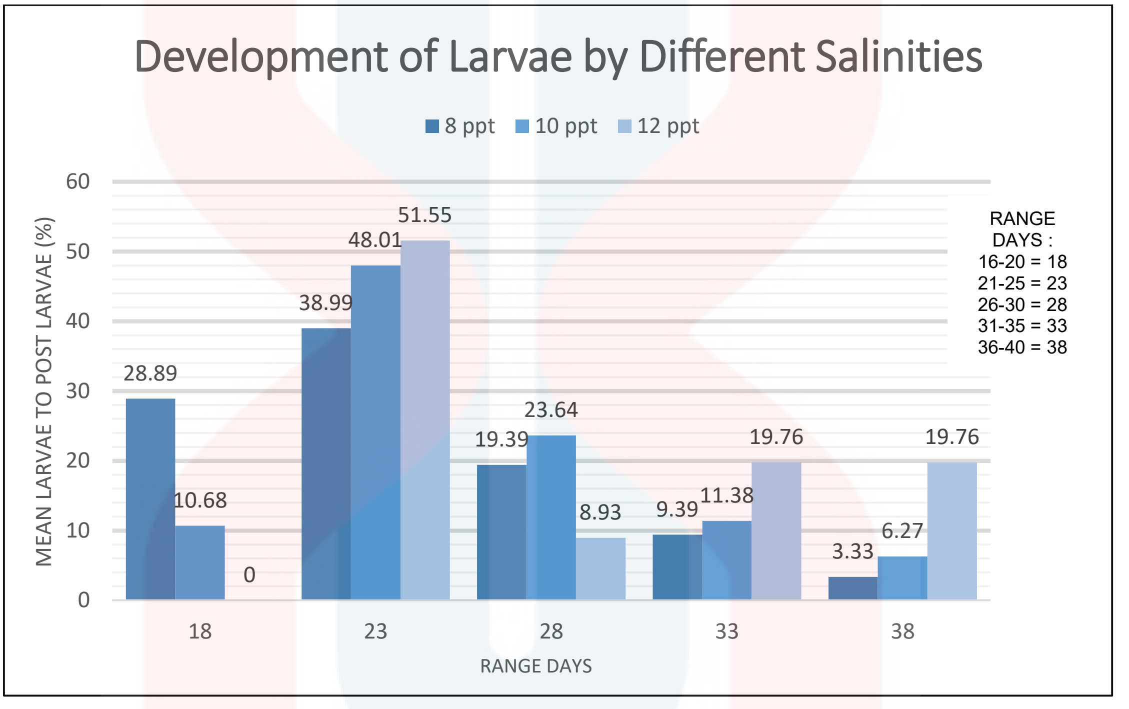

Figure 4.4 : Development larvae performance at different salinities.

Table 4.4 : Significant Development of Larvae by Different Salinity.

Range Days	Significant	Description
16-20 = 18	$\pm .882$	No significant
21-25 = 23	$\pm .291$	No significant
26-30 = 28	$\pm .619$	No significant
31-35 = 33	$\pm .387$	No significant
36-40 = 38	$\pm .025$	Significant

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4.2 Survival

In different salinity, the dead larvae at salinity 10 ppt, recorded the lowest mean, 81.33 % while at salinity 8 ppt showed the highest mean, 84 %. Then the mean survavibility was shown at 12 ppt, 83.33 %. All was not significant.

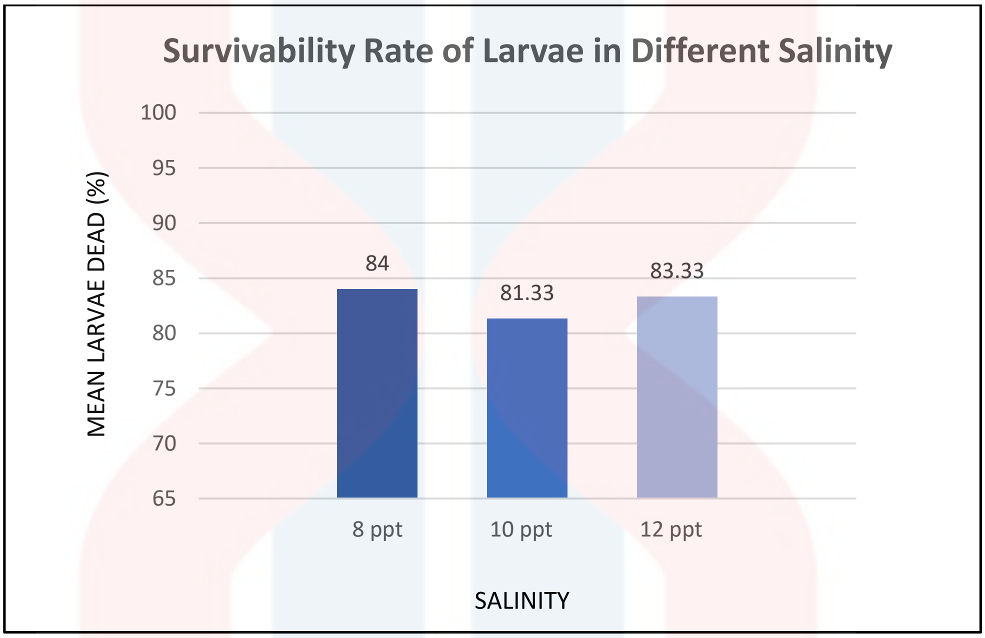

Figure 4.5 : Mean (%) for survivability of larvae in different salinity.

Table 4.5 : Mean (%) for Dead Larvae in Different Salinity

Salinity (ppt)	Mean (%)	Standard Deviation	Significant
8	84	± 8.71780	No significant
10	81.33	± 7.02377	No significant
12	83.33	± 3.05505	No significant

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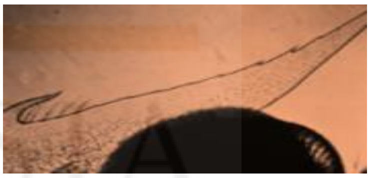
4.3 Larvae Stages

The morphological details of the various stages of larvae are described in table 4.6. In all, three larval and a postlarval stage were recorded. The newly hatched larva was the first stage zoea which underwent moulting after 2 days and formed the stage II zoea. Second moulting occurred after 4 days resulting into stage III zoea larva. Thereafter, eight moultings occurred one each on 6^{th}, 7^{th}, 10^{th}, $I3^{th}$, 15^{th}, 17^{th}, 18^{th} and 21^{th} days of hatching resulting into IV, V, VI, VII, VIII, IX, X, XI and post larva zoea larval stages respectively. The initial larval stages were transparent with few chromatoohores spread on their bodies, particularly on abdomen, telson, periopods and eyes talk. Gradually the chromatophores increased in number and branched. The post larvae metamorphosed into juveniles which ceased to have pelagic life, settled to the bottom, assumed benthic behavior and were noted to cling on submerged vegetation and other objects.

Table 4.6 : Larval stage of *M. rosenbergii* observed from 15 to day 21.

Larval stage	Days	Identification	Picture
IX	15	Endopods of pleopods with appendices internae	
X	17	3-14 dorsal, teeth on rostrum	
XI	18	Teeth on half of upper dorsal margin	

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Post Larvae	19-21	Benthic, swims forwards with dorsal side uppermost. Teeth on upper and lower margin of rostrum (also behavioural changes, mainly in swimming.	

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DISCUSSION

CHAPTER 5

5.1 Optimum Salinity for Development Stages of *M. rosenbergii* Larvae

The physical parameter of the culture condition are of critical importance for the successful rearing of any type of aquanic organism (Anger, 2001 : Maciolek, 1972 : Zheng *et al.,* 2008). Most larvae actively regulate internal ion concentrations by osmo-ionic regulatory processes, and salinity tolerance are often closely realted to their ability to maintain internal media largely independent of external condition (Anger, 2001 : Brown *et al.,* 2010). A number of previous studies have examined the improvement of larval freshwater tolerance through the effects of salinity and temperature on embryonic development and larval viability (Ching and Velez, 1985 : Brillon *et al.,* 2005 : Manush et al., 2006: Smith et al., 2009).

The salinity optimum experiment indicate that development of newly-emerged *M .rosenbergii* larvae were highest in 10 ppt at range day 23 and the optimal salinity significant at range day 33 gradually increased up to full-strenght by the mid-way point of larval development. Larvae of M. *rosenbergii* species investigated previously demonstrate a wide range of salinity requirement, from species that are able to complete their larvae development entirely in freshwater (Williamson, 1971), to those needing salinities greater than 20% for successful development (Ngoc-Ho, 1976), with a number of species of species having intermediate needs (Choudhury, 1971a, 1971b).

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One of limitations of the salinity optimum study was the difficulty in maintaining larvae in small culture volumes for extended period of time. Very heavy mortality was observed when attempts were made to rear larvae. *M. rosenbergii* require condition of constant salinity for successful development beyond stage metamorphosis into post larvae (Ling, 1961: Uno and Kwon, 1969: Takano, 1987: Nandlal and Pickering, 2006).

5.2 Survival of *M. rosenbergii* Larvae

Salinity played a vital role on rearing and survivability of larvae of freshwater prawn. Optimum level of salinity was an important factor for the growth and rearing of this species. In different salinity, result of the present study indicated that the lowest mean recorded dead larvae at salinity 10 ppt, 81.33 % of survivability of larvae while at salinity 8 ppt showed the highest mean, 84.0 %. Then the mean survavibility was shown at 12 ppt, 83.33%. The larvae survived best at 10 ppt and no significant differences occurred between treatment (one-way ANOVA : 882 and $p>0.05$). Thus it supports that the species exhibits a wide range of tolerance to abrupt salinity changes. There were the only remaining treatments containing surviving larvae at the end of the experiment. Larvae exposed to treatment salinity 8 ppt, 10 ppt and 12 ppt.

5.3 Larval stage of *M. rosenbergii* larvae

In this experiment, the *M. rosenbergii* larvae are planktonic and it swims with the tail first with their eyes looking upwards towards the surface, ventral upside-down. They are really active and will aggregate together in the dense group where there are the bright spot in the tank if the water is not moving. They are really attracted to the light (Satya and Timothy, 2005). The larvae need brackish water to survive. The salinity of the water must be reduce slowly until it reaches post larvae stage.

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The larval development was observed starting from the first day of larvae hatched. The larvae take 20-40 days to complete all 11 stages of metamorphosis but it also can complete in as fast as 18 days due to the feeding and environmental condition. The larvae are feed with *Artemia nauplii* once per day to avoid the cannibalism. In the current study, the larvae managed to live until 11 stages but only a half was lived. This happened due to the strong aeration and air bubbles that make the larvae accumulated at the bottom of the tank. The larvae were not strong enough to swim in the strong air bubble and that makes the larvae do not survive longer. So, to extend the shelf life of larvae, the aeration must not so strong and maintain other water parameter.

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CHAPTER 6

CONCLUSION

6.1 Conclusion

From the research, the optimum salinity for development stages of *M. rosenbergii* larvae, there was not significant however we found significant at range day-38. It took about 19 days for the larvae turn post larvae. The optimum parameter and condition must be maintained for the development of larvae stage. Among of survavibility for larvae, the salinity at 8 ppt – 12 ppt were not significant. The measurement of water quality parameter especially salinity, temperature, dissolved oxygen and ammonia must be constant to make sure the result is uniform and significant. The findings of this study will help to increase the *M. rosenbergii* larvae that eventually provides to the farmers.

6.2 Recommendation

As a demand species nowadays, *M. rosenbergii* research should be shared more and act as references to others to increase its production. The study should be carried out to produce better quality of larvae and increase the survival rate of larvae. Besides that, the study about controlling the optimum salinity needed for larvae rearing to complete it metamorphosis stage is required. The findings of this study can give good impact to the aquaculture industries especially giant freshwater prawn industry in East Coast region and Malaysia. It will help to fulfil the high demand of *M. rosenbergii* seed especially for the commercial hatchery.

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APPENDICES

Appendix 1 : Descriptive of survivability for *M. rosenbergii* larvae in different salinity

Descriptives

SURVIVALITY

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
S8	3	84.0000	8.71780	5.03322	62.3438	105.6562	78.00	94.00
S10	3	81.3333	7.02377	4.05518	63.8853	98.7813	74.00	88.00
S12	3	83.3333	3.05505	1.76383	75.7442	90.9225	80.00	86.00
Total	9	82.8889	5.92546	1.97515	78.3342	87.4436	74.00	94.00

Appendix 2 : Table of ANOVA of survivability for *M. rosenbergii* larvae

ANOVA

SURVIVALITY

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	11.556	2	5.778	.129	.882
Within Groups	269.333	6	44.889		
Total	280.889	8			

SURVIVALITY

	SALINITY	N	Subset for alpha = 0.05
			1
Tukey B[a]	S10	3	81.3333
	S12	3	83.3333
	S8	3	84.0000
Duncan[a]	S10	3	81.3333
	S12	3	83.3333
	S8	3	84.0000
	Sig.		.653

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix 3 : Descriptive of development for *M. rosenbergii* larvae in different salinity

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean Lower Bound	95% Confidence Interval for Mean Upper Bound	Minimum	Maximum
LTPL18	8ppt	3	28.8900	34.21252	19.75261	-56.0986	113.8786	.00	66.67
	10ppt	3	10.6833	9.27449	5.35463	-12.3558	33.7225	.00	16.67
	12ppt	3	.0000	.00000	.00000	.0000	.0000	.00	.00
	Total	9	13.1911	21.77520	7.25840	-3.5468	29.9290	.00	66.67
LTPL23	8ppt	3	38.9900	22.36379	12.91174	-16.5647	94.5447	20.00	63.64
	10ppt	3	48.0067	8.72253	5.03596	26.3387	69.6746	38.46	55.56
	12ppt	3	51.5467	12.24853	7.07169	21.1196	81.9737	37.50	60.00
	Total	9	46.1811	14.59451	4.86484	34.9628	57.3994	20.00	63.64
LTPL28	8ppt	3	19.3933	20.02758	11.56293	-30.3579	69.1446	.00	40.00
	10ppt	3	23.6433	9.05925	5.23036	1.1389	46.1478	15.38	33.33
	12ppt	3	8.9300	7.78522	4.49480	-10.4096	28.2696	.00	14.29
	Total	9	17.3222	13.37719	4.45906	7.0396	27.6048	.00	40.00
LTPL33	8ppt	3	9.3933	9.10517	5.25687	-13.2252	32.0118	.00	18.18
	10ppt	3	11.3800	11.51737	6.64956	-17.2307	39.9907	.00	23.03
	12ppt	3	19.7633	5.35892	3.09397	6.4510	33.0756	14.29	25.00
	Total	9	13.5122	9.15361	3.05120	6.4761	20.5483	.00	25.00
LTPL38	8ppt	3	3.3333	5.77350	3.33333	-11.0088	17.6755	.00	10.00
	10ppt	3	6.2700	5.69137	3.28591	-7.8681	20.4081	.00	11.11
	12ppt	3	19.7633	5.35892	3.09397	6.4510	33.0756	14.29	25.00
	Total	9	9.7889	9.01058	3.00353	2.8627	16.7150	.00	25.00

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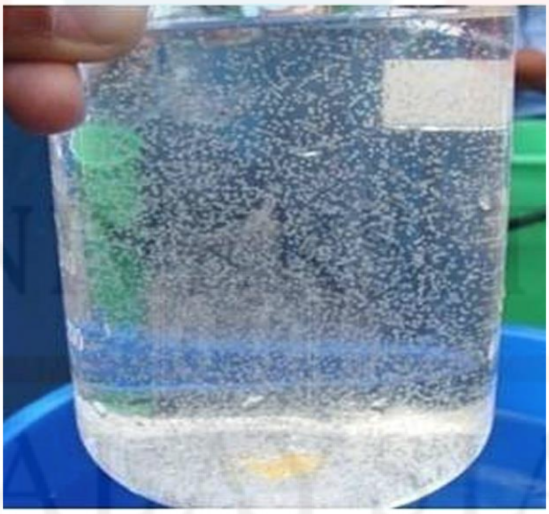
Appendix 4 : Table of ANOVA of development for *M. rosenbergii* larvae

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
LTPL18	Between Groups	1280.248	2	640.124	1.528	.291
	Within Groups	2513.025	6	418.838		
	Total	3793.273	8			
LTPL23	Between Groups	251.502	2	125.751	.519	.619
	Within Groups	1452.496	6	242.083		
	Total	1703.998	8			
LTPL28	Between Groups	344.026	2	172.013	.949	.438
	Within Groups	1087.568	6	181.261		
	Total	1431.594	8			
LTPL33	Between Groups	181.764	2	90.882	1.116	.387
	Within Groups	488.544	6	81.424		
	Total	670.308	8			
LTPL38	Between Groups	460.639	2	230.319	7.316	.025
	Within Groups	188.886	6	31.481		
	Total	649.525	8			



Appendix 4 : *M. rosenbergii* larvae at 1st week.

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