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Optimum Condition for Transporting Giant Freshwater Prawn, *Macrobrachium rosenbergii* Post Larvae

By

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A report submitted in the fulfillment 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: Hazratul Nazira Binti Hamzah

Date:

I certify that the report of this final year project entitled "Optimum condition for Transporting Giant freshwater prawn, *Macrobrachium rosenbergii* Post Larvae" by Hazratul Nazira Binti Hamzah, matric number F14A0081 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

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Date:

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ABSTRACT

Macrobrachium rosenbergii is one of the most commercially important freshwater prawn species in the countries where it is indigenous or even beyond. Once harvested, it is important to maintain the quality of the post larvae (PL) by adopting proper packaging and transportation. Trauma and stress of the PL were known to have association with handling and transport of PL will affect the survival and the quality of the PL. This experiment were carried out to determine the optimum condition to transport the PL. The parameters that conducted were the effect of stocking densities (60, 80, 100 PL in ½ /L of water), water temperatures (23 °C, 25 °C and 30 °C), presence of *artemia* as feed, and lighting factor (dark and sunlight). After reached UMK Jeli Campus, all PL were removed from plastic bags and the number of survival and dead PL were recorded. In this experiment, only water temperature showed significant difference ($p<0.05$) while other treatments showed no significant difference ($p>0.05$) in PL survival. The survival of PL stocked 60 stocking density found to be higher (96.11 %) while significantly lower rate was observed at high stocking densities of 80 and 100 PL in which 95.83 % and 94.67 % respectively. Survival of PL found to be higher at water temperature 23 °C (96.11 %) while significantly lower rate was observed at water temperature 25 °C and 30 °C in which 95.00 % and 91.67 % respectively. For treatments the presence of *artemia* as feed during transport, it shown that the survival percentage of PL with *artemia* was 97.78 % whereas survival percentage of PL without *artemia* was 94.44 %. For treatment with lighting, the survival percentage of PL that were put at dark place was 96.67 % while 92.78 % survival percentage of PL that were put at a place that exposed to sunlight. The survivability of PL can be maximized by determining the optimum condition to transport *M. rosenbergii* PL.

Keywords: Freshwater prawn, *Macrobrachium rosenbergii,* transportation, optimum condition, post larvae

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Keadaan Optimum untuk Mengangkut Udang Galah Air Tawar, Pasca Larva

Macrobrachium rosenbergii

ABSTRAK

Macrobrachium rosenbergii adalah salah satu daripada spesies udang galah yang paling komersial dan penting di kebanyakan negara sama ada di dalam ataupun di luar. Setelah dituai, adalah sangat penting untuk mengekalkan kualiti pasca larva (PL) melalui pembungkusan dan pengangkutan yang dilakukan dengan teliti. Ia adalah kerana sebarang trauma dan tekanan pada PL yang berkaitan dengan pengendalian dan pengangkutan PL akan menjejaskan kelangsungan hidup dan kualiti PL. Eksperimen ini telah dijalankan untuk menentukan keadaan optimum untuk mengangkut PL. Kajian ini menilai kepadatan stok (60, 80, 100 PL dalam 1/2 /L air), suhu air (23 °C, 25 °C and 30 °C), kehadiran artemia sebagai makanan dan faktor pencahayaan (tempat gelap dan cerah). Setelah sampai di UMK Kampus Jeli, semua PL telah dikeluarkan daripada beg plastik dan bilangan PL yang hidup dan yang mati telah dicatat. Dalam eksperimen ini, hanya suhu air yang menunjukan perbezaan signifikan ($p<0.05$) manakala rawatan lain tidak menunjukkan perbezaan yang signifikan ($p>0.05$) untuk kelangsungan hidup PL. Kelangsungan hidup PL pada kepadatan 60 didapati lebih tinggi (96.11 %) manakala kadar yang lebih rendah diperhatikan pada kapadatan yang lebih tinggi sebanyak 80 dan 100 PL di mana 95.83 % dan 94.67 %. Kelangsungan hidup PL didapati lebih tinggi pada suhu 23°C (96.11 %) manakala kadar yang lebih rendah diperhatikan pada suhu air 25°C dan 30°C di mana 95.00 %. Bagi rawatan kehadiran artemia sebagai makanan semasa pengangkutan, ia menunjukkan bahawa peratusan kelangsungan hidup PL yang mengandungi artemia adalah 97.78 % dan kelangsungan hidup PL tanpa artemia adalah 94.44 %.Untuk rawatan faktor pencahayaan, peratusan kelangsungan hidup PL yang diletakkan di tempat gelap adalah 96.67 % manakala kelangsungan hidup PL sebanyak 92.78 % untuk PLyang diletakkan di tempat yang terdedah kepada matahari. Kelangsungan hidup PL boleh dimaksimumkan dengan menentukan keadaan optimum untuk mengangkut PL *M. rosenbergii.*

Kata kunci: Udang air tawar, *Macrobrachium rosenbergii*, pengangkutan, keadaan optimum, pasca larva

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LIST OF ABBREVIATION AND SYMBOLS

ANOVA	Analysis of Variance
PPUG	Pusat Peternakan Udang Galah
FAO	Fisheries of Agriculture Organisation
PL	Post Larvae
hrs	hours
L	Liter
ml	mililiter
ppm	Part Per Million
mg/L	Milligram Per Liter
ppt	Part Per Thousand
DO_2	Dissolved oxygen
%	Survival Percentage
°C	Degree Celcius

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CHAPTER 1

INTRODUCTION

1.1 Research Background

Freshwater prawns of the genus *Macrobrachium* are free-living decapod crustaceans and usually distributed in some places in tropical streams and lakes. The genus of *Macrobrachium* contains 240 species that has been identified and *Macrobrachium* is one of the most diverse genera freshwater crustacean (De Grave et al., 2008). *Macrobrachium rosenbergii,* known as giant river prawn or giant freshwater prawn. *M. rosenbergii* live in turbid freshwater. However, their larval stages require brackish water to survive. *M. rosenbergii*, locally known as 'Udang Galah' is widely distributed in the tropical and subtropical areas of the Indo-Pacific Region, including East Pakistan, India, Ceylon, Burma, Thailand, Malaysia, Indonesia, Philippines, Cambodia and Vietnam. It is present in lakes, water reservoirs, mining pools, irrigation canals and even some paddy-fields that have direct and indirect access to the rivers (De Man, 2001).

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Prawns and other animal usually get stress during transportation. Minimizing the stress factors caused by transportation will ensure the high survivability. Several factors that need to be considered during the transportation of post larvae (PL) are temperature, dissolved oxygen (DO), stocking density of PL and distance or travel time. The optimum temperature for transporting *M. rosenbergii* PL less than 6 hours is between 20 – 24°C (New, 2002). It is important to maintain the optimum water temperature to reduce metabolic rates. Low metabolic rate could minimizes oxygen consumption by animals. The metabolic rates of prawn will vary according to the animal's age. Usually, PL have a lower metabolic rates than adult animals (New, 2002).

In addition, packaging of the prawns prior for transport usually be carried out in before sun rise. This is important to minimize heat stress. The factors responsible for mortality of the prawn during transportation include the depletion of dissolved oxygen in ambient water due to respiration, oxidation of any organic matter (BOD) load, including excreted waste of the fish by microorganism, accumulation of free carbon dioxide resulting from respiration and ammonia as excretory end product, sudden fluctuations in temperature, hyperactivity and stress due to handling and confined space as well as diseases (Mohamed, 1997). Hence, this study was conducted to evaluate the effect of stocking density, different temperature, the presence of artemia as food source and also the lighting factor due to transportation. The optimum condition for transporting PL was determined through this study.

1.2 Problem Statement

Prawns and other animal will get stress during the transportation. The stress of the prawn that cause by transport should be minimized to reduce mortality rates. This has become a major problem to the farmer that need to transport PL for a long distance. Any stress and trauma associated with handling and transport of PL will affect survival and quality of PL and resulted in decreased prawn production. Thus, the stress issue during PL transportation is the main problem being focused in this study.

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1.3 Research Objectives

The main objectives of the present study were:

1. To determine the optimum condition of the prawn post larvae during transportation.
2. To study the factors contribute to the stress during transportation of *M. rosenbergii* post larvae.

1.4 Research hypotheses

It is assumed that poor conditions during transportation can affect the stress response in the *M. rosenbergii* PL.

1.5 Research Scope

In this study, the stress of *M. rosenbergii* are handled during the transportation by determining the optimum condition for the *M. rosenbergii* post larvae. The *M. rosenbergii* were transported from Pusat Penetasan Udang Galah, (PPUG), Setiawan Perak to the Animal Husbandry Laboratory of University Malaysia Kelantan (UMK) Jeli Campus. The optimum condition for the prawn include stocking density, water temperature, presence of artemia as feed, lighting factor. The post larvae were transported in a strong transparent plastic bags that filled with water from the original post-larvae ponds and the other 2/3 of the bag is filled with oxygen. The prawns will able to survive up to 16 hours in oxygenated sealed plastic bags.

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1.6 Research Significance

Optimum condition during transportation of *M. rosenbergii* PL will be useful for a better survivability of *M. rosenbergii* PL when they reach their final destination.

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CHAPTER 2

LITERATURE REVIEW

2.1 Aquaculture Industry

Aquaculture known as aqua farming which are the farming of fish mollusks, crustacean, aquatic plants and another aquatic organism. The production of the capture fisheries has become stable over the years and the farming of the aquaculture as stated above is the fastest growing food production system globally, there is an increase in the production of animal crops which is about 9.3 % per year since 1985 (Diana, 2009). Aquaculture usually accessed by examining the impacts on natural ecosystem rather than comparing aquaculture impacts with other methods of food production (Flaherty *et al*., 1995). Aquaculture also as a major role to meet the demand for the consumption of fish and fishery product for human. Besides that, the contribution of aquaculture to the global supplies of fish, crustacean, mollusks and others has developing and there is increase from 3.9 % of total production by weight in 1970 to 36.0 % in 2006 (FAO, 2008).

2.2 General Introduction of *M. rosenbergii*

Freshwater prawn has been reared in the captivity either through introduce wild caught juveniles or by trapping them and also involves with other crustacean such as *Penaeus* spp and *Metapenaeus* spp and fish in paddy filed and pond for instance in the Indian sub-continent and Malaysia (Wickins, 1976). In 1961, a research officer at Marine Fisheries Research Institute Penang, discovered that freshwater prawn, *M. rosenbergii* larvae required brackish condition to survive.

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The giant freshwater prawn are belong to the family of Palamonidae and the genus of Macrobrachium. The orderly classification 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*

The natural distribution of *M. rosenbergii* extended from the west of Pakistan to the southern Vietnam and it is across South Earth Asia and South to Northern Australia Papua New Guinea and some Pacific Ocean Island (New and Singholka, 1985). However, the culture of exotic species to the region that have high production of wild fisheries has been a common outcome of development for aquaculture industry all over the world (Naylor *et al*., 2001). To maximize the performance of the aquaculture, the practice has commonly involves the introduction to the regions that have similar environment or surrounding to the

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native distribution of the organism. The giant freshwater prawn, *M. rosenbergii* (De Man, 1879) has been cultured widely around the world.

2.3 Biology of *M. rosenbergii*

2.3.1 Life Cycle and Larval Development

Freshwater prawn have hard exoskeleton at their outer body. The shell of the freshwater prawn usually shed to support the growth. Shedding process are called molting. When this process occurs, the body size and body weight are increased (Louis Abrama *et al.*, 1996). *M.rosenbergii* has four distinct phases in their life cycle which are egg, larvae, post larvae and adult. The life cycle of the prawn start with the breeding of male and females. Mating occur between the hard-shelled of male and soft-shelled female. The male produce sperm and deposited it into the gelatinous mass of the female that located underneath the body of female. The egg will hatch 20 to 21 days after spawning process at the temperature of 27°C. The hatched larvae swim upside down. The larvae undergo 11 molts with the different stages of metamorphosis until they turned into PL (Louis Abrama *et al*, 1996).

M. rosenbergii PL will migrate upstream into freshwater environment within one or two weeks after metamorphosis. They are able to swim against the flowing current and crawl over the rock or stones at the shallow edges of rivers (New, 2002). According to Ling, (1969), instead of walking on the substratum the PL also attach to vegetation. Post-larvae are transparent and have a light orange pink head. The PL will transform into adult freshwater prawn. Adult males are larger that adult female and the sexes can be identified easily (Brunson, 1996).

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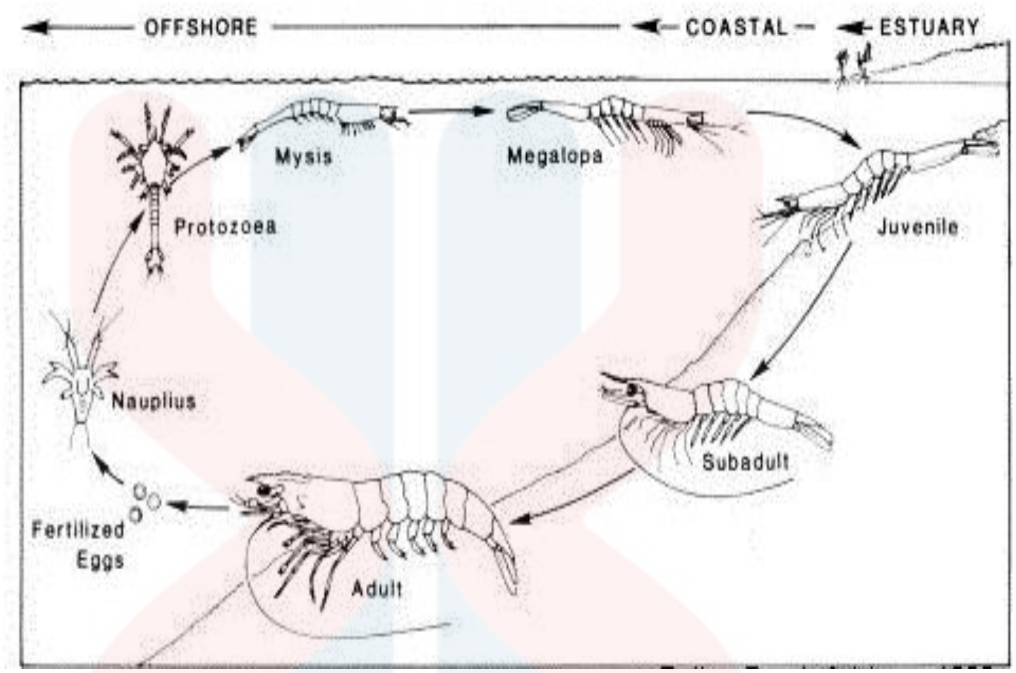

Figure 2.1 The life cycle of *M. rosenbergii* (Retrieved from: Bailey- Brock & Moss, 1992)

2.3.2 Morphology of *M. rosenbergii*

The egg of freshwater prawn are slightly elliptical and have a long axis of 0.6-0.7 mm. They are bright orange color before hatching for two or three days. Then they become grey-black. Stage 1 larvae are 2 mm long which is from the tip of rostrum to the tip of the telson. The newly post larvae (PL) is about 7.7 mm long and they are characterized by the way they move and swim as the adult prawns. The body of PL and adult freshwater prawn consist of cephalothorax and abdomen. Their bodies are divided into 20 segments which are 14 segment in the head and 6 segments in the abdomen (tail). The mature male prawn usually larger than female. The head of male is larger and the abdomen is narrower while the head of mature female and its second walking legs are smaller than adult male. The

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male genital opening are on the fifth walking legs while for female genital pores are on the third walking legs. The male can reach 320 mm long while female can reach 250 mm (Wowor & Ng, 2007).

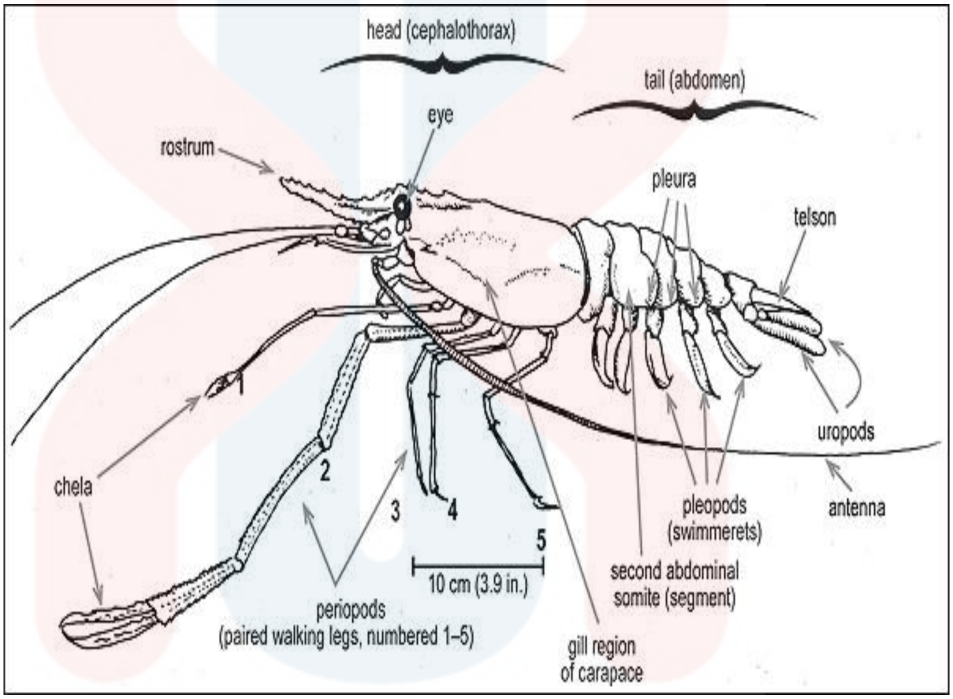

Figure 2.2: The external anatomy of *M. rosenbergii*.

Retrieved from http://extension.missouri.edu/p/G9471 on 28th March 2017

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2.3.3 Food and Feeding Habitat

Newly hatched *M.rosenbergii* larvae do not feed until after the first moult and it usually occur within 24 hours of hatching. The larvae are sustained by yolk cells during this period and it is visible in the proventriculus. The feeding limbs are folded against thorax and in the next stage these appendages are readily extended for grasping food particles (Moller, 1978). Larvae are omnivorous and mainly feed on zooplankton. Without presence of live food, they are capable of feeding on small particles of organic matter (New & Singholka, 1985).

Juvenile and adult prawn are omnivorous and they feed on variety of food item. The prawn will eat all types of food living and dead that available to them. The diet of the prawn include zooplankton, crustacean, mollusks, algae, organic material and other aquatic organism (Ling, 1969). It is important to distribute the feed uniformly all over the pond area because the freshwater prawn are aggressive and will distribute themselves evenly throughout the bottom of the pond. Prawn locate their food mostly by touch with their antennae. In farming practice, the supplementary feeds that contain individual animals and vegetables ingredients, farm-mixed feeds or the feed that compounded by manufacturer specifically for prawns or another aquatic and terrestrial animals (New & Singholka, 1985).

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2.3.4 Diseases in *M. rosenbergii* adult

There are two viral diseases that have been identified in *M. rosenbergii* which is parvo-like virus that affect the digestive tract and this diseases have been recorded more than 18 years ago (Anderson *et al*., 1990).The second viral viruses is white tail diseases (WTD) due to the clinical signs that have been observed and it was attributed to the simultaneous development of two viral different particles (Qian *et al*., 2003). The signs of the diseases include whitish coloration of the tail muscle start in some areas of the tail and extend to the tail muscle which is abdomen and lastly to all the muscle of the prawn include the head (cephalothorax) muscles. The signs above were associated with the abnormal behavior, lethargy and anorexia (Qian *et al*., 2003).

Recent research show the production trends of *M. rosenbergii* in 1993 was 3809 ton and increased by 10 000 ton for each year. There are increased in 2001 which the production reach about 128 878 tons. Unfortunately, the production decrease in 2003 and the production only about 87 143 tons because the diseases outbreak (Brunson, 1996). Then, the diseases have been identified. However, the diseases have been diagnostic and technique of treatment have been made and the outbreak disease were under controlled condition (Brunson, 1996).

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2.4 Common Methods of Transportation *M. rosenbergii* PL

In China, they used several methods of live transportation for prawn post larvae. Firstly, the anhydrous air transportation. This method relatively expensive because of the gross shipping weight. However, this method can help China to supply the prawns to the outlying market. It is when the local stock are unavailable. The other method used in China is trucking with aerated plastic barrel. This method is commonly used for transporting live animals. It is the economical method and have been used smaller supplier and usually the length of journey is less than 6 hours (Yang *et al*., 2012). According to Nandlal (2005), dual large, clear plastic bags are used to transport PL. The outside plastic bags was a backup if anything happen to the bag inside such damage and leakage. One third clean water are filled to the bags and PL are put into water and was oxygenated with pure oxygen and then sealed with rubber band.

The method that use plastic bags are effective because it considered the use of space, the materials used also affordable and easy to manage. Usually, the survival rate when using this method is about 98 % or higher for 3 hours or less travel distance (Yang *et al*., 2012). The bags 60 cm x 40 cm filled with fresh seawater and then packed with 3000-5000 PL and the density can be reduced if the travelling time is longer. After the mouth of the plastic bags are properly tighten, they are placed in the Styrofoam boxes and plastic buckets for transportation. The temperature can be reduced about 22-25 °C by placing the ice and mixed with the sawdust on the bottom, at the side and the top of the Styrofoam boxes (Yang *et al*., 2012).

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Next method by using tank on trucks. For transportation by trucks, fiber glass or plastic containers, frames or boxes lined with plastic can be used. Any size of tank can be used or transport but the recommended size is 1 m^3. The container have an open top and it is covered with sack or plastic and tied with rope in order to keep the water from splashing out and also to avoid direct exposure from sunlight. Water are filled with 70 % of its volume of the container and aerator should be provided. The post larvae also can be transported by using cooled and aerated tank. It is the ideal method for transporting freshwater prawn post larvae from the hatchery holding tanks to the pond site. However, they are rarely available and require high cost compare using the plastic bags. The aerated garbage can be used for the travelling time of up to one or two hours to the pond site. For 100 liter trash can able to hold 50 L of water and hold 50 000 PL (New, 2002).

Post larvae may be transported for short distances, up to one hour's in aerated tanks at 750/liter but for long journey inflated plastics bags in insulated containers are preferred with 125-250 PL/ liter. Better survival rates during transport can be achieved if the post larvae are at least 7 days metamorphosis, the water are slightly brackish and the temperature is reduced (FAO, 2008). In Thailand, post larvae are transported in plastic bags with stocking density of 300-400/liter (Vorasayan, 1983). The transport time can be as long as 24 hours if the temperature is low especially at night. According to Harrison and Lutz (1980), 7 day-old post larvae show higher survival after transportation than those only 1 day-old post larvae and transport PL in 15 % brackish water also can enhanced the survival of PL. The rate of survival more closely related to dissolved oxygen level than to any water quality parameter (Smith and Wannamaker, 1983).

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A study has shown that 90 % survival of PL was typically obtained if they were transported at 187-225/ liter for up to 24 hours and 112-187/ liter for 24-48 hours. Water used to transport PL sometimes fresh, sometimes brackish and temperature was 18-23°C. To buffer the water, Hydroxymethyl amino methane or any compounds are added and clinoptilolite may be put to freshwater transportation in order to absorb ammonia (New and Singholka, 1985). If the hatchery supplying PL is a long distance away, the supplier have to prepare and pack PL carefully for transportation to avoid any stress. When PL arrived at their destination, they should not be released quickly into the pond to avoid sudden changes in water temperature or water quality that can harm them and the temperature of transport water should be adjusted gradually to the temperature of pond water by floating the bags in the pond for 15-20 minutes before they being released from plastic bags (Nandlal & Pickering, 2006).

2.5 Transportation Stress In *M. rosenbergii* PL

Various approaches have been developed in order to maximize health and survival of PL during transportation that involved packaging in saw-dust or hessian or immersion in tank that have been specially designed (Fotedar, 2011). For marine prawn, anaesthetic usually being used, however they are not approved for use in some countries such as United State of America (USA). There are some codes of practice have been establish to provide technological guidelines in handling and transportation of live crustaceans (APEC, 1999). Recommended practices vary by species as crustaceans vary in their tolerance to environmental perturbations and most crustaceans transported for live market or chilled prior to transport to minimize stress. According to APEC (1999), live shrimp should be cooled to attain lethargy and the temperature should be lowered slowly because rapid chilling can cause loss of legs and claws.

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Mortality and morbidity during post-capture storage and live transport are the most frequently the results of stress response that caused by exposure to physical handling and adverse environmental condition (Borton and Iwana, 1991). The stress response is an adaptive mechanism that interact with stressor in order to maintain homeostatic state. It include a series of sequential events or responses that start with an initial neuroendocrine reaction (Borton, 2002). The primary responses involves the releases of some hormones that interfere physiological stasis. The secondary response characterized by physiological adjustments that reflected by changes in hematological features, ion and metabolite levels and also the release of heat shock or stress protein (Pickering, 1981). Tertiary response are the last stage of the sequence and it is the only obvious evidence of stress that include immune suppression that will cause increased susceptibility to the infection and can be observed by the appearance of physiological and clinical sign of diseases (Buchanan, 2000).

2.6 Factors Affecting Transportation of *M. rosenbergii* PL

Determining the optimum condition to achieve maximum survival and condition of post larvae and juveniles during transportation are very important. Transportation is a traumatic procedure because it involve the adverse stimuli which are the capture of post larvae, the transfer into the containers, then transports itself and also the transfer of the PL from the containers to the rearing unit. All the process can cause high mortality if the water quality declined during the transportation (Sperandio *et al*., 2014). The survival of the PL for transportation are depends on the stocking density, temperature, dissolved oxygen and the time of travel.

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2.6.1 Water Temperature during Transportation

There are effect of water temperature on live shipping. When the temperature of water is lowered, the oxygen consumption rate and the waste excretion rate of the prawn are also decline. Sun (2009) have shown that the low temperature can improve survival rate of *M. Rosenbergii* during the transportation. (Alikunhi 1980) has suggested for short distance of transportation of small seed, feeding and low temperature can decrease cannibalism and increase the survival rate of PL. The recommended water temperature are about 19 °C – 20 °C (Smith et al., 1983). The temperature should be lowered according to the destination and transportation method. For the short travelling distance with the hauling tanks, the temperature must be reduced only to 23 °C -24 °C.

If the ambient air temperature during the transportation with the hauling tanks is expected to be high which over 24 °C is, the water temperature must be regulated with the ice packs. If the large quantity of ice is used, it must be packed in sealed bags to avoid large salinity changes (Rome, 2003). Chen & Kou (1996) have discovered that high temperature during transportation can lead to increase the oxygen consumption and nitrogenous excretion, therefore the acceptable biomass density or travelling time for transportation should be decreased.

2.6.2 Stocking Density for Transportation

Packing density of PL per litter of water is important factors during the transportation. It is because when the packing density is more, the rate of the survival of PL is less. (Krishna Kumar and Pillai 1984) has calculated based on the experiment of seed transportation, the number of PL required for initial packing at different densities and

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also the quantity of water needed to pack them into plastic. Alias and Siraj (1988) have identified that the survival of the PL in the plastic bags 27-28 °C is remained above 85 % at the densities up to 300 L^{-1}, but it decreases significantly after the packing density increased. Singholka (1983) have used inflated plastic bags in the experiment and managed to achieve over 95 % survival with 18h of transport period with a packing density of 300/L. During the transportation from the hatchery to grow out ponds took about 24 to 36 hrs, the PL still survives in the plastic bags (New *et al*., 2009).

2.6.3 Dissolved Oxygen

Dissolved oxygen is one of the important water quality parameter for most of aquatic organism. It also can affect various physiological process. Low supply of oxygen or known as hypoxia has been identified can inhibit molting, and disturb the growth and can cause mortality to the *Penaied* prawns (Clark 1986). Dissolved oxygen level also can fluctuate in the freshwater habitat, especially in the bottom of grow out ponds where the place that the prawns spend most of their life (Cheng *et al*., 2003). In addition, for *M. rosenbergii* the survivability rate is highly dependent on the dissolved oxygen than any other water quality parameter (Avault, 1987). The dissolved oxygen levels that decreased to 1 ppm can still be tolerated (Avault, 1987). However, when dissolved oxygen level drop below 4 ppm, it will contribute to the stress to the PL. The managers of the farm should keep the dissolved oxygen level at 6-8 ppm (New, 1990).

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2.6.4 *Artemia* as Live Food Source

Artemia is also known as brine shrimp and zooplankton such as Daphnia and Copepod have been used widely as live food for marine and crustacean larval culture (Das *et al*., 2012). The demand for *artemia* cyst has increased gradually from a few metric tons annually to the approximately 800 metric tons for every year and represent about 40 % total of demand of aquaculture for feed the early stages. The *artemia* cyst can be hatched under the optimum condition, constant temperature range 25 – 28 °C, the pH of 8.0, salinity at 15-35 ppt and strong illumination of 2000 1x (Lavens & Sorgeloos, 1996). *Artemia* has high nutritive value and also high conversion efficiency.

For transportation, the *artemia nauplii* are added 15- 20 nauplii per PL as food source to the PL for every hours of transport (Rome, 2003). *Artemia nauplii* are added to the plastic bags before transport to prevent cannibalism among prawn PL. Plastic bags should be place in polystyrene boxes during transportation. Mohamed & Devaraj (1997) suggested that 5 *artemia* per ml of water will enhance the survival of PL for long journey transportation. Laven & Sorgeloos (1996) claimed that freshly hatched *Artemia nauplii* can ensure proper ingestion for the PL.

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CHAPTER 3

MATERIALS AND METHODS

3.1 Sample Collection and Transportation of *M. rosenbergii* PL

A total of 2000 *M. rosenbergii* PL were collected from Pusat Penetasan Udang Galah (PPUG) located in Setiawan Perak. *M. rosenbergii* PL were transported in the plastic bags (19 x 8 inches) containing dissolved oxygen to the Animal Husbandry Laboratory, Jeli Campus, University Malaysia Kelantan (UMK). The distance between PPUG and UMK is about 300 km which took approximately 6 hours journey.

3.2 Experimental Design

The experiment was divided into four factors which were different stocking densities of PL, different water temperatures, the presence of *artemia* as live feed and lighting factor (dark and sunlight). Tap water were used in this experiment and anti-chlorine was put into the water before fill in into plastic bags. Water quality parameters (pH, salinity and dissolved oxygen) were measured using Hanna's portable multiparameter. This study evaluated the effect of different stocking densities 60, 80 and 100 PL per ½ L of water, different water temperature 23 °C (with ice), 25 °C (with ice) and 30 °C (without ice), presence and absence of *artemia* as feed and lighting factor which is the plastic bags contain PL was put in the dark place while another one was put in place that exposed to the sunlight. Each treatment was replicated three times.

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For different stocking density, the number of PL used were 60, 80 and 100 PL per plastic bags with three replicates while other three treatment using equal amount of PL in which 60 PL per plastic bags. The corners of plastic bags are rounded with rubber bands in order to prevent the PL from getting trapped during packing. PL were counted and then put in plastic bags containing tap water according to their own treatments. The first layer of plastic bag was hold above water level and the air from inside the bag was squeeze out. Then, the bags were inflated with oxygen gas until about 2/3 of the bag and there was enough room for twisting and tying the bags. The plastic bags containing PL were quickly closed to trap the oxygen and sealed with rubber band. After that, the plastic bags were put into Styrofoam boxes. After reach UMK, mortality data on the PL samples were recorded.

Table 3.1 Parameters and treatments used in the present study

Parameters	**Treatments**		
	T1	T2	T3
Stocking densities of PL	60	80	100
Water temperatures(°C)	23	25	30
Presence of artemia	With *artemia*	Without *artemia*	-
Lighting factor	Dark place	Exposed to sunlight	-

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3.2.1 Different Stocking Densities of PL

Different stocking density of PL were investigated. Three different stocking densities that consist of 60, 80, 100 PL for every plastic bags. The plastics bags filled with ½ L water. PL were counted and inserted to plastic bags according to the stocking density that have been set. Each plastic bags was oxygenated with pure oxygen by withdrawing the hose from an oxygen tank into plastic bags and then sealed with rubber band to hold it closed and gas-tight. Then, the plastics bags containing PL were inserted in Styrofoam boxes. The treatment was replicated three times. After transportation, mortality rates of PL were recorded.

3.2.2 Different Water Temperature (°C)

This experiment evaluated the use of different water temperature during transportation. The PL were divided into three plastics bags with the same stocking density. Three water temperatures for the transportation of PL were investigated are 23 °C (with ice), 25 °C and 30 °C (without ice). The density of PL was set at 60 PL per plastic bags. The mercury thermometer was inserted into the plastic bags containing PL to monitor the temperature for the whole travelling time. Then, the plastic bags were oxygenated and strong rubber bands are used to hold it closed and gas tight. The plastic bags containing PL were transported in the Styrofoam box. To maintain the temperature, the ice were inserted for every two hours interval. The experiment was replicated three times. After the transportation, mortality rates of PL were recorded.

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3.2.3 Presence of *Artemia* as Feed

The artemia were hatched with their optimum condition at PPUG Perak. After about 15 hours, the *artemia* were harvested. The *artemia* were used as feed during transportation. In this experiment, PL were treated with and without *artemia.* The density of the PL for each plastic bags was 60 PL per plastic bag that contain ½ L of water. For treatment without *artemia,* the plastic bags contained 60 PL in ½ L of declorinized tap water. For other treatment, *artemia* were added into the plastic bags containing 60 PL in ½ declorinized tap water. Each plastic bags were oxygenated with pure oxygen and then sealed with rubber bands. The plastic bags then were transported in Styrofoam boxes. After transportation, the survival and mortality rates of the PL from each plastic bags were recorded. Both treatments were replicated three times.

3.2.4 Lighting Factor

Plastic bags containing PL were put in the dark place while another one was put in place that exposed to the sunlight. The stocking density of PL for this treatment were 60 PL per plastic bags that filled with ½ /L of water. Atmospheric air were removed and then the plastic bag were oxygenated with pure oxygen by inserting the hose of oxygen tank into plastic bags. After reach at Umk Jeli, all of the PL were removed from the plastic bags. Then, the survival and dead PL were recorded. The treatment have replicated three times.

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3.3 Data analysis

The statistic were expressed as the mean ± standard error. Mortality data and the parameter treatment were analyzed by using one way Analysis of Variance (ANOVA) available from Statistical Package for the Social Science (SPSS) software version 19.0 (IBM Corp., Armonk, New York). 5 % level of significance was used to determine the differences in comparing three stocking densities (60, 80 and 100 PL), three different water temperatures (23 °C, 25 °C and 30 °C), presence of *artemia* as feed and lighting factor. The survival rates were calculated by using formula as below:

$$\text{Survival rate (\%)} = \frac{\text{Number of live PL}}{\text{Total number of PL}} \, X \, 100\%$$

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CHAPTER 4

RESULTS

4.1 Effect of stocking density

Survival of PL at lower stocking density, 60 and 80 did not differ significantly. The survival of PL at stocking density 60 were 96.11 ± 0.56 % while lower rates of survival were observed at stocking density 80 and 100 of PL in which 95.83 ± 0.42 % and 94.67 ± 0.33 %, respectively. There was no significant difference (p>0.05) for survival percentage among stocking densities.

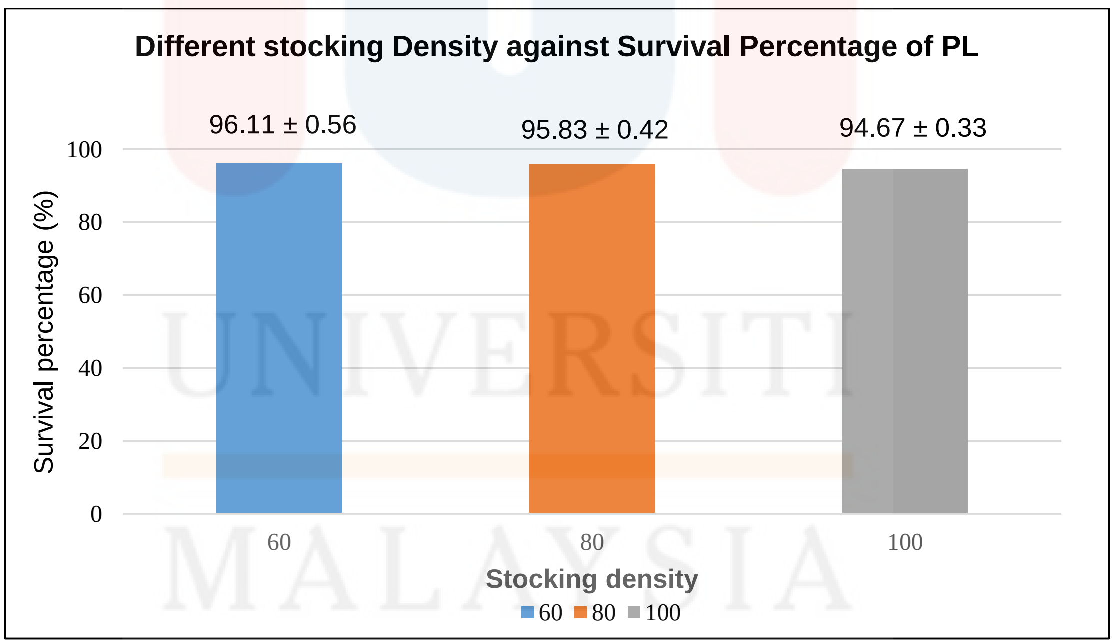

Figure 4.1: Effect of different stocking density towards survival rate of PL. The highest survival percentage is at stocking density of 60 PL. Stocking density had no significant different (P>0.05) impact on survival of PL. The statistic were expressed as mean ± standard error.

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4.2 Effect of water temperature

Survival rate of PL were higher at water temperature 23 °C (with ice) compared to those stocked at 25 °C (with ice) and 30 °C (without ice). The survival of PL at water temperature 23 °C, 25 °C and 30 °C in which 96.11 ± 0.56 %, 95.00 ± 0.96 % and 91.67 ± 0.96 %, respectively. There was significant difference ($p<0.05$) among water temperature treatments.

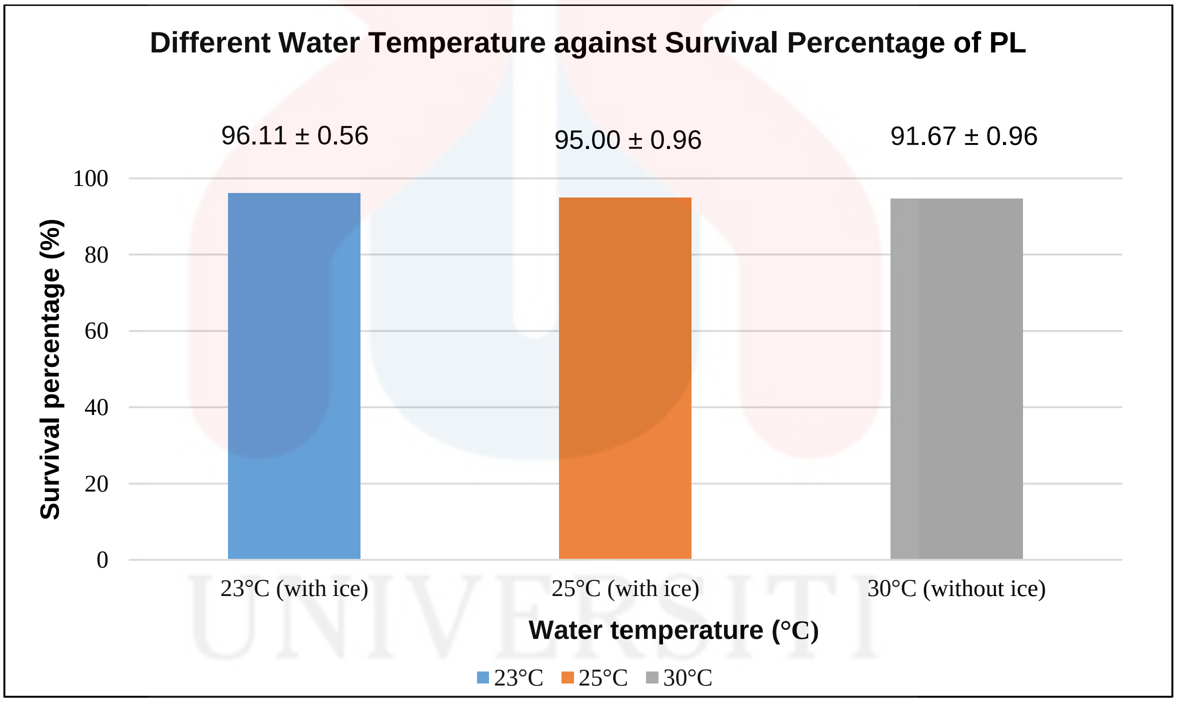

Figure 4.2: Effect of different water temperature towards survival rates of PL. The highest survival percentage found to higher at water temperature 23 °C (with ice) and the least survival percentage of PL were at water temperature 91.67 % (without ice). The statistic were expressed as mean ± standard error.

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4.3 Effect of presence of *artemia* as feed

In this experiment, PL in the plastic bags were treated with and without *artemia* during transportation. There was no significant difference (p> 0.05) on survival of PL which averaged 96 % overall. The survival of PL that contained *artemia* as feed (97.78 ± 1.11 %) found to be higher compared to PL without *artemia* (94.44 ± 2.00 %).

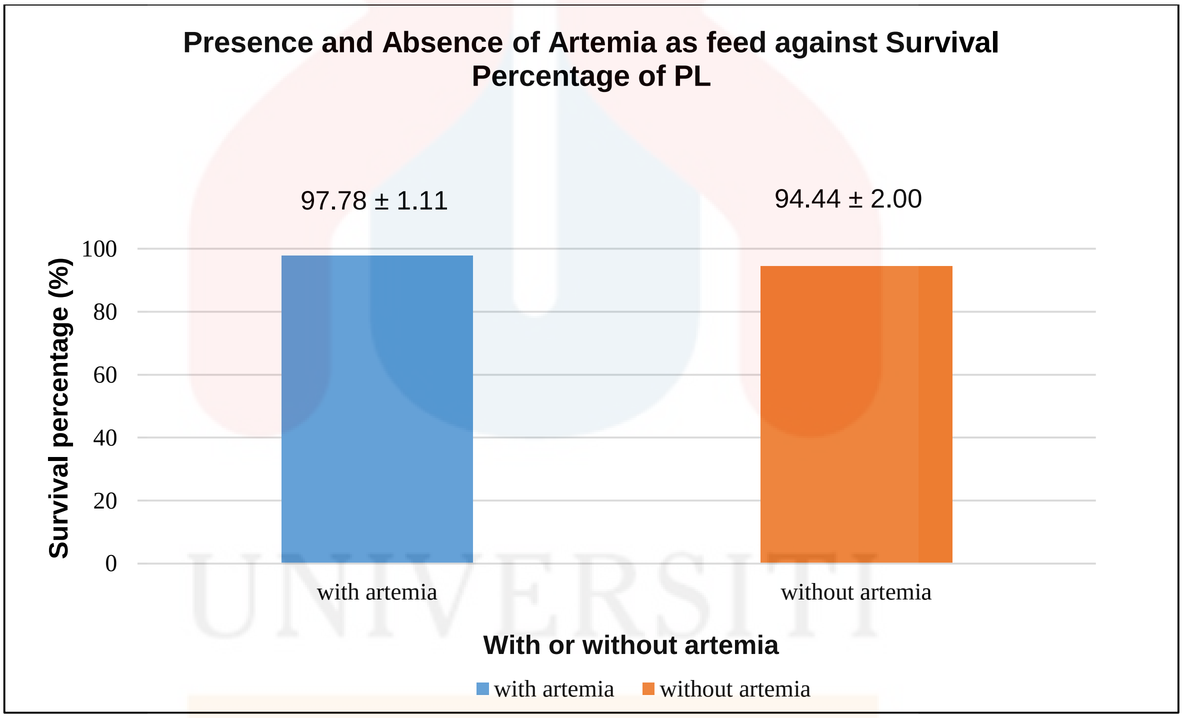

Figure 4.3: Effect of presence and absence of *artemia* as feed during transportation towards survival rates of PL. Survival of PL with *artemia* as feed found to be slightly higher compared to PL without *artemia* during transportation. The statistic were expressed as mean ± standard error.

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4.4 Effect of lighting factor during transportation

Based on figure 4.4 below, it was shown that survival of PL that were put in the dark place found to higher (96.67 ± 0.96 %) compared to the PL that were put in place that exposed to sunlight (92.78 ± 1.47 %). The effect of lighting factor during transportation had no significant difference (p> 0.05) among treatments.

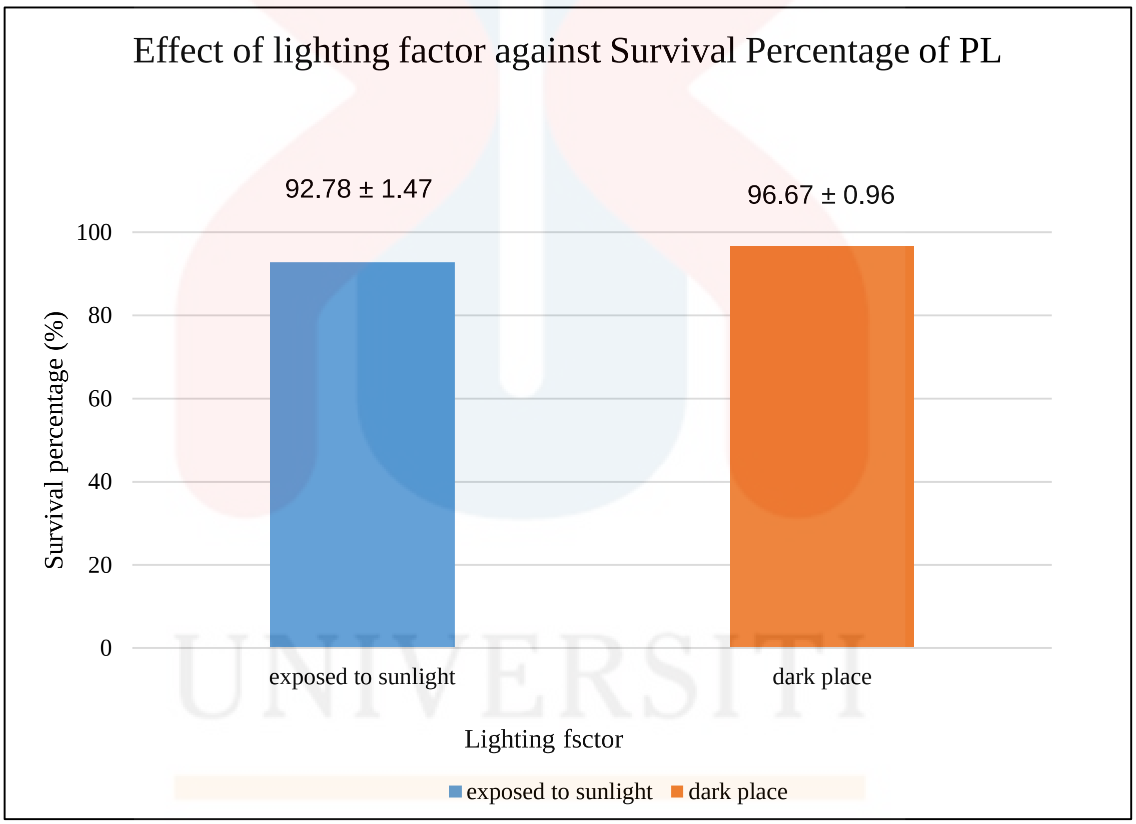

Figure 4.4: Effect of lighting factor towards survival rates of PL. Survival of PL located at dark place found to higher compare to PL that exposed to sunlight. The statistic were expressed as mean ± standard error.

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CHAPTER 5

DISCUSSION

Transportation of aquaculture species involve many aquaculture operations from one facility to another, during restocking practices, from a hatchery to rivers, lakes and ponds. Transportation is known to cause stress to the species and leads to physiological responses (Pankhurst, 2011). In order to maintain health and maximize long-term survival of aquaculture species for transportation, several common stressor should be considered which included loading and handling operations that consist of handling, confinement, unfavorably high densities and degraded water quality condition (Sutphin & Wu, 2008). The transportation of live organisms that do not take into account the health and welfare of animal can result in high mortality. Mortality could occur because transport is traumatic procedure that involve adverse stimuli succession including capture, transport and storage of animals (Robertson *et al*., 1988; National Research Council, 2006). Loading and transportation of aquaculture species at high densities can cause low water quality condition, mechanical abrasion and physiological stress which can result in poor survivability of the species (Urbinati, 2004).

In this study, effect of stoking density showed that there was no significant difference ($p>0.05$) in survival of PL on different stocking densities, 60, 80 and 100 PL per ½ L water. This experiment does not affect survival of PL during transportation because the stocking densities tested still within the optimum range to transport PL. Furthermore, due to some unavoidable limitations such as lack of facilities and financial problem, only small number of PL were used in this experiment. The PL need to be counted carefully

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according to the stocking densities that have been determined. Therefore, there is no effect of stocking density on the survival rate of PL. According to New (2002), a 100 L container able to hold 50 000 PL in 50 L of water and the baffles should be inserted in the container in order to prevent excessive water movement during transportation. In short journey, larger and open plastic bags about 1 m^3 that contain 500 L of aerated water can hold about 500 000 PL. For longer distances, double plastic bags contained 1/3 water and 2/3 oxygen can be use and 250-400 PL can be filled in each liter of water and a 45 x 80 cm plastic bag holding 8 L of water can be filled with 2000-3000 of PL (New, 2002).

The effects of different water temperature in this study showed significant different ($p<0.05$) impact on the survival of PL. Three water temperatures used in which 23 °C, 25 °C and 30 °C and the survival rate found to be higher at temperature 23 °C (with ice) and the least is at 30°C. It is because water temperature 23 °C is the optimum temperature for transporting PL that can contribute to the higher survival. Ice packs were used and placed in the box containing plastic bags, so it will contact with air and help reducing water temperature. Based on the result, survival percentage were least at water temperature 30 °C and no ice packs were used and it may cause the temperature rise during transportation. Without ice, the low water temperature cannot be maintained and it will contribute to the stress to PL and then caused the mortality of PL in the plastic bags. Maintaining water temperature at 23 °C is important to minimize the metabolic activity of the prawn, thus decreasing the usage of oxygen.

Temperature is a controlling factor in physiology of poikilothermic species (Dove et al, 2005). The changes of water temperature can cause stress to living organism and adversely affect their health (Yu *et al.*, 2009). Stress induced by water temperature changes were associated with enhanced generation of reactive oxygen species (ROS) and oxidative stress (Lushchack & Bagnyukova, 2006). Low water temperature should be maintained and avoiding disturbances during transport may maximize organism survival (Whiteley & Taylor, 1992) and low production of ROS due to decreased shrimp metabolism. Metabolic activity can be reduced when the temperature during transport is low (New, 2002).

Mohamad and Devaraj (1997) stated that water temperature plays major role in the rate of metabolic including O_2 consumption, CO_2 production and excretion of NH_3 and also random swimming activity of the poikilotherms. When water temperature containing PL increases, swimming activity of PL will increase and result in high metabolic rates and then it will cause dissolved oxygen decreases (Mohamad & Devaraj, 1997). To overcome the problem, water temperature should be reduced to 17 °C to 18 °C because it will help to reduce metabolic rates, swimming activity and cannibalism.

The presence and absence of *artemia* as feed in this study showed no significant different ($p>0.05$). The survival rate of PL transported with artemia found to be higher compared to PL without *artemia*. It is because when *artemia* was added as feed, cannibalism among PL during long distance transportation can be avoided. The survival percentage of PL transported without *artemia* as food source was lower, perhaps due to cannibalism that occur among them. During growth, cannibalism is a common and

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problematic within post larvae. The dead PL would be eaten by others. According to Krishna Kumar and (Pillai 1984), when the travelling time of PL increases, the survival rate would be decreased when 8 days old post larvae were transported for 24, 36 and 48 hours. Poor survival of early PL is caused by frequent moulting and behavior of cannibalistic of the early post larvae stages.

According to Alikunhi (1980), *artemia nauplii* that freshly hactched can be added as feed to the PL because it can help to reduce cannibalistic behavior among PL during transportation. Some pieces of nylon net also can be used in order to guard PL from attacking each other. Adding 5 *artemia* per ml of water for long travelling time were suggested because it can enhances survival of PL. Addition of phytoplankters like *Chaetoceros* sp. would reduce NH_3 and toxicity, but light required for photosynthesis. In the absence of light for photosynthesis, the phytoplankters would compete with PL for oxygen for respiration and addition of substratum that contained denitrifying bacteria bring down the ammonia level (Mohamad & Devaraj, 1997).

According to Primavera (1983), several factors such as size (age), number of fry and duration of transport or distance would contribute to increased activity thereby require corresponding inputs for instance oxygen and low water temperature because it will help to reduce metabolism or activity of the PL so that they spend less energy, consume less oxygen, do not require feeding and they would produce less waste. If they are fed, water becomes polluted and if they are hungry, they would cannibalize each other.

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In this study, the effect of lighting factor during transportation had no significant effect on PL survival. The survival of PL that were put in the dark place found to higher (96.67 %) compared to the PL that were put in place that exposed to sunlight (92.78 %).This is may be due to condition of weather during the travelling time which is in cloudy state, so it does not significantly affect PL survival. However, PL that transported in the boxes (dark place) show higher survival because low water temperature can be reduced. PL that were placed exposed to the sunlight during transport show less survival compared to dark place may be due to the stress response of PL because of raising in water temperature in the plastic bags. The containers should be kept in the shade, away from sun and other sources of heat during transportation and if possible the transportation should be done late afternoon or early morning in order to avoid high daytime temperatures (Primavera, 1983).

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CHAPTER 6

CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

Factors that contribute to the stress on *M. rosenbergii* PL such as the different stocking density, different water temperature, presence of *artemia* as food source and the lighting factor during transportation have been determined. The present study showed that water temperature has a significant effects on PL survivability while no significant effects on PL survival for effect of stocking density, presence of artemia as feed and lighting factor. Survivability of PL could be maximized if all parameters used in the present study were kept optimum.

6.2 RECOMMENDATION

As for recommendation, in order to achieve high survival of post larvae during transportation, the optimum condition should be determined first. Several methods for transporting PL also can be practiced by using tank and plastic bags that contain oxygen. The use of new water also should be avoided for packing and it is because when new water is used, most of the PL will moult during transportation and cannibalism among PL may occur. Sampling and counting of PL should be accurate in order to avoid overcrowding or underutilizing the transportation activity. Transportation is acknowledged to be successful when the survival after stocking is more than 90%. I also want to recommend that more research about transportation of prawn should be conducted in future because transportation are very important to ensure successful of aquaculture farming.

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Appendix A

Table A.1: The survival percentage of PL against different treatments

Treatments	Survival percentage of PL (%)			
	R1	R2	R3	Average
Stocking density				
i) 60	95.00	96.67	96.67	96.11
ii) 80	96.25	96.25	95.00	95.83
iii) 100	95.00	94.00	95.00	94.67
Water temperature (°C)				
i) 23 (°C)	96.67	95.00	96.67	96.11
ii) 25 (°C)	93.33	95.00	96.67	95.00
iii) 30 (°C)	90.00	93.33	91.67	91.67
Artemia as feed				
i) With artemia	96.67	96.67	100	97.78
ii) Without artemia	98.33	91.67	93.33	94.44
Lighting factor				
i) Place exposed to sunlight	95.00	90.00	93.33	92.78
ii) Dark place	96.67	95.00	98.33	96.67

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Table A.2: Analysis of variance (ANOVA) on the different stoking densities during transportation

Source of variance	Sum of Squares	df	MS	F Tabular 5%	Sig
Between groups	3.532	2	1.766	2.970	0.127
Within groups	3.568	6	0.595		
Total	7.100	8			

ns- not significant

Table A.3: Analysis of variance (ANOVA) on the different water temperature during transportation

Source of variance	Sum of Squares	df	MS	F Tabular 5%	Sig
Between groups	32.123	2	16.062	7.424	0.024
Within groups	12.982	6	2.164		
Total	45.105	8			

s- significant

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Table A.4: Analysis of variance (ANOVA) on presence or absence of artemia as feed during transportation

Source of variance	Sum of Squares	df	MS	F Tabular 5%	Sig
Between groups	16.700	1	16.700	2.125	0.219
Within groups	31.430	4	7.857		
Total	48.130	5			

ns- not significant

Table A.5: Analysis of variance (ANOVA) on lighting factor during transportation

Source of variance	Sum of Squares	df	MS	F Tabular 5%	Sig
Between groups	22.698	1	22.698	4.907	0.091
Within groups	18.504	4	4.626		
Total	41.202	5			

ns- not significant

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Table A. 6: Main effect means* of PL survival (%), dissolved oxygen (mg/L), salinity (ppt) and pH in plastic bags filled with *M. rosenbergii* post larvae at 60,80 and 100 PL per ½ L water for five hours of journey.

	Stocking density during transport			
	Initial	60 PL	80 PL	100 PL
Survival (%)	100	96.11 ± 0.56 a	95.83 ± 0.42 a	94.67 ± 0.33 a
Dissolved oxygen (mg/L)	2.05	1.67 ± 0.02 a	1.75 ± 0.05 a	1.69 ± 0.33 a
Salinity (ppt)	0.30	0.25 ± 0.08 b	0.24 ± 0.01 ab	0.07 ± 0.03 a
pH	7.05	6.84 ± 0.09 a	6.93 ± 0.06 a	7.03 ± 0.05 a

Table A. 7: Main effect means* of PL survival (%), dissolved oxygen (mg/L), salinity (ppt) and pH in plastic bags filled with 60 *M. rosenbergii* post larvae per ½ L water at water temperature 23°C, 25°C and 30°C after five hours of journey.

	Water temperature for transportation			
	Initial	60 PL	80 PL	100 PL
Survival (%)	100	96.11 ± 0.56 b	95.00 ± 0.96 b	91.67 ± 0.96 a
Dissolved oxygen (mg/L)	2.60	1.72 ± 0.02 a	1.72 ± 0.01 b	1.65 ± 0.02 a
Salinity (ppt)	0.25	0.02 ± 0.01 a	0.15 ± 0.01 b	0.17 ± 0.03 b
pH	7.15	6.87 ± 0.09 a	6.98 ± 0.03 a	6.90 ± 0.11 a

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Table A. 8: Main effect means* of PL survival (%), dissolved oxygen (mg/L), salinity (ppt) and pH in plastic bags filled with 60 PL per ½ L with and without artemia as feed after five hours of travelling time.

	Presence of artemia		
	Initial	With artemia	Without artemia
Survival (%)	100	97.78 ± 1.11	94.44 ± 2.00
Dissolved oxygen (mg/L)	2.00	1.76 ± 0.02	1.69 ± 0.01
Salinity (ppt)	0.20	0.09 ± 0.02	0.15 ± 0.02
pH	7.10	6.87 ± 0.08	6.94 ± 0.07

Table A. 9: Table A. 7: Main effect means* of PL survival (%), dissolved oxygen (mg/L), salinity (ppt) and pH in plastic bags filled with 60 PL per ½ L that were placed in the dark and light place after five hours of travelling time.

	Lighting factor		
	Initial	Dark place	Light place
Survival (%)	100	96.67 ± 0.96	92.78 ± 1.47
Dissolved oxygen (mg/L)	2.69	1.64 ± 0.02	1.72 ± 0.02
Salinity (ppt)	0.27	0.22 ± 0.01	0.16 ± 0.02
pH	7.05	6.79 ± 0.11	6.78 ± 0.11

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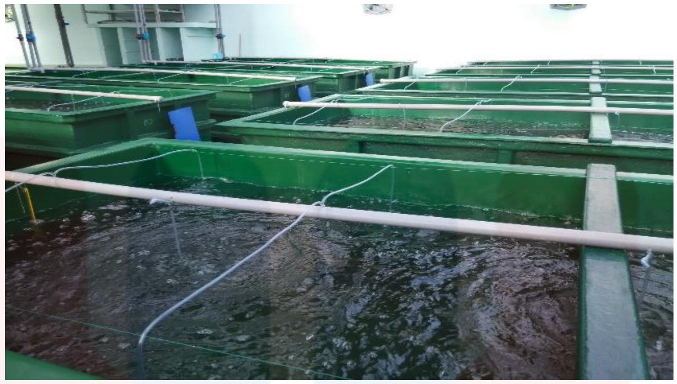

Figure A. 10: The tank of post larvae at PPUG

Figure A. 11: The plastic bags used to fill PL for transportation

Figure A. 12: The method of harvesting artemia

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Figure A.13: Filling oxygen by withdrawing the hose from oxygen tank

Figure A. 14: Styrofoam boxes used for PL trasportation

Figure A. 15: Styrofoam boxes contain PL safely arrived at UMK

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