THE CORRELATION BETWEEN FECUNDITY WITH LENGTH AND WEIGHT OF *Macrobrachium rosenbergii* AT BALAI RINGIN AND MALUDAM, SARAWAK.

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DECLARATION

I hereby declare that no portion of the work referred to in this dissertation has been submitted in support of an application for another degree or qualification to this university or any other institution of higher learning.

________________________

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The Correlation Between Fecundity with Length and Weight of *Macrobrachium rosenbergii* at Balai Ringin and Maludam, Sarawak.

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ABSTRACT

This study was conducted to determine the correlation between fecundity with length and weight of *Macrobrachium rosenbergii* at Balai Ringin and Maludam from August 2013 to January 2014. Prawns were collected using three layer gill net and a traditional method called ‘Selambau’ after an overnight of net deployment. A total of 34 berried female samples collected. The fecundity of 27 samples and fertility of 7 samples has been estimated through gravimetric and volumetric approach respectively. With the fecundity and fertility estimated, the relationships with the female body size have been expressed in a linear regression with positive correlations. The number of eggs produced from females of *Macrobrachium rosenbergii* in Maludam river is much higher of about 48% for weight dependent and 3% higher for length dependent than Batang Kerang floodplain’s females. Meanwhile, the number of hatched larvae produced for Batang Kerang floodplain’s females is much lower than the number of eggs produced of the same area, of 32% lower for weight dependent and 37% lower for length dependent.

Key words: Maludam; Batang Kerang floodplain; *Macrobrachium rosenbergii*; giant freshwater prawn; fecundity; fertility; gravimetric; volumetric; body relationship

ABSTRAK


Kata kunci: Maludam; dataran banjir Batang Kerang; *Macrobrachium rosenbergii*; udang gergasi air tawar; kesuburan; gravimetric; isipadu; hubungan badan
1.0 Introduction

*Macrobrachium rosenbergii* (de Man, 1879) is a giant freshwater prawn that is widely distributed in most of the tropical and subtropical areas of the Indo-Pacific Region, including Malaysia, Thailand and Philippines. This freshwater prawn belongs to the Palaemonidae Family, where *Macrobrachium* is the largest genus in the family. This crustacean is commonly found along the coastal river system, where the adult lives in freshwater but a brackish environment is required for the larvae to develop (Mohd-Shamsudin et al. 2013). Locally known in Malaysia as ‘Udang Galah’ or in Sarawak as ‘Apek Sepit Biru’, this long-legged freshwater prawn is commercially important because of its size as well as its eating flesh qualities and it is getting more attention from the aquaculturists due to its high market demand (Habashy, 2011).

Many studies have been done on the biological, morphological and behavioral aspect of *M. rosenbergii* that contribute in aquaculture development (Rashid et al., 2013; Jee & Kook, 1991; Mejia-Ortiz et al., 2001; Cavalli et al., 2001). One of the works done on biological aspect of *Macrobrachium* sp. prawn is the study of fecundity on ovigerous female by Bhuiyan et al. (2007). The term fecundity is referred to the number of eggs borne in the brood pouch during a single pawning act. Meanwhile fecundity – size relationship of *M. rosenbergii* is referring to the correlation of the number of eggs with their body size, the length and also body weight of the prawn. The information of fecundity and its correlation with the body size is important in estimating the reproductive potential of brood prawns, which in turn, can greatly help the management strategies of prawn hatcheries (Sharma & Subba, 2005). There are numerous works has been done on fecundity of *M. rosenbergii*, such as in Bangladesh by Rashid et al. (2013), Malaysia by Jee & Kok (1991), Mexico by Mejia-Ortiz et al. (2001) and Belgium by Cavalli et al. (2001). However, with the increasing development of aquaculture, the study of the
relationship of fecundity with length and weight of *M. rosenbergii* in Malaysia is vital, especially in Sarawak coastal river system.

Giant river prawn samples were collected from Batang Kerang floodplain and Maludam River. The main objective is to compare the established correlation between fecundity with length and weight of *M. rosenbergii* between the two sites, Batang Kerang floodplain, Balai Ringin and Maludam River, Maludam. The specific objectives of the study are as below:

i. To identify all berried female samples of *Macrobrachium rosenbergii*.

ii. To culture the eggs of *Macrobrachium rosenbergii* until all are hatched.

iii. To estimate the fecundity and fertility of each samples on each sites.

iv. To compare the relationship of fecundity and fertility with length and weight between two sites.
2.0 Literature Review

2.1 *Macrobrachium rosenbergii* (de Man, 1879)

*Macrobrachium rosenbergii* (de Man, 1879) are referred to as freshwater prawns in Australia but as freshwater shrimp in the United States of America (USA). This difference is because the use of those terms is geographically dependent. In Malaysia, this genus is referred as freshwater giant prawn. This freshwater prawn species is the major commercial species in farming. It is an indigenous species in South and Southeast Asia and it has been exported into many tropical and subtropical areas.

The giant freshwater prawn can be found in inland freshwater areas namely rivers, lakes, canals and ponds, and also estuarine areas. The giant freshwater prawn require brackish water in the early stages of the life cycle although it completes in saline inland water and extremely turbid water conditions (New, 2002).

*Macrobrachium rosenbergii* belongs to the Caridea family, a group of prawns which carry their eggs attached to modified abdominal appendages for a period of incubation. The term spawning refers to the release of eggs from the genital pore prior to attachment to the pleopods. Unproductive spawning refers to eggs, which were initially attached to the pleopods but which later became detached (Wowor & Choy, 2001).
2.1.1 Morphology of *Macrobrachium rosenbergii*

![Figure 1: The external features of Macrobrachium rosenbergii (New, 2002).](image)

Figure 1 showed the external features of *Macrobrachium rosenbergii*. According to New (2002), *Macrobrachium rosenbergii* can be differentiate from other species by the following characteristics:

i. It is the largest species in *Macrobrachium* genus, where the adult female’s body length can reach up to 29 cm while male’s up to 33 cm

ii. Its rostrum has 8-10 ventral teeth and 11-14 dorsal teeth

iii. Tip of the telson reaches beyond the posterior spines

iv. The adult male has a very long second chelae, have blunt spines and are elongated

v. In adult male, a velvet-like fur covers one of the finger in the second chelae
2.1.2 Life Cycle of *Macrobrachium rosenbergii*

There are four stages in the life cycle of the giant freshwater prawn. This include and they are adults, postlarvae, larvae and eggs stages. During mating, the male will discharge gelatinous semen, called spermatophore onto the underside body of the female. According to New (2002), ideal mating will only occur with ripe females that have just finished the pre-mating moult. This usually happens at night, thus resulting in either hard shelled and soft shelled males (New, 2002). Mating of giant freshwater prawn occurs all around the year, although sometimes there are peaks, that relates to environmental factors. In tropical areas, the mating season relates with the rainy season, meanwhile in temperate areas the mating occurs in the summer.

Within the few hours of mating, the eggs are discharged out from the gonopores and led by the ovipositing setae of the female into the brood chamber. Ovipositing setae is the stiff hair located at the root of the pleopods of a female. Meanwhile, at the same time, the eggs will be fertilized by the spermatophore that has been attached earlier onto the exterior body of the female. The female held the eggs in the brood chamber and are stuck onto the ovigerous setae. The eggs are kept well aerated by the result of vigorous movements of the pleopods (Gupta et al., 2012).
The period of time that the female carry the eggs is normally not more than three weeks. The body size of a female affect the number of eggs they can lay per one spawning. A fully mature female of *M. rosenbergii* can lay up to 80 000 - 100 000 eggs in one spawning. However, the female in their first broods or that are in their first year, usually lay eggs around 5 000 to 20 000 only (New, 2002). Normally female of *M. rosenbergii* becomes mature when they reached body weight of 15-20 g but it has been observed that a female with the weight 6.5 g is found to be berried.

The vigorous movements of the pleopods of the female will disperse the larvae as the eggs hatches. This process is normally completed in one or two nights. *M. rosenbergii* larvae are planktonic and they swim actively on their tail first with the ventral side uppermost. According to New (2002) *M. rosenbergii* larvae requires brackish water condition for survival. The larvae that hatch in freshwater will not survive unless they reach brackish water within a few days. In hatchery conditions, giant freshwater prawn larvae have been found to complete their larval phase in as shortly as 16 days. Larvae eat

*Figure 2: The eggs of Macrobrachium rosenbergii on the berried females will attach inside the pleopod until they hatch; as they ripen, it change from orange (right) to grey/black (left) (New, 2002).*
continuously and their diet is zooplankton, a very small worm, and other aquatic invertebrates that are in the larval stages (New, 2002).

On the completion of the larval phase, freshwater prawns will metamorphose into postlarvae. Starting from this phase, the freshwater prawns resemble a miniature adult prawn and are now able to crawl. Even so, according to Harpaz (1997) when a postlarva of *M. rosenbergii* do swim, it is often in a normal and forward direction. Postlarvae have a great tolerance on salinities at a wide range. This is because in this phase, the postlarvae begin to migrate into freshwater condition on the upstream in one or two weeks following their first metamorphosis (New, 2002).

### 2.2 Culture of Larvae

There are many aspects to be concerned regarding the culture of larvae for hatchery. The factor that affects the growth of the eggs of *Macrobrachium rosenbergii* are the condition of tank, air supply, water distribution, water discharge, presence of light, filtration equipment, and also miscellaneous equipment such as pails, weighing scales, nets, tools, flexible tubing, brushes, postlarval transport equipment namely bags, tanks, and portable air supply, spares for electrical equipment, disease prevention drugs and chemicals, spares for PVC pipe work and valves, refrigerator, feed preparation equipment, stereo microscope, salinometer, pH meter, beakers, heaters, glass jars and various chemicals (New, 2002).

The berried females should be hold in brackishwater of around 5 ppt salinity, at temperature of 25-30°C and ideally at pH 7.0 - 7.2. A slight salinity will give a better egg hatchability and a research by New (2002) shows that control of pH significantly improves the hatching rate. Temperatures below 25°C is ideal for fungal growth on the eggs and also
will cause unbalanced time for eggs development. Meanwhile, temperatures of above 30°C will promote the development of protozoans and other micro-organisms (Gupta et al., 2012). Light does not affect egg-hatching rate but direct sunlight penetration must be avoided. According to New (2002), there is no need to feed the females when the females are being kept for a few days only for larval collection.

In hatcheries, a collecting device is usually used to harvest the newly hatched larvae from the broodstock tank. Then after the eggs have hatched, a coarse dip-net is used to remove the females. An easier way to remove the female is by putting the females into cages with coarse mesh placed within the tanks. When the females are in the larvae rearing tank, the level of the water must be around 30 cm, the salinity and pH must be about 5 ppt and around 7.0 - 7.2 respectively. When the females has been removed, the water level must be raised to the normal level, which is around 70 - 90 cm and the salinity should be adjusted back to the normal larvae rearing level, which is around 12 ppt. When the larvae can be seen in the tank and there is no eggs on the underside of the female, it means the eggs are hatching, and which it mostly occurs at night (Harpaz, 1997).

2.3 *Artemia*

The success on the commercial production of *Macrobrachium rosenbergii* post larvae and seeds of other crustacean decapods depends on the efficient use of available food sources. The dependence on live food imposes difficulties to define adequate and economical food management during the larval phase. Newly hatched *Artemia* nauplii constitute the principal live food used in the larviculture of crustaceans of commercial value. *Artemia* nauplii presented as the microcrustacean food source to the *M. rosenbergii*
has the advantages of easy handling and high protein content (De Barros & Valenti, 2003). The *Artemia* cysts are available to buy nowadays.

There are several things need to consider when hatching the *Artemia* cysts. Those are the temperature, illumination, aeration, pH, salinity and cyst density. The best hatching equipment to use is a conical-shaped container and it must be well aerated from the bottom. This is because, if cylindrical or square-bottomed containers used, there will be dead spots in the container, which *Artemia* cysts will accumulate and then suffer from the lack of oxygen. A container with transparent or translucent wall is better because it will help with the inspection of the cysts when harvesting. The ideal oxygen level must be more than 2 mg/l, preferably around 5 mg/l (Lavens & Sorgeloos, 1996). Thus the intensity of the aeration must be enough to maintain the oxygen levels. Meanwhile, the temperature should be in the range of 25 - 28°C. This is because below 25°C the cysts will hatch more slowly and if it is above 33°C, the metabolism of the cysts will be irreversibly stopped. The salinity ranging of 5 - 35 ppt will give optimal hatching, where at 5 ppt salinity the nauplii hatches faster. Besides that, the pH must be kept above 8 so that the hatching enzyme will functions at its optimal (Lavens & Sorgeloos, 1996). Strong illumination is essential and the maximum cysts density should only be at 5 g/l. This is to minimize any mechanical injury of the nauplii that may occur and hence avoid the suboptimal water conditions (Lavens & Sorgeloos, 1996).

### 2.4 Ooctye Treatments

Ooctye is the term used for the eggs produced by the berried female animals (New, 2002). According to Lowerre-Barbieri and Barbieri (1993), it is crucial to have a method of ooctye preservation which does not damage or destroy oocytes and has a determinate
effect on oocyte size. There are many different methods of treatments that can be applied onto the oocytes and the first one is the Gilson’s solution. It is a solution comprised of 100 mL 60 % ethanol, 880 mL glacial acetic acid and 20 g of mercuric chloride. The benefit of using this solution is its ability to harden oocytes while chemically separates from the ovarian tissue (Lowerre-Barbieri & Barbieri, 1993). Despite the benefits, a number of problems have been associated with this method, including the degeneration of hydrated oocytes, substantial and continuous oocyte shrinkage, a relatively long fixation period of several days to a few weeks and the extreme toxicity of mercuric chloride (Lowerre-Barbieri and Barbieri, 1993). The other method of oocyte treatments is by using different concentrations of alcohol (Rakka & Ganias, 2011). Rakka and Ganias (2011) tested four different types of treatment on the oocytes, which are 25%, 50%, 70% of alcohol and the other one is 10% formalin. It was found that the treatments that caused the greater changes in oocyte size were those of 50% and 70% alcohol while solutions of 10% formalin and 25% alcohol had marginal effect on the oocyte size. Thus it is safer to treat the oocyte with 10% formalin, as this concentration does not greatly affect the size of the oocyte.

2.5 Fecundity

Fecundity may be defined as the spawning potential of fish for a particular season or alternatively, the number of ripening eggs in a female prior to the next spawning period. Fecundity values are often used to calculate egg and fry survival, and ultimately, recruitment rates. According to Rashid et al. (2013), fecundity was defined as the number of eggs laid per hatching that was found to adhere to the female pleopods. Thus, fecundity data are important to theoretical and empirical studies of early life-history strategies and are essential for management policies directed toward fish stocks.
There are several approach in estimating fecundity, which are gravimetric, volumetric, combined gravimetric and automated particle counting method, stereometric method, and auto-diametric method. Different approach has different advantage and disadvantage. The gravimetric method is currently the most common method used to estimate fecundity. It is based on the relation between ovary weight and the oocyte density in the ovary. This method can be used to estimate batch fecundity, total fecundity and potential annual fecundity (Hunter et al., 1989). The volumetric method is based on the same principles as the gravimetric method, but uses ovarian volume and the subsample volume instead of ovary weight and subsample weight (Simpson, 1951). The combined gravimetric and automated particle counting method is a variation of the gravimetric method with the major difference being that an automated particle counter is used to enumerate the number of oocytes in a subsample (Kraus et al., 2000). The stereological method is based on the Delesse principle, which states that the fractional volume of a component is proportional to its fractional cross sectional area (Murua et al. 2003). An underlying assumption of this principle is that the component is distributed randomly and evenly through the tissue. The auto-diametric method estimates the potential fecundity from the mean vitellogenic oocyte diameter and the total ovary weight using a calibration curve that relates mean oocyte diameter to oocyte density (Thorsen and Kjesbu, 2001).

2.6 Fertility

Measure of reproduction is called the fertility. The difference between fertility and fecundity is that fertility means the actual production of live offspring while fecundity only means the ability to produce live offspring. Fertility depends on factors of sexual behavior, nutrition, timing, way of life, endocrinology, instinct and emotions (Siswanto, Muhamad,
Omar & Karmawati, 2009). According to Da Silva, Sampaio and Santos (2004), to determine the fertility, ovigerous female prawns were stored in individual 10 L glass tanks maintained under strong aeration and once it hatches, the larvae were siphoned and counted. According to New (2002), the newly hatched can be counted by using the volumetric method of fecundity estimation, where it uses ovarian volume and the subsample volume instead of ovary weight and subsample weight as described by Simpson (1951).

2.7 Relationship of Fecundity-Size and Fertility-Size

The most common body size to relate with the correlation of fecundity of an organism is body weight and length. This is because according to King (1998) the absolute fecundity is positively correlated with its size such as body weight. Commonly, the trends of relationship between fecundity and body parameters, length and weight, were estimated by the formula: \( F = a + bX \). Where, \( F = \) fecundity, \( L = \) body length, \( W = \) body weight, \( b = \) slope and \( a = \) constant. The coefficient of correlation \( (r) \) of each of the relationships was also assessed (Rashid et al. 2103). The formula is known as the linear regression expressed in a linear graph.

Statistical analysis is commonly used to compare the relationship and determine whether the estimation made is significantly different between the variables involved. Examples of statistical test found commonly used are ANOVA, Student t-Test and Coefficient of Variation. These test and many other statistical tests are crucial to support the finding and to determine whether the finding turned out as expected or otherwise. Even so, there are a few statistical software’s that is available and much easier to conduct the test, such as IBM SPSS Software.
3.0 Material & Method

3.1 Sampling Site

Prawn sampling were carried out at Batang Kerang Floodplain, Balai Ringin and Maludam River, Maludam by using 3-layers gill net with 10 cm mesh size on both sides of the outer layers and 4 cm of the inner layer. Another method used to catch prawns were ‘Selambau’, a traditional method still used in Batang Kerang floodplain. Sampling stations at Batang Kerang floodplain and Maludam River were determined using GPS (Garmin) on the day of sampling. Field sampling were carried out several times throughout the period from August 2013 to January 2014. The gill nets and ‘Belat’ were placed at a suitable depth at the selected stations and left for about 1-2 hour.
Figure 3: Maps showing the Batang Kerang floodplain (above) and Maludam River (bottom) sampling site.
3.2 Identification and Measurement

All collected berried female prawns were identified using keys identification of *Macrobrachium rosenbergii* from Rowel & Holthiew. The alive berried females were measured and stored in a plastic containing aeration and brackish water collected from the river. Length measurements were carried out by using a long ruler with centimeter (cm) unit. The total length was measured from the start of the rostrum till the end of the telson as shown in Figure 4. The samples were wrapped up of tissues to remove the excess water for weighing using a weigh balance to the nearest of 0.01 g. Then the samples were photographed for future reference. Then all samples were grouped together in a plastic bags equipped with a portable aerator and filled with freshwater each, meanwhile for the dead samples, immersed in 10% Formalin with their correspond labels for transportation to laboratory in FRST, UNIMAS.