



Faculty of Resource Science and Technology

**Investigating the Effect of Mixed Meals on Egg Development of A Mud Crab,
*Scylla olivacea***

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Declaration

I, **Lee Winnie**, declare that this final year project report entitled **Investigating the Effect of Mixed Meals on Egg Development of A Mud Crab, *Scylla olivacea*** and the work presented in the report are both my own, except as cited in the references and have been generated by me as the result of my own original research. I confirm that:

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Abstract

The genus *Scylla* is commonly known as mud crab or mangrove crab, inhabit muddy substrate in estuaries and mangrove areas. Aquaculture of mud crabs is expanding due to increasing demand on crab meats in Asia. Mud crab wholesale prices may exceed MYR 80+/kg during peak season, as many people consider it a delicacy. The study of egg development crabs by applying alternate feeding technique are still poorly discovered. Hence, the study regarding egg development of mud crab by applying alternate feeding are important in order to provide farmers with a consistent supply due to the shortage in Malaysia and even other countries around the world. The study was conducted at Puncak Innovasi Sdn. Bhd., Kampung Seri Lalang, Batu Enam, Kluang, Johor with the Recirculation Aquaculture System (RAS). The source of brood stocks, female mud crab *Scylla olivacea* had been provided by ABCrabs Trading, Kluang. Alternate feeds such as squid, mussel, fish and cockle were fed in this study. The stages of ovarian maturity and the weight of mud crabs were recorded as proxy of egg development because it is correlated to the egg development. One-way ANOVA had been used to study the data collected. The repeated measure ANOVA test showed that the mixed feeds did not give significant effect on egg development of mud crab, *Scylla olivacea*.

Keywords: *Scylla* species, brood stocks, egg development, alternate feeds, one-way ANOVA

Abstrak

Genus *Scylla* biasanya dikenal sebagai ketam lumpur atau ketam bakau, mendiami substrat berlumpur di kawasan muara dan bakau. Akuakultur ketam lumpur berkembang kerana permintaan yang meningkat terhadap ketam di Asia. Harga borong ketam lumpur mungkin melebihi MYR 80+/kg pada musim puncak, hal ini kerana banyak orang menganggapnya sebagai makanan istimewa. Kajian mengenai ketam pengembangan telur dengan menggunakan teknik pemberian makanan alternatif masih kurang ditemui. Oleh itu, kajian ini adalah penting untuk memberi petani bekalan yang konsisten kerana kekurangan di Malaysia dan bahkan negara-negara lain di sekitar dunia. Tapak kajian dijalankan di Puncak Inovasi Sdn. Bhd., Kampung Seri Lalang, Batu Enam, Kluang, Johor dengan Sistem Akuakultur Recirculation (RAS). Sumber stok brood, ketam lumpur betina *Scylla olivacea* telah dibekalkan oleh ABCrabs Trading, Kluang. Makanan alternatif seperti sotong, kupang, ikan dan kerang telah diberi makan dalam kajian ini. Tahap kematangan ovari dan berat ketam lumpur dicatat sebagai proksi perkembangan telur kerana ia berkorelasi dengan perkembangan telur. ANOVA sehalu telah digunakan untuk mengkaji data yang dikumpulkan. Ujian ANOVA ukuran berulang menunjukkan bahawa makanan campuran tidak memberi kesan yang signifikan terhadap perkembangan telur ketam lumpur, *Scylla olivacea*.

Kata kunci: Spesis *Scylla*, stok brood, perkembangan telur, makanan alternatif, ANOVA sehalu

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List of Abbreviations

AM	Before midday
PM	After midday
Temp	Temperature
NH ₄	Ammonia
DO	Dissolved oxygen
pH	Potential hydrogen

1.0 Introduction

1.1 Problem Statement

The aquaculture sector is forecasted to grow with 4.46% CAGR (compound annual growth rate) for the year 2019-2022. The main driver of growth is due to the rising population (which is expected to reach 8 billion at the year 2022), along with the decline in the captured fisheries. The food production will have to increase by 70% within the next 40 years to feed the population. Mud crabs (*Scylla* spp.) aquaculture have also been forecasted to grow due to the high demand in Asia. Mud Crab wholesale prices can reach up to MYR 80+/kg at peak season, as it is considered a delicacy for many. Hence, investors have joined the mud crab industry aiming at filling the gaps of the industry.

Furthermore, the mud crab culture sector is also experiencing a supply shortage especially from the wild source. Mud crabs are vulnerable to overfishing due to their high pricing. Mud crabs larger than 500 grams have been difficult to come by in Malaysia, which was not the case previously. In other countries, such as Thailand, India, and Sri Lanka, have had similar issues. Traders in Sri Lanka have also started seeing the effects of overfishing, with big crab population starting to decline. Apart from overfishing, mud crabs are also threatened by habitat loss. Mud crabs are widely found in mangrove habitats, which are also being degraded as a result of clearance, firewood production, and pollution. More than 35% of the world's mangrove forest is already gone and is classified as one of the most threatened ecosystems. Countries that are still sourcing mud crabs directly from the wild include Indonesia, Malaysia, Bangladesh.

1.2 Research Question

The shortage in wild stocks has driven the need for aquaculture of the *Scylla* species. Despite the industry attempts to culture mud crab, the outlook remains bleak due to lack of

commercial hatchery. The aim of the hatchery is to be able to supply the farmers with a consistent supply of instar stage 1 (C1) or instar stage 2 (C2) crablets. Therefore, as part of the development plan, this project were cooperated with aquaculture business, Puncak Innovasi Sdn. Bhd. and a mud crab hatchery was set up with RAS (Recirculating Aquaculture Systems).

Therefore, RAS system were planned and designed for the broodstocks maturation tank and formulation of alternate optimal diet for the broodstocks maturation process. Moreover, the optimal water quality for the broodstocks maturation process were determined for enhanced growth of mud crabs and enhanced egg development of mud crabs. The research question of the study is does mixed meal has significant affect on egg development of mud crab cultured under Recirculating Aquaculture System (RAS).

1.3 Hypothesis

H₀: There is no effect of mixed meals on the egg development of *Scylla olivacea*.

H₁: There is effect of mixed meals on the egg development of *Scylla olivacea*.

1.4 Objectives

The gaps of study of enhanced egg development of mud crabs by using alternate feed are still needed to be filled. Hence, the study regarding the rate of egg development of mud crabs are important in order to provide farmers with a consistent supply due to the shortage in Malaysia and even other countries around the world.

This projects aims to achieve below objectives:

1. To investigate the effect of mixed feeds for egg development of mud crabs, *Scylla olivacea*.
2. To describe the water quality of the experiment.

2.0 Literature Review

2.1 Genus *Scylla*

Scylla is a genus of decapod crustacean that found in many parts of the Indo-West Pacific. *S. serrata* is the most widespread species, but it is the only species that has been found and recorded in the Western Indian Ocean, the South Pacific islands and Japan. *S. tranquebarica* and *S. olivacea* have distributions centred on the South China Sea and spreading into the Indian Ocean and the western Pacific respectively, whilst *S. paramamosain* emerges to have a more restricted distribution, largely circumscribed to the South China and Java Seas (Keenan, 1999).

Mud crabs are mainly established in muddy habitats in estuarine and protected coastal environments, and big populations are normally found in distinguished mangroves, particularly in estuaries. On the other hand, the factors influencing the local organization as well as exuberance of the four mud crab species which included *S. serrata*, *S. olivacea*, *S. tranquebarica* and *S. paramamosain* are likely to be complicated. At the larval or juvenile stages, four *Scylla* species may have differed in their salt tolerance or proclivity suggested by Keenan et al. (1998).

2.2 Taxonomic Classification

The Linnean system classify the organism into eight ranks or hierarchy from domain to species. The mud crab is classified in the infraorder Brachyura of family Portunidae and the genus of *Scylla* (Keenan et al., 1998; Ng et al., 2008).

The genus was revised by Estampador (1949a) which three species and one synonym of *Scylla* were identified in the Philippines utilising burrowing habits, colouring, morphological features, and chromosomal structure: *S. tranquebarica*, *S. oceanica*, *S. serrata* and *S. serrata* var. *paramamosain*. The genus *Scylla* was also studied genetically,

of three unique species which including *Scylla serrata*, *Scylla ocellata*, and *Scylla tranquebarica*, were discovered (Furuya and Watanabe, 1996). In the late 1990s, Keenan et al. (1998) was using genetic and morphometric analyses to modify the taxonomy, and four new species were found viz. *S. tranquebarica*, *S. paramamosain*, *S. olivacea* and *S. serrata*. The later investigation came to the conclusion that the four species could be distinguished based on morphological characteristics such as teeth appearance, colour, spination of the carpus and cheliped propodus. The capacity to differentiate the four species using external features gives a stable fundamental for a better knowledge of the biology, ecology, and management of mud crabs (Le Vay, 2001).

2.3 Morphological Characteristics of *Scylla*

There are a few major key characteristics that can be used to distinguish *Scylla* spp. such as the arrangement and height of spines on a frontal lobe, as well as the presence of spines on the cheliped's propodus and carpus (Keenan et al., 1998). *Scylla serrata* which also called giant mud crabs have smooth carapace with solid transverse crest; usually green to olive-green in colour with marbled pattern; deep gastric groove in the shape of a H; sharp teeth on the frontal margin of carapace; usually marbled last legs which also called swimming legs and sharp granules on the palm and carpus, which are frequently spiniform (Forsskål, 1775). Moreover, for *Scylla olivacea* which have another name called orange mud crabs, the colour of carapace of *S. olivacea* is orange brownish to brownish green and sometimes orange to yellow colour. It also have smooth and more evenly convex carapace but with low transverse crest. It have a gastric groove in the shape of H but shallow not deep as *S. serrata*; rounded teeth on the frontal margin of carapace; and the middle pair of frontal lobes is more rounded than the lateral ones, and they extend somewhat forward (Herbst, 1796).

The colour of *Scylla paramamosain* is between *S. serrata* and *S. olivacea* which normally green to light green in colour of carapace, palm is typically green to greenish blue, and the bottom surface and base of the fingers are typically pale yellow to yellowish orange. In addition, the sharp tooth on the frontal margin is similar to *S. serrata*; palms with prominent, sharp spines are common in *S. paramamosain* (Estampador, 1949) which is opposite of *S. olivacea* because palms of *S. olivacea* normally are decreased, blunt spines. Furthermore, carapace colour of *Scylla tranquebarica* ranges from brown to practically black, and the chelipedal carpus with the palm both have well-developed spines on their outside surfaces. *S. tranquebarica* have more excessively triangular teeth on the frontal margin compared to the other three species, and sharp granules on the palm and carpus is same as *S. serrata* but it's never spiniform (Fabricius, 1798).

Commonly, the carapace of these four species of *Scylla* spp. is glabrous carapace and it is wider than it's long. While the shape of carapace is round and somewhat convex in form but smooth. Front teeth come in a variety of shapes because varies between species, from rounded lobes to pointed spines as the above mentioned for each species. While the width of the front different a bit for every species. Besides, genus *Scylla* have huge and unequal chelipeds that the walking legs may shorter than and the walking legs are same in shape for the first three pair while the forth pairs are peddle-shaped dactylus (de Hann, 1833). Female abdomen of mud crab is round and broad in shape, but male abdomen of mud crab is narrow in shape with 3-5 joined segments (Keenan et al., 1998).

2.4 Life cycle of *Scylla*

Mud crabs (*Scylla* spp.) have a catadromous life cycle in which adults spawn in the open ocean but juveniles move inshore. External characteristics such as the form of the abdominal flap can be used to determine sexes morphological easily. Even though the morphology of the flap is comparable in mature and immature males, the female flap

exhibits noticeable separation in both phases (Srinivasagam, 2000). Male mud crabs have an inverted 'T' shaped abdomen, whilst female mud crabs have a semi-circular abdomen. Furthermore, males have a greater chelate and are generally trimmer for body shape than females. Chelipeds can sometimes be used to detect sex from front because male chelipeds are bigger than female chelipeds.

Internally, a pair of testes, vas deferentia, and ejaculatory ducts are present in the male mud crab reproductive system, while outwardly, a pair of pleopods and auxiliary reproductive organs are found on the inner side of the abdomen. The ejaculatory ducts open into the genital papilla, which is a tiny genital papilla. Non-motile sperms and seminal plasma make up the ejaculate. The sperms are stored in the vas deferentia, and the pleopods aid the sperms move via the ejaculatory ducts (Quinitio et al., 2010). When the carapace width gets to 90 mm⁶, both male and female become sexually mature. Three phases of sexual maturity of male mud crabs can be identified: (i) The lack of a pronounced vas deferens and an immature-creamy/transparent colour (ii) The maturing-creamy white vas deferens takes up 1/4 of the body cavity, while (iii) the maturing-milky white vas deferens takes up the whole body cavity (Srinivasagam, 2000).

A pair of ovaries, seminal receptacles, a pair of oviducts, four pairs of pleopods (externally), and several auxiliary structures make up the female reproductive system. The seminal receptacles make up the majority of the oviduct, which opens to the outside via the female genital opening at the sixth thoracic segment (Quinitio et al., 2010). Ovarian maturing is divided into three phases: (i) immature, with a yellowish/transparent colour and no prominent seminal receptacle; (ii) maturing, with a pink colour and a seminal receptacle that occupies 1/3 of the body cavity; and (iii) mature, with an orange red prominent seminal receptacle that occupies the entire body cavity (Srinivasagam, 2000; Quinitio et al., 2010).

Female mud crabs are mating in the estuary habitat before migrating to the ocean which the place breeding occurs (Ong, 1966). Spawning appears to take place all year round, with occasional periodic surges. For tropical populations, these climax appear to be tied to the particular period of rainfall, but reproduction in temperate locations is more closely linked to the temperature, with spawning activity peaking in the summer months (Heasman et al., 1985). The zoeal larvae grow in the open ocean till they have progressed to the megalopa level, at which point they return to the estuary habitat (Keenan, 1999). The mud crab's life cycle may be divided into many phases such as larval stage (zoeal stage), megalopa stage, juvenile crab, young adult, mature crab (Australia, 2013; Quintio et al., 2010; Smallwood, 2013).

2.5 Hatchery and Nursery

Most of the shrimp hatcheries may be transformed into mud crab hatcheries for the development. To hatch mud crabs, there are a few facilities need to prepare such as broodstock tanks, maturation tanks, algal culture tanks, spawning tanks, *Artemia* hatching tanks (organisms that rich in protein), larval rearing tanks, reservoir tanks, and natural food tanks. Not only that, equipment and accessories are required for hatchery and nursery operations of mud crabs. Water quality need to be test by using the equipment such as refractometer and pH meter. Besides, refrigerators, weighing scales, drainers, beakers, test tubes and nets among other things, are critical in hatchery operations (Quintio, 2008).

Important criteria must be addressed while choosing a location for the crab hatchery. For example, electric power supply for the culture system, availability of freshwater and seawater, and suitable environment (Quintio, 2003). Water quality levels that are suitable for mud crabs broodstock are: 30-34 ppt salinity for *S. serrata* (lower salinity can be tolerant by *S. tranquebarica*, *S. paramamosain*, and *S. olivacea* however for

maturation and spawning, full strength saltwater is still preferable), temperature between 27 to 31°C, pH between 7.5 to 8.5, natural photoperiod and light intensity, dissolved oxygen more than 4 ppm and unionized ammonia less than 1 ppm (Quinitio, 2008). In hatchery operations, continuous aeration is essential to keep food particles and natural food suspended while maintaining ideal dissolved oxygen levels (Quinitio, 2008).

2.6 Nutrition Requirement of Mud Crab

The uniformity of feeding regime is the most important aspect of mass larval rearing of aquacultured species. *Scylla* mass rearing feeding regimens have yet to be established. During the development of mud crab larvae, nutrition has been recognised as a possible cause of mass death (Balasubramanian et al., 2014). When larvae were fed solely *Artemia nauplii*, they had a maximum survival rate of 26% from zoea to first crab instar were reported by Heasman and Fielder (1983), but Marichamy and Rajapakyam (1991) reported a maximum survival rate of 80 percent when larvae were given both rotifer and artemia nauplii.

Feed management is the most important aspect of successful aquaculture since feed is the primary input in crustacean aquaculture. Feed makes between 40-50 percent of overall operational expenses (Trino et al., 1999). Locally accessible inexpensive protein sources (trash fish, mollusks) can be provided at a rate of 8-10% of biomass in the grow out culture management. The crabs can be given a diet that includes bycatch (waste fish) and fresh mollusk or crustacean flesh such as green mussel, clam, or oyster, cause *Scylla* species are known to be predators, at least while they're fully grown (Hill, 1979). When the mud crabs' natural productivity is exhausted, "supplementary" feeding is necessary (Christensen, Macintosh, Phuong, 2004).

3.0 Methodology

3.1 Study Site

The study site was performed at Puncak Inovasi Sdn. Bhd., Kampung Seri Lalang Batu Enam, Kluang, Johor. The Recirculation Aquaculture System (RAS) for brood stock maturation tank, spawning tank and larvae tank have been planned and designed before starting the hatchery. Moreover, the materials needed for the hatchery have been purchased. The brood stock source have been provided by ABCrabs Trading, Kluang which live mature female mud crabs *Scylla olivacea* have been obtained from ABCrabs Trading.

3.2 Hatchery

After getting the crabs, the crabs would be disinfected with 150 ppm formalin about 30 minutes before putting them into the brood stock maturation tanks, this method is proposed by Qunitio et al. (2008) with slightly alteration. The purpose of disinfection was to eliminate bacteria and pathogens on the surface of crabs. The crabs were then acclimatized with the salinity started from 15ppt for 1 hour and increased the salinity by 2ppt after an hour until the salinity reached the same with the hatchery system at Puncak Inovasi Sdn. Bhd. Acclimatization is a natural process in which crabs gradually alter their physiological responses to changing salinity environments. It is the crabs' capacity to perform physiological adjustments in order to reduce the stress of a new climatic salinity environment. This helped the crabs to increase their salinity tolerance and survival rate under stress. The breeding cycle of mud crab is shown in Figure 1. For this project, only maturation stage of crabs were used. Therefore, the design of the RAS system for the maturation tanks is shown in Figure 2.

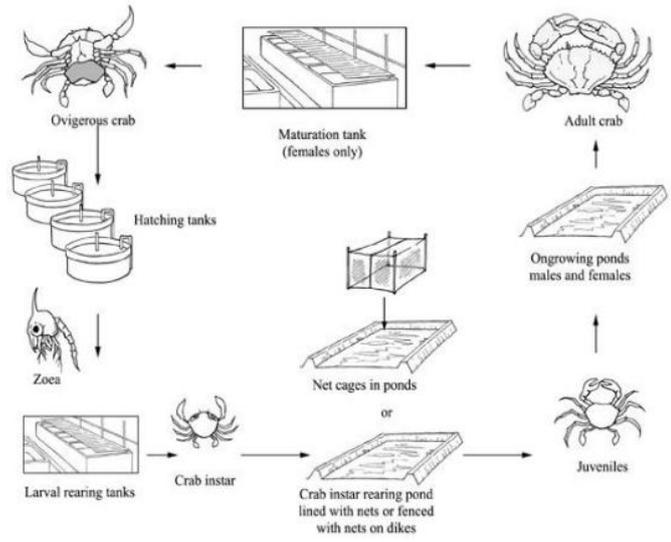


Figure 1: Induced breeding cycle of mud crab (Modified from the Protocol of Quintio, 2010).

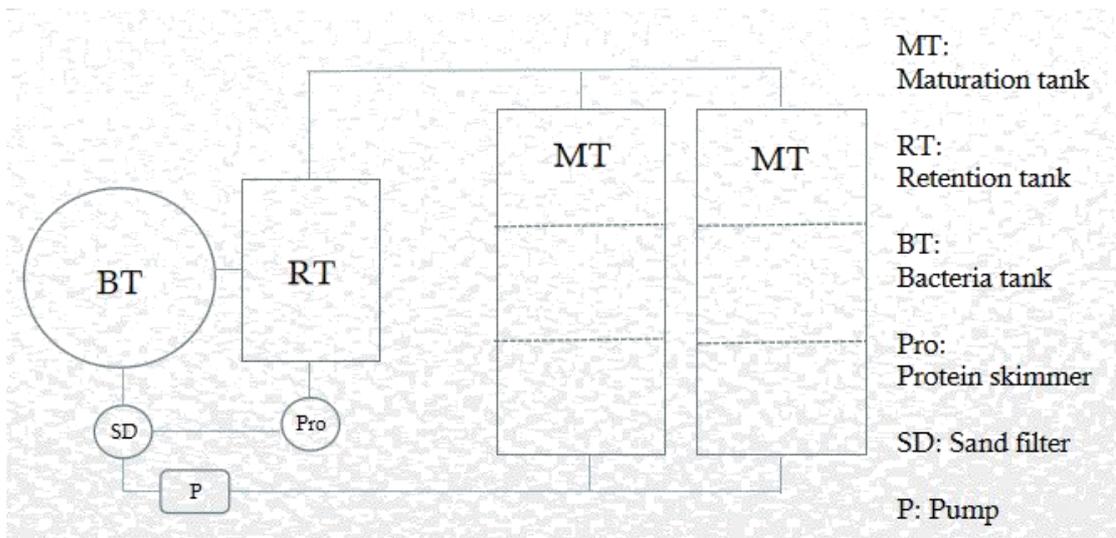


Figure 2: Design of experiment.

The induced maturation and breeding regimen should be completed within a week, suggested method by Quintio et al. (2008). Water quality for brood stock maturation process should be in an optimal range which show in the table below. The study had been done on the basis of completely randomized design with repeat measures. The environmental parameters in each tank were maintained at the same range as possible (Table 1) because the RAS system is a recirculation system which the saltwater cycle through the whole system.

Table 1: Optimal water quality for crab brood stock (Quinitio et al., 2008).

Parameter	Range
Temperature	27-30 °C
Salinity	20-34 ppt
Dissolved oxygen	>4 ppm
pH	7.5-8.5
Unionized ammonia	≤1 ppm
Photoperiod	natural
Light intensity	natural

3.3 Feeds and Feeding

Alternate feeding technique was applied in this experiment. The brood stocks were fed with feed at a rate of 15% body weight once daily after scooping out the leftover feed in the morning when the gonadal maturity stage of the samples was I, then dropped to 5% of their body weight when the gonadal maturity stage reached stage IV, method suggested by Herlinah (2015) with modification such as the mud crabs were fed twice a day which once in the morning and once in the afternoon. There were four types of feeds namely mussel, squid, cockle and trash fish that were given to the crabs at alternate times for one month (Table 2). Uneaten feeds should be scooped out from the tank and were recorded the weight of leftover feed each day after cleaning. The random sequences of feeding for 4 weeks are shown in the table below.

Table 2: Alternate feeding technique applied in the study.

Week Day	1		2		3		4		5		6		7	
	AM	PM												
1	Mussel	Cockle	Fish	Squid	Cockle	Mussel	Fish	Squid	Mussel	Fish	Cockle	Fish	Mussel	Cockle
2	Squid	Fish	Cockle	Mussel	Squid	Cockle	Fish	Squid	Mussel	Fish	Cockle	Mussel	Fish	Squid
3	Squid	Mussel	Cockle	Fish	Cockle	Squid	Mussel	Fish	Cockle	Mussel	Squid	Fish	Cockle	Mussel
4	Fish	Squid	Mussel	Cockle	Fish	Cockle	Squid	Mussel	Fish	Cockle	Mussel	Squid	Fish	Cockle

Mussel

Cockle

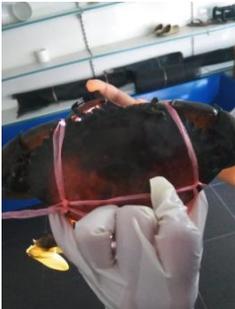
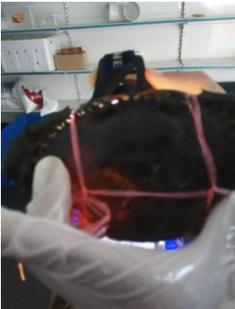
Fish

Squid

3.4 Data Collection

Weight of the feed were recorded everyday twice before feeding and weight of leftover feed were also recorded everyday twice after feeding. The gonadal maturity levels were observed by using visual observation every seven days to determine the stage of egg development, this method was similar with the method suggested by Herlinah (2015) with modification that observation would be done every seven days through light penetrating through the carapace to see the emptiness which reflected to the egg development. Moreover, the weight of the crabs was recorded every seven days too as proxy of egg development with the assumption that it was correlated to the egg development by feeding with mixed meals. The ovarian maturity stages could be identified through visual observation by light penetrating which showed in the table below (Table 3).

Table 3: Percentages of light penetrate through the carapace. The stage of egg determined by the percentage of light seen on the carapace with more light indicate early stage.

Stages	1	2	3	4
Pictures				
Percentages of light penetrate through (%)	> 75	51-75	26-50	0 - 25

3.5 Statistical Analysis

Descriptive statistics were used to summarize the data collected of mixed meals on egg development of a mud crab, *Scylla olivacea* by using measurement stages and weight. Repeated measures ANOVA was used to compare the difference of egg development among weeks. The data was tested normal prior to the analysis of variance.

4.0 Results

4.1 Body weight of berried mud crabs

In this experiment, egg development was measured based on the stage (see Tables 3 & 4), and the body weight of the berried crabs (Table 5). While the “stage” is a nominal data type, the body weight (g) is a continuous data that would usually provide a robust inference. Therefore, body weight of each berried mud crab was used as metric of measurement of its egg development. Additionally, the increase of the body weight is expected to proportionate to the egg development.

The stage of egg shows a gradual increase from Week 1 to Week 4 (Table 4), which take about 28 days before the berried mud crabs transferred into a spawning tank. Similarly, the mean of body weight of berried crabs has increased gradually with the exception in Week 2 that probably an error during the measurement (Table 5). The normality test of Shapiro-Wilk shows the stage data were not normally distributed, whereas the body weight of berried crab was normally distributed (Tables 4 & 5).

Table 4: Descriptive statistics for stages.

	Week 1	Week 2	Week 3	Week 4
Mean stage	1.429	1.714	2.286	2.571
Standard Deviation	0.535	0.488	0.488	0.535
Shapiro-Wilk	0.664	0.600	0.600	0.664
P-value	0.001	< .001	< .001	0.001

Table 5: Descriptive statistics for weight.

	Week 1	Week 2	Week 3	Week 4
Mean	347.143	362.857	352.857	365.714
Standard Deviation	56.188	68.243	54.989	42.762
Shapiro-Wilk	0.849	0.916	0.898	0.912
P-value	0.120	0.442	0.318	0.412